FoodIntegrity Handbook

A Guide to food authenticity issues and analytical solutions

edited by Jean-François Morin / Michèle Lees

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FOODINTEGRITY HANDBOOK

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Foreword by Mr. Van Goethem

Director DG Health and Food Safety, European Commission

The past half a century has seen a revolution in the way that food is produced, processed and marketed. Today, EU citizens are accustomed to choice, convenience, quality, and competitive prices when it comes to the food they buy.

The complex nature of our globalized food supply chains and the economic motivation to provide cheaper food products have contributed to the growing problem of food fraud, with recent scandals such as horse meat in beef products drawing worldwide attention. Fraudsters are becoming increasingly inventive in the deceptive tactics they are deploying to take advantage of the sophisticated nature of food supply chain. Because of its complexity and worldwide reach, reining in food fraud requires a collaborative effort between industry and government agencies. Preventive systems identifying problems at an early stage, preparedness at all levels and coordination are essential.

In this respect, the European Commission has taken action in creating the EU Food Fraud Network. EU Member States, supported by a dedicated IT system, can now rapidly exchange information on potential cross-border fraud. The Knowledge Centre for Food Fraud and Quality created on 13 March 2018 and operated by the Commission's Joint Research Centre complements the EU Food Fraud Network by providing an interface between science and policy-making.

At industry level, many companies have already implemented ways to counter global fraud threats, but more needs to be done. Industry alarms should go off whenever a commodity suddenly floods the market at a too-good-to-be-true price. New analysis tools are appearing to help alert industry and regulators in real time to potential problems. Some of the responsibility also falls upon consumers to remain vigilant and speak up when they witness what they believe to be fraudulent practices. Manufacturers can support this effort by helping consumers identify issues, giving them the resources to identify fraudulent products so that they know what to look for to avoid these products. Transparency and data-sharing between national governments, agencies and industry is key to detect and prevent fraudulent practices. The Food Integrity Project contributes to this goal by gathering experts from industry, academia, research institutes, technology providers and a global network of stakeholders. It is an international focal point for harmonisation and exploitation of research and technology for insuring the integrity of European food.

Thanks to thorough and detailed guidance, this Food Integrity Handbook will be an important resource to all of us when looking for information on food authenticity issues and will help to create a trusted food sector. The authors and all those who contributed to the handbook deserve special recognition for their work.

Foreword by Pr. Christopher Elliott OBE

Founder of the Institute for Global Food Safety
Queen's University of Belfast

The first handbook on food authenticity was published 20 years ago and it is quite remarkable to track the events that have unfolded over this period of time. Food authenticity or food fraud or as sometimes referred as food crime has become a widely discussed issue in many parts of the world. The melamine scandal in China dating back a decade now seems to have been the trigger to alert many stakeholders in governments, food industries and more importantly the general public of the impact cheating in the global food supply system can have.

The new edition of the handbook has sought to address the growing complexities of food and drink authenticity. It seems the ingenuity of those who set out to cheat us all knows no bounds. It also seems that virtually everything we eat and drink has some vulnerability to fraud and that individuals and potentially organised crime gangs will try to exploit these.

A major element in the fight against fraud is the development, validation and implementation of novel methodologies that can detect and often quantify the level of cheating that has occurred. There have been many innovations in analysis over the past two decades and the handbook gives some excellent examples of these and how they can be applied.

Another interesting and very worthwhile addition to this handbook is the horizon scanning which the authors have conducted. What will be the major challenges over the next 20 years and how will analytical science provide some of the solutions.

Introduction

The first “handbook” of this type dealing with food authenticity was published in 1998, the result of European collaboration through the EU-funded Concerted Action FAIM[1] which brought together over fifty scientists from food research institutes, from industry, from regulation authorities and from private laboratories. Their aim was to review the authenticity issues current at that time and to investigate the availability of analytical methods to address those concerns. The FAIM handbook contributors are given a special mention in the Acknowledgements section at the end of this book.

Twenty years on, through another, albeit much larger, European funded project, Food Integrity[2], a similar group of scientists have collaborated to produce an updated handbook on food authenticity issues and related analytical methods. A lot has changed in twenty years. The Food Integrity Handbook is not simply a revised version of the earlier FAIM book but does complement it in several ways. It has retained a very similar structure, which is repeated throughout the different chapters. On the other hand, it deals with a wider range of food products; it includes chapters on eggs and egg products, nuts, nut products and seeds, plant-derived sweeteners, spices, wines, spirit drinks, tea, flavourings and gelatine, in addition to the main food commodities - cereals, coffee, dairy products, fish and meat products, fruit juices, honey, oils and fats – dealt with in the FAIM Handbook. In addition this new Handbook does not have a separate chapter on the use of Chemometrics in food authenticity studies, which in the FAIM book reviewed some of the most important and useful concepts in multivariate chemometric/statistical methods. Two decades ago such concepts were still fairly new in food science whereas today they are widely used in the analytical field.

The Food Integrity Handbook has been written for food business operators and is primarily aimed at quality control managers working in food production and to those actors involved in the food supply chain. It may also be useful to young scientists starting their career in food science and to students and researchers with little prior knowledge of the area. The first section of this book provides the definitions of Food Authenticity and the different concerns that constitute Food Fraud, compiled in connexion with the work being undertaken in the Authent-Net project[3] to establish a European voluntary standard (a CEN Workshop Agreement, or CWA) entitled "Authenticity in the feed and the food chain - General principles and basic requirements".

[1] FAIM : Food Authenticity – Issues and Methodologies. Funded by the European Commission’s Agro-Industrial Research Programme under project No AIR2-CT94-2452. 1994-1998.

[2] Food Integrity - Assuring quality and authenticity in the food chain. Funded by the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No 613688. 2014-2018.

[3] Authent-Net – Food Authenticity Research Network. Funded under the European Union´s Horizon 2020 research and innovation programme under grant agreement No 696371.

Since this Handbook is intended as a simple, searchable guide to food fraud / food authenticity issues organised by product type, each of the food group chapters follows a similar structure, making it easier for the reader to find the information they are looking for. The format starts with a general overview of the product, with a short introduction to the industry sector, its importance in the global market, and how it may have changed in recent times as a result of consumer pressure or the implementation of new technology. Food products may be marketed in different forms, some of which may be linked to a specific manufacturing process or method of agricultural production. This type of knowledge is important in order to explain certain fraudulent practices, and essential when interpreting data from analytical tests.

Most food products are defined by a set of specifications, or "standard of identity", whether in legislation or in industry sector guidelines. These specifications, which often include specific compositional characteristics, form the basis for describing a foodstuff and for highlighting any deviations from this composition that could be due to mislabelling and/or economic adulteration. Current standards of identity or related legislation, both in the European Union and on the international level are provided for the different food commodities where available.

The prime focus of this Handbook is of course food authenticity and the analytical solutions available to address existing concerns. These are described in detail for each food product, starting with food fraud problems that are currently facing the food industry or have occurred in the past. Although the main motivation for food fraud is economic, there is increasing concern among both regulatory authorities and consumers about the potential health risk of a fraudulent practice. For example a cheap extender might be used that is allergenic, as in the case of nut protein in cumin spice. Where relevant, examples of the potential threat to human health are provided.

Then follows a review of the analytical methods used to test for authenticity. In this section care has been taken to highlight those methods that are most commonly used, and in particular, those that are officially recognised. In recent years, increasingly sophisticated techniques and instrumentation have been developed to detect adulteration and misrepresentation. These latest methods are included where they are accessible to routine use. When comparing the original FAIM Handbook and this present one, two major areas of analytical investigation stand out as different. One of these is the use of DNA techniques which, for a majority of the food products dealt with in the FAIM handbook, were only mentioned under the section on "potential methodologies" as techniques requiring further efforts before being accepted as routine procedures. As can be seen in this book, DNA techniques are now routinely and officially used for authentication purposes. One example is the verification of specific Basmati rice varieties from India and Pakistan that, under EU Regulations, are granted a zero rate of import duty on presentation of an authenticity certificate based on DNA analysis. The other major difference is the breakthrough in untargeted methods. These approaches employ various spectroscopic and/or chromatographic techniques, which can provide an entire analytical profile of a food product which can then be used to judge its authenticity. The most established in this area is based on NIR (Near Infrared) spectroscopy which is now widely used in various sectors and is particularly suitable as a rapid method for the at-line and on-line use (see for example the chapter on Cereals). A further example employs ^{1}H-NMR (Nuclear Magnetic Resonance) screening which, provided that appropriate statistical models have been established from authentic material beforehand, can evaluate a large number of analytical parameters related to quality and authenticity, simultaneously and in a few minutes (see the example given in the chapter on fruit juices).

One of the purposes of this Handbook is to help small and medium companies with setting up food fraud mitigation plans and therefore a section on additional tools for mitigating food fraud risk has been included towards the end of the document. This section provides information on the

different approaches to evaluate food fraud vulnerability, the importance of traceability in the food supply chain, and a “best practice” example of sector specific food fraud mitigation.

But what will the future look like in the next twenty years? The authors of each of the food product chapters have been asked to give their expert opinion on potential authenticity issues to look out for in the future, such as those that may not be economically viable now but may become so due to changing geopolitical situations, the effects of climate change and so on. They also provide an insight into where current research on authenticity techniques is heading and which analytical methods are on the horizon.

Food Fraud has been around a long time but following several highly mediatised incidents such as the milk and infant formula contaminated with melamine in 2008 and the horsemeat scandal in 2013, all authenticity issues have become big news. Regulators and customers now require food operators to keep abreast of any potential risks and to regularly assess their raw material and ingredient supply chains for vulnerability to food fraud. It is hoped that this Food Integrity Handbook will be a useful companion to help the food industry achieve this aim.

Definition of food fraud and food authenticity

The notion of food fraud and food authenticity received increased focus as a field of investigation by the research community and the food industry in the late 2000s, following highly mediatised crises such as the melamine scandal in China in 2007 and Horsegate in 2013, with adverse impact on vulnerable consumers for the former. These incidents led stakeholders to request a clear definition of food fraud including the identification of the different types of fraud as a first step toward combatting these practices.

Some early work was carried out in the United States by the Grocery Manufacturers Association [1], Michigan State University [2] and the US Pharmacopeial Convention (USP) [3]. After the horsemeat scandal, a series of high level reports was published by some national health authorities [4–6]. They all highlighted the importance of the standardisation of the terms related to food fraud.

In 2012, a 'Food Fraud Think Tank' was set up with the support of the Global Food Safety Initiative (GFSI), an public-private initiative, to explore food fraud issues. It published a document on food fraud mitigation in which it provided some definitions of the different types of fraud [7]. This collaborative work, some members of which took part in the FoodIntegrity project over the following years, is the basis of this chapter. Most of the definitions used in the following pages refer to it.

During another European research project named Authent-Net [8], a standardisation initiative within the framework of a "CEN/CENELEC Workshop Agreement" (CWA) has been launched in order to set up a first consensus-based terminology of authenticity and food fraud [9]. This working group has received input from scientists, industry organisations and other ongoing research projects, and in particular from FoodIntegrity. It has made terms and concepts related to food fraud clearer and more accurate, thus enriching the GFSI definitions. It is expected that this work will lay down the basis for future internationally standardised definitions.

The Codex Alimentarius Commission has established an electronic working group (eWG), chaired by the Islamic Republic of Iran and co-chaired by Canada and the European Union, whose mission has included the clarification of the definitions of food integrity, food authenticity, food fraud and economically motivated adulteration (EMA) in relation to Codex Committee on Food Import and Export Inspection and Certification Systems (CCFICS) texts. They have published a position paper [10] where key elements identified underlying these notions have been identified and definitions developed. This position paper will be used as a basis for initiating new work in this area, so as to provide guidance on how to assure the authenticity of food by minimising vulnerability to fraud and mitigating the consequences of food fraud.

Food fraud definition

In the vast array of issues which can be faced in the whole food supply chain, the GFSI Food Fraud Think Tank considered four categories related to food integrity (cf. Figure 1). Distinguishing between these categories requires putting oneself in the place of the person at the source of the issue. Is the action deliberate or unintentional? If unintentional, it is a food safety issue, when consumer health can be harmed, or simply a food quality issue.

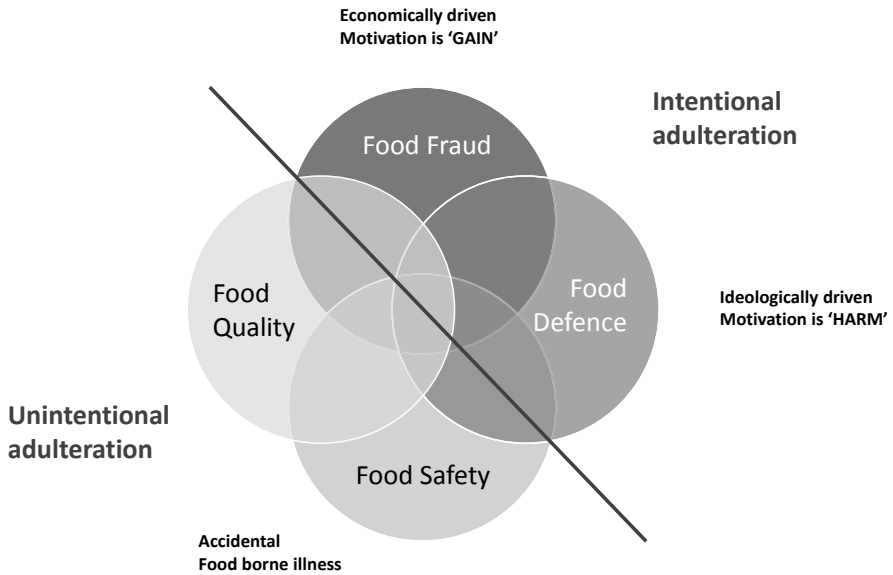

Figure 1: Difference between food fraud, food defense, food safety and food quality. Food fraud. Adapted from Food Fraud Think Tank [7]

But when the action is intentional, then the behaviour can be considered a crime. When the motivation of the criminal is to harm people, the type of action falls in the field of "Food defence" according to the GFSI Food Fraud Think Tank. It can be even qualified as terrorism when the action aims at gravely disturbing public order. When the intention is only economical, the action can be considered as food fraud.

The notion of economic gain has been endorsed in the CWA: it is stated that "financial gain is the most common motivation for food fraud", as well as the intentional factor. The definition of food fraud that has been agreed on in this document is:

Food fraud: an action *"intentionally causing a mismatch between food product claims and actual food product characteristics, either by deliberately making claims known to be false or by deliberately omitting to make claims that should have been made."*

As most food products are produced and sold according to relevant regulations and requirements, food fraud also occurs when some aspect of the production violates these requirements or regulations.

In the same way, the definition of an "Authentic food product" given by the CWA is very close to that of food fraud:

An authentic food product is *"a food product where there is a match between the actual food product characteristics and the corresponding food product claims; when the food product actually is what the claim says that it is."*

It should be noted that the definition of food fraud developed by Codex Alimentarius position paper identified as key elements: deliberate intent, deception, financial gain and misre-presentation. The document provided by the eWG considers food fraud as being intimately linked to food integrity: food fraud is '*any deliberate action of businesses or individuals to deceive others in regards to the integrity of food to gain undue advantage*'.

The document also distinguishes 'food authenticity' and 'food integrity': both are a status of a food product, but the former is the state of being *'not altered or modified with respect to expected characteristics including, safety, quality, and nutrition*', while the latter is the state of being '*genuine and undisputed in its nature, origin, identity, and claims, and to meet expected properties*'.

The different types of food fraud

Based on the definition of food fraud the GFSI Food Fraud Think Tank developed, it identified a series of seven different types of frauds, as shown in Figure 2. Some of these terms have been defined in the CEN Workshop Agreement.

One of the most common frauds is **adulteration**. According to the CWA, it is:

"A type of food fraud which includes the intentional addition of a foreign or inferior substance or element; especially to prepare for sale by replacing more valuable with less valuable or inert ingredients."

This practice is sometimes referred to as Economically Motivated Adulteration (EMA). This term is defined in the Codex Alimentarius position paper. It is recognised as '*a subset of food fraud*'.

Different types of adulteration can occur in food products. Their definition is given below in italic.

Substitution is the "*process of replacing a nutrient, an ingredient or part of a food (often one with high value), with another nutrient, ingredient or part of food (often one with lower value).*" Examples of substitutions are substituting low value fish species for how value fish species when selling processed products (fillets, fish pies, etc.), substituting milk protein with hydrolysed leather protein or sunflower oil partially substituted with mineral oil.

Dilution is *'the process of mixing a liquid ingredient (solute) with high value with a liquid of lower value*'. The action addition of water to Not-from-concentrate (NFC) fruit juice or to milk is an example of this.

Unapproved enhancement is the *"process of adding unknown and undeclared compounds to food products in order to enhance their quality attributes"*. The melamine in milk falls under this category, as adulteration with melamine in milk products aimed at enhancing nitrogen content in

already diluted milk. Use of unauthorised additives, such as Sudan dyes in spices, is another example of unauthorised enhancement.

Concealment is the *"process of hiding the low quality of food ingredients or products"*. Injecting poultry with hormones to conceal disease is an example of this, as well as meat treated with carbon monoxide.

The other types of food frauds identified by the GFSI Food fraud Think Tank have not been specifically defined in the CWA. However, they are commonly used in a number of scientific publications, including this book. A definition of these terms was drafted by the FoodIntegrity experts when they designed the FoodIntegrity Knowledge Base (see the dedicated chapter of this book).

Grey market: this term includes production, theft, and diversion involving unauthorised sales channels for products. An example of this is the sale of excess unreported product when there are production agreements or quotas for the product and the product in question is deliberately produced in excess of these. A fish product originating from illegal, unreported, and unregulated (IUU) fishing is another example. This term also applies when there is a geographical restriction on the sale and distribution of the product, and the product in question is deliberately sold or distributed in other areas; this is often referred to as “grey market” sales.

Counterfeit is a case when where Intellectual Property Rights (IPR) infringement is in effect. This could include any or all aspects of the other product or packaging being fully replicated, for instance the process of copying the brand name, packaging concept or processing method for economic gain. Imitation wines and spirits with fake labels of a popular brand is a classical example (see the chapter on Spirits).

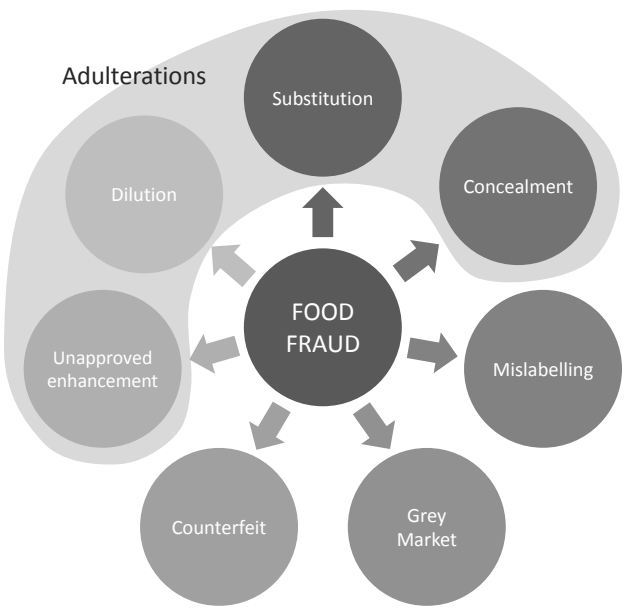

Figure 2: Terminology of food frauds. Adapted from Food Fraud Think Tank [7]

Mislabelling is a special case of food fraud. It concerns the process of putting false claims on packaging for economic gain. Selling farmed salmon as wild salmon, or conventional fresh produce as organic are examples of this fraud. Expiry date modifications fall under this category. However, mislabelling may apply to all forms of food fraud: to be efficient, a fraudulent product must indeed be "mislabelled" to be purchased by a buyer. But the expression is mainly used to indicate distortion of the information provided on the label.

The Codex Alimentarius position paper has also identified seven different types of food fraud. Although their designations are slightly different ('*simulation*' is used instead of *'concealment*', for instance), they overlap and are consistent with the definition of the GFSI and CEN Workshop Agreement.

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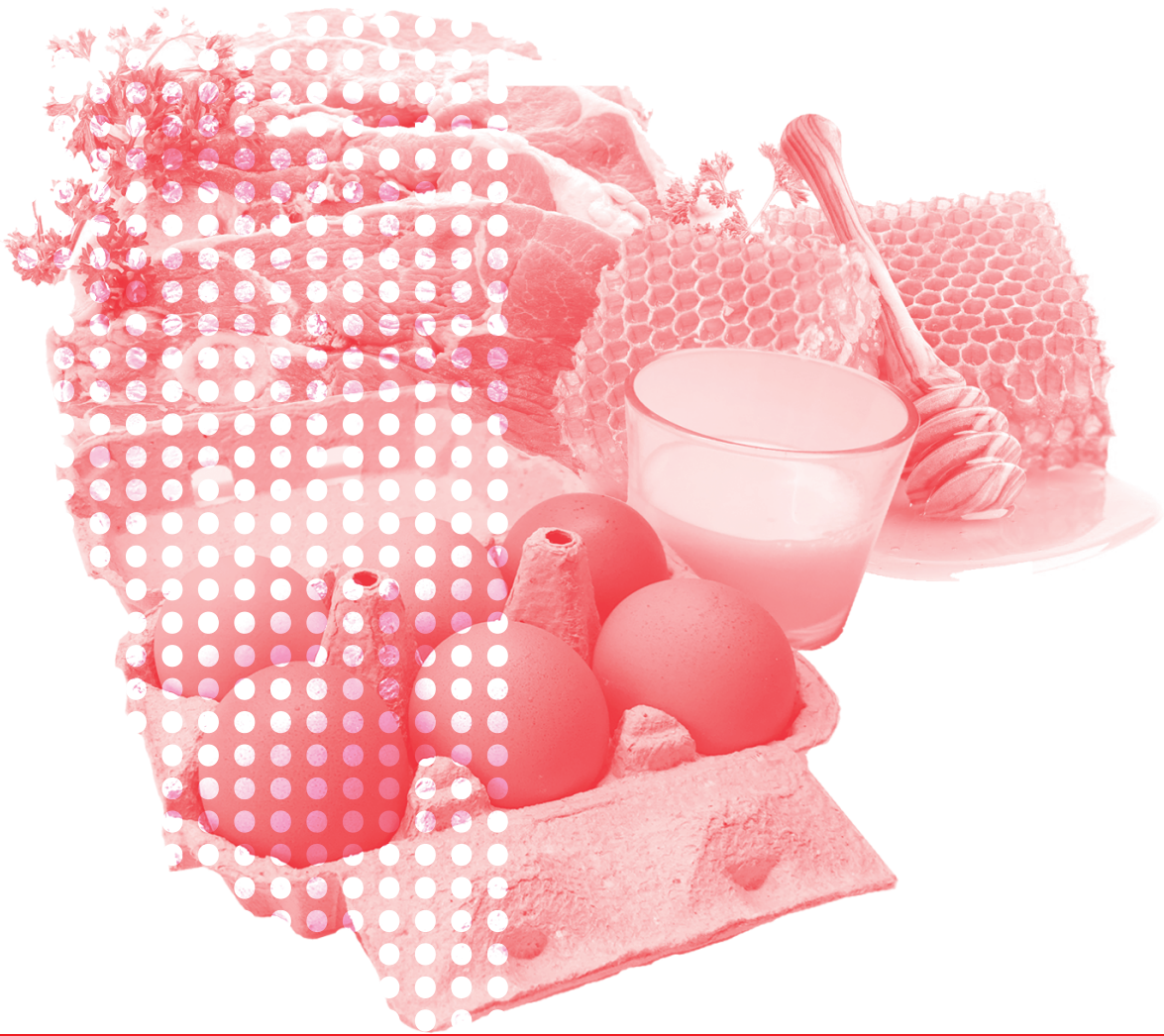
Animal Products



Milk and milk products

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General overview of the products

Milk is a nutritious food that plays an important role in the diet of particular groups, such as the new-born, children, the elderly and pregnant women. In addition to those groups, milk is consumed worldwide by a large part of the population, either alone or in the form of dairy products. According to the most recent data available from the Food and Agriculture Organization [1], the world production of milk increased from 724 million tonnes in 2010 to 798 million tonnes in 2018. Considering the data for cow, goat, sheep, camel and buffalo whole fresh milk, currently, Asia is the main producer in the world, mainly due to a high production of buffalo milk besides that of cow's milk. However, cow's milk remains the most consumed worldwide, corresponding to 82.6 % of the total fresh milk production in 2016. Europe is the major producer of this milk (32.7 % of world production in 2016), with most of it being produced in the European Union (EU). The dairy sector is of great importance to the EU since its value (close to 55 billion EUR) represents around 15 % of the total EU agricultural output (average 2011-2013) [2]. Although Germany and France are the most significant producers, followed by the United Kingdom, the Netherlands, Poland and Italy, a striking feature in the EU dairy sector is that milk is produced in every single Member State, without exception. The EU dairy industry is renowned for the quality of its products, being considered a major player in the world dairy market and a leading exporter of many dairy products, most notably cheeses [3]. In the EU, approximately 50 % of milk is used for cheese production, though a wide variety of other products is also produced, such as butter, yogurts, ice creams, among others. In 2013, the EU produced 9.3 million tonnes of cheese, 46.2 million tonnes of fresh dairy products, 2.1 million tonnes of butter, 1.1 million tonnes of skimmed milk powder (SMP) and 0.7 million tonnes of whole milk powder (WMP) [2]. In addition to these, a wide range of new products is nowadays being offered by the dairy industry, from products targeting special groups of consumers (such as products with low lactose content or lactose free, for lowering blood cholesterol, etc.) to dairy-based ingredients for other food industries.

https://doi.org/10.32741/fihb.1.milk

1. Product Identity

1.1. Definition of the product and manufacturing process

According to the Codex Alimentarius, milk is the normal mammary secretion of milking animals, without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing, while a milk product refers to a product obtained by any processing of milk, which may contain food additives, and other ingredients functionally necessary for the processing.

Milk and milk products encompass a wide range of products consumed worldwide including liquid milk, fermented milks and products thereof, cheeses, butter, ghee and dairy fat spreads, condensed milk, evaporated milk, cream, milk and cream powders, whey products and casein. Liquid milk, including raw milk and products such as pasteurised, skimmed, ultra-high-temperature (UHT) and fortified milk, is the most consumed, processed and marketed dairy product [4]. Fermented milk, obtained using suitable microorganisms, is generally used to produce dairy products such as yoghurt and kefir, among others. Cheese is the ripened or unripened soft, semi-hard, hard, or extra-hard product, obtained through the coagulation of milk protein by rennet, other suitable coagulating agents or processing technologies, and in which the whey protein/casein ratio does not exceed that of milk [5]. During this process, whey is also obtained, corresponding to the liquid part that remains after the separation of the curd. Whey can be used for several purposes such as the preparation of whey cheese, whey powder, whey drinks and for different industrial purposes [4]. Butter, ghee and dairy spreads are fatty milk products in the form of a water-in-oil emulsion. Cream is the fluid milk product comparatively rich in fat, in the form of an emulsion of fat-in-skimmed milk, obtained by physical separation from milk [4,6], and can give raise to a wide range of products such as whipping cream, whipped cream, acidified cream, among others. Condensed and evaporated milks are both obtained from the partial removal of water from whole or skimmed milk, with the first being frequently used in the form of sweetened condensed milk. Milk powders are obtained from the dehydration of milk and include several products such as whole milk powder, partly skimmed milk powder, skimmed milk powder and cream powder.

1.2. Current standards of identity or related legislation

1.2.1. Codex Alimentarius

Codex has developed several specific standards for milk and milk products. A compilation containing all Codex standards and related texts adopted by the CAC up to 2011 has been carried out [7]. It includes the standards of milk, milk powders, condensed milks, creams, butter and all sorts of cheeses. It also includes other general texts for milk and milk products such as the General standard for the use of dairy terms (CODEX STAN 206-1999) [8]; the Code of hygienic practice for milk and milk products (CAC/RCP 57-2004) [9]; the Guidelines for the preservation of raw milk by use of the lactoperoxidase system (CAC/GL 13-1991) [10] and the Model export certificate for milk and milk products (CAC/GL 67-2008) [11].

Codex has also developed several texts on food labelling, methods of analysis and sampling, food import and export, and certification systems that apply horizontally to all food products (including milk and milk products), such as:

- General standard for contaminants and toxins in food and feed,
- General standard for food additives,
- Guidelines for design and implementation of national regulatory food safety assurance programmes associated with the use of veterinary drugs in food producing animals,
- Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for residues of veterinary drugs in foods,
- Maximum residues limits for pesticides.

1.2.2. EU Legislation

General principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety have been stated in Regulation (EC) No 178/2002 [12]. The general rules for food business operators on the hygiene of foodstuffs have been laid down in Regulation (EC) No 852/2004 [13]. Specific hygiene rules for food of animal origin are covered by Regulation (EC) No 853/2004 where a section is specifically dedicated to raw milk and dairy products (Annex III, Section IX). Finally, Commission Regulation (EC) No 1664/2006 deals with measures for certain products of animal origin intended for human consumption, in particular the testing for raw and heat-treated milk [14].

Rules on the common organization of the market in milk and milk products for drinking milk (Council Regulation (EC) No 1153/2007) are now considered in Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 [15].

Milk can easily be contaminated by micro-organisms that are naturally present in the environment or which originate from diverse human activities. Therefore milk and dairy products have been extensively covered in EU legislation. The European Parliament and Council have established specific rules for the organisation of official controls on products of animal origin intended for human consumption (Regulation (EC) No 854/2004) [16].

Regarding quality evaluation, the Commission has published methods for the analysis and quality evaluation of milk and milk products eligible for public intervention and aid for private storage (Commission Implementing Regulation (EU) 2018/150 of 30 January 2018) [17]. More specifically**,** the Commission has issued new rules on caseins and caseinates intended for human consumption with EU Directive 2015/2203 [18].

Requirements on microbiological criteria have been amended by Commission Regulation (EC) No 1441/2007 concerning microbiological criteria for foodstuffs with regards to milk and dairy products and Commission Regulation (EU) No 365/2010 on microbiological criteria for foodstuffs as regards *Enterobacteriaceae* in pasteurized milk and other pasteurized liquid dairy products and *Listeria monocytogenes* in food grade salt [19, 20].

The Commission has also ruled on maximum residue levels for several pesticides in or on certain products (Commission Regulation (EU) 2018/686 and 2018/687 of 4 May 2018) [21,22]. Very recently, a Commission Implementing Regulation (EU) 2018/555 concerning a coordinated multiannual control programme of the Union for 2019, 2020 and 2021 has been established to ensure compliance with maximum residue levels and to assess consumer exposure to pesticide residues in and on food of plant and animal origin; the date of entry into force is however unknown at the date of writing this text (pending notification) [23].

1.2.3. European Dairy Association (EDA)

The European Dairy Association (EDA) is the European milk processors' platform for exchange throughout all parts of Europe and across all types of dairy companies, cooperatives and privately-owned dairies, world dairy leaders and enterprises. Recently, the EDA has issued its sectorial Guidelines for the voluntary indication of the origin of dairy products as an industry reference in the implementation of the new rules laid down in the EU Commission Implementing Regulation on voluntary origin labelling of foods. EDA also edited in June 2018 Guidelines on the principles and enforcement of the Protection of Dairy Terms. It takes place one year after the "Tofu Town" judgement by the European Court of Justice, in which the EU Court ruled that purely plant-based products cannot be marketed with designations such as 'milk', 'cream', 'butter', 'cheese' or 'yoghurt'. The new dairy industry guidelines intend to address the use and misuse of protected definitions, designations and sales descriptions of milk and milk products within the European Single Market and to serve as a tool to facilitate their enforcement at national level.

1.2.4. ISO Standards

The Technical Committee 34 of ISO (International Organization for Standardization) (ISO/TC 34) is responsible for the development of International Standards on topics connected to food and feed products. ISO/TC34/SC 5, created in 1970, focuses especially on standards for milk and milk products. It has published 184 ISO Standards. Their scope is the standardisation of methods of analysis and sampling, covering the dairy chain from primary production to consumption. The standards are used to determine, for example, the nitrogen content (ISO 8968-4: 2016) [24]. With reference to the melamine crisis in 2008, ISO and the International Dairy Federation (IDF) worked together on the edition of ISO/TC 15495 (ISO/TS 15495: 2010), which gives guidance for the quantitative determination of melamine and cyanuric acid content in milk, powdered milk products, and infant formulae by electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS) [25]. In 2013, ISO/TC 34 published guidelines for the application of mid-infrared spectroscopy in milk and liquid milk products (ISO 9622: 2013). It gives guidelines for the quantitative compositional analysis of milk and liquid milk products, such as raw milk, processed milk, cream and whey, by measurement of the absorption of mid-infrared radiation. The guidelines specified are applicable to the analysis of cow's milk and to the analysis of milk of other species (goat, ewe, buffalo, etc.) and derived liquid milk products, provided adequate calibrations are generated for each application and adequate control procedures are in place [26].

1.2.5. US Regulation

In the United States (US), the Department of Agriculture (USDA) and the Food and Drug Administration (FDA) regulate milk production and its guidelines are some of the strictest in the industrialised world. Farmers, processors and government agencies all work together to ensure the milk is safe and of the highest quality. The US FDA edits Guidance Documents and Regulatory Information including Coded Memoranda Issued by the Milk Safety Branch, Interstate Milk Shipments and Dairy HACCP.

The Pasteurized Milk Ordinance (PMO) of the US FDA serves as the basic milk sanitation standard for National Conference on Interstate Milk Shipments (NCIMS) members (all 50 states and Puerto Rico). Pasteurized Milk Ordinance, revised in 2015, presents the most current developments in milk sanitation for 'Grade A' milk and milk products [27].

2. Authenticity issues

2.1. Identification of current authenticity issues

Milk has been considered one of the seven foods most vulnerable to economically motivated adulteration. Due to the high demand for milk and the value of some dairy products, fraud in the dairy industry has become a widespread problem and a real concern for many consumers and authorities since adulteration invariably reduces product quality and may introduce hazards that can jeopardise health. Over the last decades, there has been an increasing interest in the quality evaluation and authentication of milk and milk products, in order to ensure consumer protection, avoid unfair competition among producers and improve general confidence in the sector.

2.1.1. Undeclared addition of certain ingredients

The practice of milk adulteration invariably reduces its quality and may introduce hazardous substances into the dairy supply chain threatening consumers' health [28]. Milk adulteration typically involves dilution and/or addition of inexpensive, low-quality and sometimes dangerous products to increase volume, mask inferior quality or replace the natural substances in milk for economic gain [29].

2.1.1.1. Adulteration with water

Water is the most commonly and simplest case of adulteration in milk. Addition of water not only reduces the nutritional value of milk, but also poses a health risk to the consumer [28]. However this is closely monitored by dairy companies when they purchase their milk. In addition, since many dairy companies pay for milk on the basis of its compositional quality any water addition would, to some extent, be self-defeating [30].

2.1.1.2. Adulteration of nitrogen content

Nitrogen-rich adulterants constitute also a well-known issue in milk adulteration which has received much attention in recent years owing to a series of food safety incidents [28]. These include the addition of nitrogenous compounds to increase the apparent protein content. This type of adulteration is very usual because the non-protein nitrogen cannot be distinguished by the Kjedahl and Dumas methods that are commonly used for determining total protein content in dairy products. Melamine, urea and whey are the main adulterants for this purpose due to their high nitrogen content and low cost. Melamine (2,4,6-triamino-1,3,5-triazine) is a nitrogen-rich organic compound commonly used to increase the apparent protein content of liquid and powdered milk and thus their economic value. Whey/whey protein is a very cheap by-product of cheese manufacturing that somehow resembles skimmed milk as it retains some milky aspect and flavour and is added to liquid milk not only to increase volume but also protein content. Urea is also extensively used in frauds because of its low cost [29]. Urea is added to milk to provide whiteness, increase the consistency of milk and standardise the solid-not-fat content to the value expected for the natural milk. Soya constitutes also a common source of nitrogen-rich adulterants. Low grade soya powder is a common vegetable protein used to increase the protein content of the adulterated milk, due to its lower price and easy availability in the market. Soya protein has good water holding and binding capacity and therefore can improve the texture of a product (e.g. cheese) [31]. Soya milk is also added to bovine milk either for sale as fluid milk or in the preparation of skimmed milk powder (SMP) and cheese for revenue maximisation. This is because of its similar properties to cow's milk [28].

2.1.1.3. Adulteration of the fat content

Fat is one the major components of milk and generally constitutes 3-5 % (m/m) of cow's milk. Triacylglycerols constitute about 97-98 % of the fat in milk and are important components that provide the characteristic flavour and texture. Major adulterants are vegetable oils (e.g. soybean, sunflower, groundnut, coconut, palm and peanut oil) and animal fat (e.g. cow tallow and pork lard). Detecting adulteration with vegetable oils is often difficult because of the variation in the chemical composition of these oils. Detecting adulteration with animal fat is also difficult because its chemical composition is similar to milk fat [29]. Moreover, it must be considered that lipid composition can naturally vary according to the different seasons and feeding regimes. On the other hand, a characteristic lipid profile can be associated with a particular product, produced in a certain period and geographical region with a specific feeding regime. In this sense, it can be challenging for the counterfeiter to imitate that specific composition, which can facilitate the detection of fraud in the case of suspect samples that can be matched against specific samples retained at the dairy industries [32].

2.1.1.4. Synthetic or reconstituted milk

Synthetic milk is an excellent imitation of natural milk containing vegetable oil, urea and emulsifier. It has the fat, nitrogen content and frothiness as well as similar specific gravity to natural buffalo milk. When mixed with natural milk in varying proportions, it becomes identical in milky aroma [28]. Synthetic milk is reported to be used for the adulteration of dairy milk at 5-10 %. In addition to this fraud, because detergents are essential components for the emulsification of fat added to the preparation of synthetic milk, they are considered a new class of milk adulterants. The presence of detergents in infant milk formula can sometimes be detected by means of colour and smell. Long-term consumption can cause serious deleterious health effects such as heart and digestive problems [29]. Adulteration of fresh milk with reconstituted milk containing cheap powdered milk is also a malpractice in common use [28].

2.1.1.5. Adulteration with preservatives

This practice involves the addition of substances to decrease microbiological growth and thus increase the product shelf life. This group includes several substances such as hydrogen peroxide, formaldehyde, hypochlorite, salicylic acid, and even potassium dichromate. These substances are toxic for humans and their monitoring is required for quality control [29,33].

2.1.2. Species substitution

Among the several possible adulterations in milk and milk products, one of the most frequent regards the species of origin, namely the substitution of high valued milks (such as sheep, goat or buffalo) by less expensive cow's milk, to reduce production costs and increase profits [34,35]. This is explained by seasonal oscillations and lower production yields of ovine, caprine and bubaline (or more exotic species such as camel or donkey), which raises the economic values of these types of milk and products thereof. Species substitution, besides having a negative economic impact, is also a problem for several groups of consumers because of other reasons such as religious, ethical or cultural objections.

In several EU countries, in particular those from the Mediterranean area, and other parts of the globe, namely the Middle East, a variety of valued cheeses are traditionally produced from goat's, sheep's, the mixture of both or buffalo milk. Traditionally produced cheeses are regarded as specialities and generally attain higher market prices and therefore are more prone to adulteration. Moreover, recently, in some countries, there has been a growth in the market for

milk species other than cow, in particular for goat's milk, due to its superior nutritional features and other aspects such as its attractive odour and taste, and superior digestibility when compared to cow's milk [36]. Additionally, according to some authors, goat's milk can be a possible alternative to cow's milk because it is considered less allergenic [37]. In this case, the undeclared presence of cow's milk could be a potential health risk for allergic consumers. Nevertheless, due to protein similarity, people allergic to cow's milk proteins can be affected by milk from any species, which demonstrates the importance for correct labelling.

2.1.3. Geographical origin (PDO, PGI products)

To recognise and support the potential of certain foods, in 1992 the EU created different labels, including the Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI), to promote and protect the names of quality foods from misuse and imitation. The PDO label covers agricultural products or foods that are produced, processed and prepared in a specific geographical area, using recognised know-how, therefore ensuring a strong link to the territory. Besides PDO, the PGI label also has a specific link to the region where the product comes from, however it only requires that at least one of the stages of production, processing or preparation occurs in that area, allowing the ingredients used in production to come from another region. In 2010, products with geographic indication (GI), namely PDO or PGI, had an estimated wholesale of EUR 54.3 billion, with agricultural products and foodstuffs corresponding to 29 % of this amount (EUR 15.8 billion). Among PDO products, cheeses account for a third of total turnover [38]. Presently, there are 189 PDO cheeses registered on the EU Database of Origin & Registration (DOOR), from a total of 14 EU countries, with Italy, France, Spain and Greece being the ones with higher number of products each (50, 45, 26 and 21 PDO cheeses, respectively). A PDO label has also been attributed to other dairy products, such as butter (e.g. Beurre d'Isigny and Beurre de Bresse (France), Mantequilla de Soria (Spain), Beurre rose (Luxembourg), Beurre d'Ardenne (Belgium)) and cream (Crème de Bresse and Crème d'Isigny, France).

Currently, consumers are increasingly interested in traditional, local and higher quality products, which in turn encourages agricultural producers to use geographical indications to differentiate and capitalise on the value of their products, thereby improving competitiveness and profitability. Thus, premium foods, frequently face competition with fraudulent products, which discourages producers, disappoints consumers and severely affects the agri-food industry and market. This is the case of PDO cheeses for which consumers frequently pay 1.5 times as much for GI products than for non-GI products [39]. In fact, the high market value of the PDO cheeses and their reputation worldwide make these products very prone to adulteration. Cheese is considered the 3rd GI food with higher infringing rates (10.6 %) corresponding to losses estimated in EUR 644.7 million [39]. The avoidance of economic losses due to mislabelling/fraud related to geographical origin is therefore a driving force behind the authentication of dairy products.

2.1.4. Rennet origin

During cheese production, the conversion of milk to cheese curd is usually made through an enzymatic coagulation process, either using animal, vegetable or microbial coagulants. Among those, animal rennet, which corresponds to enzymes (mainly chymosin and pepsin) secreted in the fourth stomach of unweaned ruminants (calves, lambs or kids), is frequently used in traditional cheese production [40, 41]. The use of rennet generally has a significant role in the sensory output of the produced cheese because it also contains lipolytic enzymes that release free fatty acids during ripening, therefore affecting the final characteristics of the product [40]. According to the specifications of several added-value cheeses, in particular various PDO labelled cheeses from

Southern European countries, specific animal or vegetable rennet should be used [41]. In general, lamb or kid rennet is preferred in the case of some sheep and/or goat PDO cheeses, such as Roncal cheese in Spain, Pecorino Romano and Fiore Sardo cheeses in Italy and Feta cheese in Greece, among others. In the case of the Italian PDO cheese Pecorino Romano, the specifications mention that, besides using exclusively lamb rennet paste, the fourth-stomachs used to produce this rennet should also come from animals raised in the PDO geographical area [42]. On the other hand, other PDO cheeses such as Azeitão, Serpa and Évora cheeses in Portugal, prefer the use of specific vegetable rennet, namely that from *Cynara cardunculus*. Portuguese sheep milk PDO cheeses, when compared to other cheeses from the same species, generally present a creamy semi-soft texture and exquisite flavour, these characteristics being attributed in part to the vegetable coagulant used, which is very proteolytic [43]. Thus, when specifications of PDO cheeses stipulate the origin of rennet used for manufacturing, the use of another type of rennet, such as those from microbial origin, constitutes an adulteration and the characteristics of the final product may even be different, since the use of a specific rennet is frequently associated to particular characteristics of the cheese.

2.1.5. Technological processes (heat processing, freezing) and maturation

Heat processing is frequently used in the dairy industry because it provides a guarantee of the microbiological safety of raw milk as well as enhancing its stability, being also used in the production of some milk products such as SMP. Different technological processes are currently available, ranging from the use of mild temperatures, such as pasteurisation, to more severe heat treatments, such as UHT. Depending on the temperature or heat processing technology applied, natural milk components, such as vitamins, can be degraded or novel substances formed. The extent of chemical changes that milk and milk products undergo during processing and storage depends on the intensity of the heat treatment applied to milk [44]. Therefore, higher concentrations of Maillard compounds than those lawfully expected can be due to either excessive or repeated heat treatments, thus indicating milk of inferior microbiological quality, or fraudulent use of milk powders [45]. Thermal processing of milk is also an important parameter to check in the case of cheeses traditionally prepared from raw milk since pasteurisation of milk can alter the indigenous milk microflora, affecting the final organoleptic characteristics of the product. Additionally, milk freshness is also a concern as regards high-quality milk products such as some PDO cheeses that must be produced from fresh milk. An example is Mozzarella di Bufala Campana cheese, for which the use of frozen material is prohibited. However, due to the seasonality of water buffalo milk production (which reaches a peak during winter, while mozzarella consumption is higher in the summer) as well as a rapid decrease in product quality, adulteration can occur by the use of frozen curd or frozen milk [46].

Another important aspect in some cheeses is the ripening period, during which several biochemical processes occur. Among those, proteolysis is one of the most important for the development of flavour and texture. Therefore, to guarantee the organoleptic characteristics of some cheeses, a minimum ripening period may be established, such as with the Spanish Manchego PDO cheese produced from sheep's milk, that requires a ripening period for at least two months, although the most prized cheeses are aged longer [47]. Thus, accelerated cheese ripening or mislabelling of the ripening period is also an authenticity issue to consider.

2.2. Potential threat to public health

Melamine (2,4,6-triamino-1,3,5-triazine) is a nitrogenous heterocyclic compound with several industrial uses, since it is a chemical intermediate in the manufacture of amino resins, laminates, coatings and plastics [48]. Melamine is not approved for direct addition to human food nor to animal feeds. However, since it is a nitrogen-rich compound (about 66 %), it has been fraudulently added to milk and infant formula to increase the apparent protein content. In 2008, melamine was detected in the infant formula of 22 dairy companies in China, resulting in 294 000 affected babies, more than 50 000 hospitalisations and 6 confirmed deaths [49,50]. Melamine itself has a low acute toxicity because it is absorbed from the gastrointestinal tract and rapidly excreted from the body [51]. However, in the presence of cyanuric acid impurities, melamine precipitates in the kidneys in the form of crystals, which can lead to kidney failure and even to death [52]. In the 2008 scandal, the melamine used contained only traces of cyanuric acid, however this compound was able to form complexes with uric acid, which is present in larger amounts in the urine of infants than adults, affecting kidney function [49]. In response to this scandal, a tolerable daily intake (TDI) of 0.2 mg/kg body weight was established by EFSA according to that established by the World Health Organization [49].

The use of other compounds to increase nitrogen content, such as urea, also presents a public health problem particularly for pregnant women, children and sick individuals. Urea in milk overburdens the kidneys as they have to filter out more urea content from the body and this can cause problems such as indigestion, acidity, ulcers and osteoporosis [31].

The use of preservatives to extend the shelf-life of milk also presents a significant risk to human health. Preservatives such as hydrogen peroxide and formaldehyde are probably the most frequent ones, but substances such as hypochlorite, salicylic acid and dichromate have also been reported [29]. The addition of low amounts of hydrogen peroxide, although permitted in some countries, is forbidden in others because of its toxic effects, such as irritation of mucous membranes, gastro-intestinal complications, which can lead to gastritis and inflammation of the intestine [32]. Hydrogen peroxide activates the natural enzyme lactoperoxidase, which has antimicrobial activity. The Codex Recommended Code of Practice CAC/GL 13-1991 allows its addition in small quantities, but only in countries that do not have dairy industries with a suitable refrigeration infrastructure [32]. Addition of formaldehyde or dichromate to milk is critical because of the associated toxicity and carcinogenicity [29].

The consumption of adulterated milk and milk products with cheap food materials, such as whey or soya proteins, can also impose serious health problems in particular in vulnerable groups such as infants and children. In these groups, where milk products are frequently the entire or major source of nutrition (e.g. milk formula such as infant milk powders), severe or even fatal effects can occur if the nutritional balance of the food is compromised due to adulteration of the product [53]. Additionally, adulterants such as soya that belong to one of allergen groups whose presence must always be declared in processed foods (Directive 2007/68/EC; Regulation (EU) No. 1169/2011) can also be a problem to sensitised individuals [54, 55].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Addition of water

The proportion of extraneous water added to milk can be estimated by cryoscopy to determine the freezing point of milk, according to standard ISO 5764|IDF 108:2009. This standard specifies a reference method by using a thermistor cryoscope for the determination of the freezing point of raw bovine milk, heat-treated whole, reduced fat and skimmed bovine milk, as well as raw ovine and caprine milk [56]. Calculation of the amount of extraneous water is subject to daily and seasonal variations but, generally, for polled milk from the same farm the variation range is quite narrow [32].

3.1.2. Species identification

According to EU legislation [17], isoelectric focusing of γ-caseins after plasminolysis should be used as the reference method to guarantee that cheese made exclusively from ewes' milk, goats' milk or buffalo milk or from a mixture of ewes', goats' and buffalo milk does not contain cows' milk casein. In this method, samples should be analysed together with reference standards containing 0 % and 1 % cows' milk, being considered positive if both bovine γ_2- and γ_3-caseins (obtained by plasminolysis), or the corresponding peak area ratios when applying densitometry, are equal to or greater than the level of the 1 % reference standard. The method can be used for detecting either raw or heat-treated cow's milk and caseinate in fresh or ripened cheeses made of ewes', goats' and buffalo milk or their mixtures, though it is not suitable for the detection of milk and cheese adulteration by heat-treated bovine whey protein concentrates. It is not adequate for species quantification, especially in ternary mixtures due to the similarities between some species, such as ovine and caprine [28]. Also according to this legislation, routine methods for detecting cows' milk casein in ovine, caprine and water buffalo cheeses may be used provided that (i) the detection limit is a maximum of 0.5 %, (ii) there are no false-positive results and that (iii) cows' milk casein is detectable with the required sensitivity even after long ripening periods, as may occur in usual commercial conditions [17]. However, the reference method is considered laborious and requires specific equipment, not always available in small dairy industries. Therefore, other approaches based on immunochemical methods are frequently used for routine screening, namely lateral flow immunocromatographic tests and enzyme-linked immunosorbent assays (ELISA). Currently, both options are commercially available in kit format for detecting cow's milk in sheep's and goat's milk and cheese, based on the detection of bovine immunoglobulin G (IgG). These approaches are suited for rapid screening, however since IgGs denature with thermal processing, adulteration with UHT cow's milk will give false negative results. More recently, a commercial kit has become available which is based on a competitive ELISA using a mouse monoclonal antibody (Mab) raised against bovine κ-casein that allows screening both raw and heat treated cow's and buffalo milk in the milk and cheese of other species and sources.

3.1.3. Lipid analysis

As mentioned previously, adulteration of milk and milk products can include milk fat substitution by vegetable or other animal fat, or even the addition of these to skimmed milk to sell this as full-fat milk. To detect such adulteration, a lipid profile analysis is generally performed, either by the determination of fatty acids based on standard ISO 15885|IDF 184:2002 after obtaining the methyl

ester derivatives (standard ISO 15884|IDF 182:2002) or by the determination of triacylglycerides (standard ISO 17678|IDF 202:2010). The principle of fatty acid analysis relies on the preparation of the methyl esters of milk fat fatty acids (FAME) by base-catalysed methanolysis of the glycerides and transesterification [57]; the obtained FAME are then separated and determined by capillary gas-liquid chromatography with flame ionisation detector (GC-FID) [58]. The purity of milk fat extracted from milk or milk products can also be determined based on triacylglycerides analysed by GC-FID [59]. The presence of vegetable or animal (beef tallow and lard) fat can be inferred using suitable equations to calculate S-values, which should comply with those established for pure milk fat. Nevertheless, some cases can result in false positives when applying this method, particularly when the animals are given exceptionally high feed of pure vegetable oils, such as rapeseed oil; milk products from individual cows; milk fat subjected to technological treatment (e.g. cholesterol removal) or obtained from skimmed milk or buttermilk; and some cases of fat extracted from cheese as the ripening process can affect fat composition.

3.1.4. Adulteration of nitrogen content and addition of reconstituted milk

Several milk products, by definition, should not contain proteins other than those naturally present in milk. However, as mentioned, some non-milk protein sources, such as soya, are attractive as potential adulterants due to their low price. The detection of vegetable proteins added to milk products, namely the addition of cheaper soya and pea protein isolates to low-heat milk powder, can be achieved using capillary electrophoresis in the presence of sodium dodecyl sulphate (SDS-CE) as described in ISO 17129|IDF 206:2006. However, the method is not suitable for detecting the presence of hydrolysed plant proteins in milk powder. An alternative option uses ELISA kits to screen for the presence of soya proteins [60].

The addition of non-food proteins to milk, powdered milk products and infant formulae, can be achieved based on the quantitative determination of melamine and cyanuric acid (mg/kg of product) by electrospray ionization liquid chromatography tandem mass spectrometry (LC-MS/MS), according to standard ISO/TS 15495|IDF/RM 230:2010 [61].

According to EU legislation [17], the detection of rennet whey in skimmed-milk powder can be performed by using the reference method based on the determination of the caseinomacropeptides by high performance liquid chromatography with ultraviolet detector (HPLC-UV). As well as for species identification purposes, there are available on the market as competitive enzyme immunoassay kits to screen for the presence of bovine rennet whey in bovine milk and milk products. As an example, one of such ELISA kits is based on the detection of an epitope located on the glycomacropeptide (CMP) part of κ-casein, which is released during cheese production, and therefore indicates fraud when detected in milk powder.

HPLC-UV is also the technique proposed by the Chinese Ministry of Agriculture (Chinese standard NY/T 939-2016) for the detection of furosine and lactulose, used to detect the addition of reconstituted milk in pasteurized or UHT milk [62]. A method based on the determination of lactulose content by HPLC-UV is also proposed by ISO and IDF but to distinguish milk sterilized by UHT from in-bottle sterilised milk (ISO 11868|IDF 147:2006) [63]. Lactulose is not present in raw milk, being formed by epimerisation of lactose due to heat treatment. The extent of the isomerisation is related to time and temperature, and can therefore be used to evaluate the severity of the heat treatment [64]. Determination of alkaline phosphatase and lactoperoxidase, two naturally occurring enzymes in raw milk, is also used to evaluate the use of thermal processing [65, 66].

3.1.5. Spectroscopy (MIR)

In the particular case of milk and milk products, FT-MIR spectrometry is the worldwide method of choice for composition and quality controls during routine liquid milk testing. In 1961, a patent application for a FT-MIR method determining fat, protein and lactose in milk was introduced [67]. The first apparatus, an IRMA (Infrared Milk Analyzer, Grubb Parsons, Newcastle upon Tyne, UK) using a monochromator, was based on the principle of measuring direct absorption of the infrared energy at specific frequencies by carbonyl groups in the ester linkages of the fat molecules, by peptide linkages between amino acids of protein molecules, and by the O-H groups in lactose molecules. A second generation of infrared instrumentation has adopted the change from wavenumber selection by diffraction grating to optical filters [68] and was largely used by central milk laboratory testing, where samples of milk from both tanks and individual cows were tested. FT-MIR supplies complementary chemical information and allows a high throughput with high sensitivity in a short response time from a very small quantity of sample [69]. In 1993, the first purpose-built FT-MIR instrument based on Fourier Transform Infrared (FT-MIR) technology was marketed (Anadis MI-200) [70]. With the introduction of FT-MIR, new applications have been developed because of the use of the full spectrum of the sample. In this way, FT-MIR has been applied for the determination of more and more milk components such as free fatty acids [71], protein composition [72], minerals [73], ketone bodies [74], lactoferrin [75] and fatty acid profile [76,77]. Recent studies have been performed using these milk components predicted by FT-MIR in order to predict physiological indicators of the animal [78-80].

More recently, the FOSS company (Foss, Hillerød, Denmark) has developed an Abnormal Spectrum Screening Module (ASM) where new milk samples are automatically compared to the spectra of the natural (not contaminated) historical dataset obtained with the MilkoScan™ FT120 (www.foss.fr/industry-solution/products/milkoscan-ft1/), then outliers are detected by a combination of the residuals from the PCA on natural samples and the Mahalanobis distance.

3.2. Alternative methods

3.2.1. DNA-based methods

During recent years, analytical methods relying on DNA analysis have rapidly progressed as alternatives to overcome the limitations of protein analysis and have been successfully applied for milk authenticity testing. DNA-based methods present several advantages, specifically the ubiquity of nucleic acids in every type of cells and their superior stability to proteins. Most DNA-based methods rely on the polymerase chain reaction (PCR) technique due to its high specificity, sensitivity, simplicity and rapidity, allowing the identification of species of origin even in complex and processed foods, such as dairy products. Although both nuclear and mitochondrial genes can be targeted as species-specific DNA markers, the latter has been preferred because of the high number of copies per cell and sequences are highly conserved within different animal species.

Several PCR-based methods have been developed and applied to the authentication of milk and milk products, such as heat-treated dairy products, cheeses, yogurts, butter and milk-based sweets [81]. The methods include mainly PCR with restriction fragment length polymorphism (RFLP), species-specific PCR, multiplex PCR and real-time PCR. The detection of cow's milk in milk mixtures [82], in goat's and sheep's cheeses [83,84] and in buffalo cheeses [85,86] was successfully achieved with species-specific PCR with sensitivities down to 0.1 %. The use of two or more sets of primers in the same reaction allows multi-species detection based on multiplex PCR. Bottero et al.

(2003) and Gonçalves et al. (2012) proposed multiplex PCR assays to detect cow, goat, sheep and buffalo species in dairy products [87,88]. The development of duplex PCR assays enabled the detection and quantification of cow's milk in sheep's [34] and goat's [35] cheeses.

Real-time PCR has been the technique of choice in many laboratories for species identification and food authentication, including for dairy products. The combination of high sensitivity, specificity, reproducibility and quantitative analysis are major advantages of real-time PCR. Additionally, the amplification of short DNA fragments (100-200 bp) is a major benefit when analysing highly processed foods [89]. Several authors have proposed real-time PCR assays with TaqMan probes to detect cow's milk in dairy products [90-94]. The simultaneous detection of several species in dairy products has also been succeeded by multiplex real-time PCR assays [95,96].

3.2.2. Other protein-based methods (chromatography/mass spectrometry)

The evaluation of proteins and/or the sequence of peptides by mass spectrometry (MS) or liquid chromatography (LC) coupled to MS, is increasingly being used as reliable biomarkers for dairy product authentication. This has been possible due to several technological advances that allow for accurate analysis of proteins and peptides, namely the use of soft-ionisation techniques, such as electrospray ionisation (ESI) and matrix-assisted laser desorption ionisation (MALDI). MALDI time-of-flight mass spectrometry (MALDI-TOF-MS) provides informative fingerprints of milk proteins for dairy authentication, and is also a simple, fast, sensitive and highly reproducible technique. LC-MS techniques are advantageous in terms of high selectivity and sensitivity, which makes them useful as confirmatory techniques. However, the development of specific LC-MS methodologies is laborious and requires skilled technicians and costly equipment. Besides the already mentioned use of LC-MS as a reference method in the analysis of nitrogen-based adulterants, namely melamine, in the last years, LC-MS and MALDI-TOF-MS have demonstrated their usefulness in the detection of other types of fraud in dairy products, such as species identification, accessing freshness, addition of rennet whey, etc. based on the analysis of specific peptides as biomarkers [97-101]. For instance, the use of specific peptides as biomarkers for milk species identification presents advantages over other protein-methods whose results are affected by thermal processes since the sequence of peptides is related to the genetically determined primary structure of proteins, which is generally resistant to processing.

3.2.3. Spectroscopy (NIR, Raman)

There are various types of analytical methods applied to food authentication that can provide information concerning its physical and chemical characteristics, including major and minor constituents. However, most of these methods are often tedious, time consuming and use reagents that may be harmful for the environment. In the food sector, and especially in the milk and milk products area, with the increasing demands being made by consumers and legislators, there is a general need for methods that are suitable for process and quality control and are simple, rapid and reach the required accuracy, repeatability and sensitivity. Fingerprint methods are the ideal candidates to replace these analytical procedures. The term "fingerprinting" can be defined as a variety of techniques that can measure the composition of foodstuffs in a non-selective way. Among these methods, vibrational spectroscopy methods based on infrared and Raman spectroscopic techniques, use the information from major compounds present in food products [102-104]. Organic compounds absorb radiation at specific wavelengths or frequencies, thus giving rise to spectral signatures which are characteristic of the food composition and may be considered as «fingerprints» of the food. However, these signatures also include interference due

to variation occurring as a result of natural events (e.g. weather, climate, disease etc.) during growth or the production of primary foods or to batch-to-batch variations in processed foods or food ingredients. Interrogation of signals from sufficiently large and characteristic sample collections by mathematical techniques can detect primary foods which are not what they claim to be or processed foods that do not comply with a declared specification. Vibrational spectroscopy methods are suitable for implementation in factories and milk parlours as they allow on-line control and the screening of a high number of samples by unit of time. Fingerprinting methods are also of interest to regulatory bodies because they enable rapid preventative action to be taken. It should be noted that, despite the many studies demonstrating their potential, the application of fingerprinting methods in routine analysis and food authenticity surveillance still remains limited [105].

Until now, untargeted analysis has been associated mainly with direct analysis techniques, such as mass spectrometric-based metabolomics or isotope-assisted methods. Only a few studies have linked untargeted analysis with vibrational spectroscopic methods [106]. Moore et al. [107] developed non-targeted screening tools to detect adulteration in skimmed milk powder using NIR spectroscopy and Xu et al. developed a method for the untargeted detection of protein adulteration in yogurt by removing unwanted variations in pure yogurt [108]. In all these cases, the approach involved building statistical models based on the measured fingerprints of a large representative set of normal and abnormal samples, and then applying these models to unknown samples in order to characterise them. More recently, Fernández Pierna et al. have developed a moving window based PCA method using vibrational spectroscopic data. The PCA spectral score residuals are evaluated and used to define thresholds to be applied to the spectral score residuals of unknown samples [109]. The method was applied to study milk contaminated with melamine. Since the discovery of melamine contamination in infant milk formula in China, strict regulations have been enforced throughout the world and many papers have been published on the use of such methods as wet chemistry, chromatography, mass spectrometry and vibrational spectroscopy to detect melamine in both raw and powdered milk. In this study, liquid UHT milk was contaminated with melamine at various levels ranging from 0.01 % to 1 % (100 – 10 000 ppm) and measured using Fourier Transform Mid-Infrared (FT-MIR) spectrometry. Samples spiked at levels higher than 100 ppm were easily detected using this method, which would not have been possible using classical techniques such as Mahalanobis distance, usually applied as an outlier detection method.

3.2.4. Isotope-ratio mass spectrometry (IRMS) and elemental analysis

The authentication of the geographical origin of milk and dairy products is difficult to achieve because it needs to consider not only the variability inherent to a product of animal origin but also that of environmental conditions [110]. So far, the techniques employed for geographical authentication and/or differentiation of PDO dairy products are mainly based on stable isotope fingerprinting determined by IRMS or its combination with elemental composition most frequently determined by inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectroscopy (ICP-AES). Stable isotopes mostly depend on climatic or geographical conditions, being affected by biological/environmental interactions in addition to hydrological and climatic variations, while the elemental composition is mainly affected by geology and pedological characteristics of the soil [111]. Isotopic analysis has been applied to the discrimination of several different cheeses with distinct geographical origin [112] and was officially adopted in 2011 as a reference method for verifying the authenticity of PDO Grana Padano cheese [113]. Isotopic analysis has also been proposed as a useful parameter to access the addition of

maize in the animal's diet and corresponding mislabelling of dairy products declared as being produced by pastured animals or PDO cheeses for which the diet of the animal has an established maximum amount of maize in the diet [114]. The analysis of mineral and trace elements coupled with the development of classification models based on chemometrics have also been applied for the differentiation of the type of milk production, namely organic versus conventional [115].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Cryoscopy	Freezing point	Addition of water
Spectroscopy	Spectroscopic profile	Addition of water; melamine; addition of vegetable lipid or proteins; adulteration (non-targeted approach)
Isoelectric focusing	bovine γ2- and γ3-caseins	Bovine milk in sheep´s, goat´s and buffalo cheese
Lateral flow immunocromatographic tests	Bovine immunoglobulin G	Raw bovine milk in sheep's and goat's milk and cheese
ELISA	Bovine immunoglobulin G	Raw bovine milk in sheep's and goat's milk and cheese
ELISA	Soybean proteins	Vegetable proteins (soybean)
Competitive ELISA	Mouse monoclonal antibody raised against bovine κ-casein	Raw and heat treated bovine and buffalo's milk in the milk and cheese of other species
Competitive ELISA	Glycomacropeptide of κ-casein	Bovine rennet whey in bovine milk and milk products
GC-FID	Fatty acids, Triacylglycerols	Vegetable or other animal fat
SDS-CE		Vegetable proteins
LC-MS/MS	Melamine, cyanuric acid	Addition of non-food nitrogenous compound
HPLC-UV	Caseinomacropeptides	Addition of rennet whey
HPLC-UV	Furosine and lactulose	Addition of reconstituted milk
Species-specific PCR, real-time PCR, PCR-RFLP, LAMP, NGS	Molecular markers	Adulteration regarding species origin
Mass spectrometry (LC-MS and MALDI-TOF-MS)	Specific peptides	Adulteration regarding species origin; assessing freshness; addition of foreign proteins
IRMS	Isotope fingerprinting	Geographical origin
ICP-AES, ICP-MS	Trace Metals	Geographical origin

5. Conclusion

During the last decade, cultural and social shifts have occurred in developed societies with consumers becoming increasingly aware about subjects such as biodiversity, climate change and ecological footprint. Therefore, one can expect a growth in the number of consumers willing to spend more money on certain food products, such as specialties produced according to traditional processes and organic foods. In this sense, possible adulterations in the dairy industry that may occur in a near future include mislabelling of the organic origin of milk and milk products and of the breed origin of milk used in the production of some PDO products, such as cheeses. Several PDO milk products, mostly cheeses, besides requiring the use of milk from specific animal species also specify the animal breed. Among several other examples, Spanish Manchego cheese must be produced from sheep's milk of the Manchega breed and the Portuguese Terrincho cheese produced from sheep's milk of the Churra da Terra Quente breed. Since some traditional breeds specified in PDO products are less productive compared to others that are more frequently used, a possible fraud could imply the use of milk from the same animal species but from a different breed to that specified for a particular milk product.

Looking into the future, there are several trends in milk and milk products authentication, one of those being the use of untargeted approaches such as spectroscopic techniques. In recent years, food safety has become an increased concern for consumers due to several important crises related directly or indirectly to human health. Most of the studies published have attempted to develop analytical procedures based on spectroscopic techniques to characterise/authenticate milk or milk products and at the same time detect the presence of any possible known contaminant or adulterant before reaching the food chain. Until now, statistical tools have been used to interpret multivariate data obtained from the spectroscopic analysis of different products and this has led to the creation of some decision rules. These enable verification of compliance against specifications in order to decide whether to reject or accept the product. However, the challenge will be to exploit the huge amount of information contained in the data generated by such spectroscopic techniques but taking into account the concept of data-driven discovery or untargeted analysis. New crises of adulteration/contamination with illegal ingredients other than known ones continue to occur from time to time. By relying solely on targeted analysis methods, adulteration could get out of control and analysis would become trapped in a cycle of 'adulteration, targeted analysis, and new adulteration', and so on. In contrast to targeted analysis, which uses information from known possible unusual ingredients, an untargeted experiment registers all information within a certain correlation/similarity, including data from new products. Untargeted detection methods are therefore required for screening products for a range of known and unknown adulterants. Untargeted analysis will mean alerts can be given more rapidly and fraud detected more easily

Vibrational spectroscopic methods are based on measuring the amount of electromagnetic radiation absorbed by a sample according to the Beer-Lambert law and can be very useful when authenticity and quality controls need to be established at both the laboratory and the industry levels, as they can be applied at the point where products are delivered to factories or during the production process. They are rapid with almost no sample preparation; they do not use chemical reagents and do not require skilled staff. However, fingerprinting methods are not confirmatory techniques, and therefore are not used in official control and have no weight in a judicial court. Nevertheless, such methods could to be interesting for regulatory bodies as they would enable preventative actions to be taken rapidly. Spectroscopic methods are increasingly presented as new approaches for at-line, on-line and in-line control of authentication of food products. As

mentioned, these techniques are already routinely used in the industry to control both raw materials and finished products for specific production standards as a common authenticity issue and it is expected that they will be increasingly used in a near future. The main limitation of the spectroscopic approach is the requirement for large datasets to calibrate any given instrument. Also, a main challenge facing the spectroscopists is to extract the information in such a way that it can be used in qualitative and quantitative analysis. NIR spectra can contain thousands of absorbance values at defined wavelengths (i.e. variables) and the challenge is to characterise the spectral data set and isolate the variables that can be correlated with the information of interest (i.e. authenticity issue) [116]. To achieve this goal, a wide range of chemometric tools are at the disposal of the analyst who has to select the most appropriate according to the specific aims of the method and the characteristics of the dataset. Among the many methods proposed for the authentication of food products, spectroscopic methods seem to be the preferred ones to flag suspicious samples before, during and after the production of a food product. The real future challenge for spectroscopic techniques will be the demonstration of their daily use in the industry and the marketplace for food product authentication.

More recently, other novel and advanced techniques, such as real-time PCR coupled with High Resolution Melting (HRM) analysis, Loop-Mediated Isothermal Amplification (LAMP), next generation sequencing (NGS) and biosensors have emerged and are being applied to milk authenticity testing [81]. By relying on isothermal amplification of DNA, LAMP presents several advantages, namely its simplicity, speed and the fact that is does not require specialised equipment such as thermocyclers [117]. This, as well as the possibility of being integrated on microfluidic devices, allows for its portability, giving this technology great potential for use as a screening tool. NGS technologies have changed the way in which DNA can be analysed by increasing sequencing throughput by several orders of magnitude. NGS combined with DNA barcoding has been termed metabarcoding, which relies on the use of universal PCR primers to amplify, massively, one or more taxonomically informative targets. Recently, Ion Torrent NGS technology was successfully applied for the identification of species in dairy products by sequencing targeted mtDNA fragments [118]. Although the cost of NGS platforms is still very high, this technology presents several advantages regarding species identification for food authentication, and its use is expected to increase in the near future.

Due to their small size and high integration, biosensors are simple to operate with and generally capable of fast measurements. Therefore one can also expect their increased used in the dairy industry with multiple applications [119].

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Eggs and egg products

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General overview of the product

Eggs are a key component in human and animal diets thanks to the large content of high-quality proteins and vitamins. For this reason, eggs are widely used as ingredients in the industrial preparation of different types of foods (foams, emulsions, pastry and bakery products); these mixtures falling under the denomination “egg products”.

The eggs sector is one of the most important agricultural industries all over the world since, unaffected by weather, it is suitable for all different climate regions.

Figure 1: Global distribution of egg production in 2013 on country development base (Source: FAO database; design: Ursula Welting)

The main producing countries are the European Union (EU), China and North-Central America. Given the perishable nature of both shell eggs and liquid egg products, the flow of trade in these goods between different countries is limited and most of the production is fully dedicated to internal markets. This may vary in case of big issues such as the outbreak of Avian Influenza in the

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US that led the entire country to import liquid products from the EU. Powder products are a different case, especially albumen which has specific properties for use in bakery products. These can be commercialised without concern for their expiry dates, but may face some trade restrictions between countries (i.e. not all European countries can export to the US).

Most suppliers operate a supply chain in which the different steps are vertically integrated: the feed-mill, the shelling and heat treatment plant and farms (either fully owned or under an agistment contract). There has been a steady decrease in spot market purchasing of eggs due to traceability requirements.

The increasing importance of animal welfare has put the entire sector under even more pressure and it has had to adapt to new regulations especially in the EU (2012) and also to consumer demand to eradicate the use of cages by suppliers. In response to these issues, the market has reacted in order to tackle the increasing demand for suitable farms with better animal welfare, and in addition to ensure a more transparent supply chain and thus prevent any other possible scandals that have affected the market in the past.

1. Product Identity

1.1. Definition of the product and manufacturing process

The concept of egg products is related to all the forms of presentation of the egg: yolk, albumen or a mix of both. The term “Egg Products” refers to processed or convenience forms of eggs obtained by processing shell eggs: egg products include whole eggs, egg whites, and egg yolks in frozen, pasteurised and refrigerated liquid and dried forms available in a number of different product formulations. In particular, the food industry is interested in high quality egg products in a liquefied form, obtained from eggs shelled within 4 days and which have undergone homogenisation and pasteurisation: their use is mainly related to the preparation of egg pasta and bakery products [1].

1.1.1. Farming systems

Eggs used in the production of egg products for the food industry come from farms where an “intensive” farming of hens is usual, following different modalities depending on the structure of the farms and their management. Battery cages, Cage-free (Free-range, Barn) and Organic are the most common types of farming systems utilised.

1.1.1.1. Battery cages

These comprise a housing system used for various animal production methods, but primarily for egg-laying hens. The name comes from the arrangement of rows and columns of identical cages connected together, in a unit, as in a battery. Although the term is usually applied to poultry farming, similar cage systems are used for other animals. Battery cages are the predominant form of housing for laying hens worldwide, but have generated controversy between advocates for animal rights and industrial producers. These housing systems reduce aggression and cannibalism among hens; on the other hand, they are barren, restrict the hens’ movements preventing natural behaviour, and finally, increase rates of osteoporosis. The introduction of the European Union Council Directive 1999/74/EC which banned conventional battery cages in the EU from January 2012 for welfare reasons, has meant that the number of eggs from battery cages in the EU Member States is decreasing.

1.1.1.2. Cage-free

In the EU, this type of egg production includes barns, free-range, organic (in the UK, systems must be free-range if they are to be labelled as organic) and aviary systems. Non-cage systems may be single or multi-tier (up to four levels), with or without outdoor access.

In free-range systems, hens are housed to a similar standard as the barn or aviary. In addition, they have constant daytime access to an outside range with vegetation. Each hen must have at least 4 m^2 of space.

The European Union Council Directive 1999/74/EC stipulates that non-cage systems must provide the following:

- A maximum stocking density of 9 hens/m^2 of "usable" space
- If more than one level is used, a height of at least 45 cm must exist between the levels

- One nest for every seven hens (or 1 m^2 of nest space for every 120 hens if group nests are used)
- Litter (e.g. wood shavings) covering at least one-third of the floor surface, providing at least 250 cm^2 of littered area per hen
- 15 cm of perching space per hen.

In addition to these requirements, free-range systems must also provide the following:

- One hectare of outdoor range for every 2,500 hens (equivalent to 4 m^2 per hen; at least 2.5 m^2 per hen must be available if rotation of the outdoor range is practiced)
- Continuous access during the day to this open-air range, which must be "mainly covered with vegetation"

1.1.2. Transformation process

After laying, the eggs are sorted in order to separate out damaged, dirty, or broken ones and to classify them (in function of size) according to the characteristics defined by law (A category, B category).

A classification of the eggs also in terms of freshness, in other words the time from laying to the transformation into egg products or the shipping to the retail market, is done. According to this classification, "extra-fresh" eggs or "fresh" eggs are clearly distinguished from "conventional" eggs.

Eggs for industry use, entering the process for transformation to liquid egg products, are destined to management by food transformation factories where from eggs in shells, they turn into pasteurised refrigerated egg products. The flow diagram of this transformation process is in Figure 2.

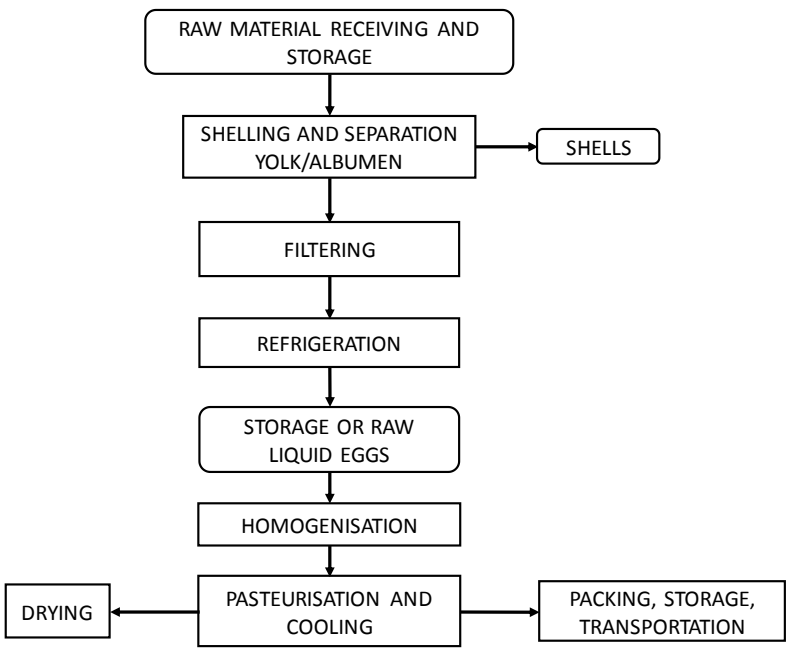

Figure 2: Flow diagram whole eggs

Liquid egg products coming from this process are commonly delivered to food companies in refrigerated tanks and their quality and food safety characteristics (chemical, physical parameters included in related technical specifications) are carefully controlled by the producers before their release and by the customers on reception and before use.

1.2. Current standards of identity

The following legislation in the EU relates to eggs and egg products.

178/2002 [2] – This regulation lays down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

852/2004 [3] – This regulation and its annexes define a set of food safety objectives that food operators must meet.

853/2004 [4] – This regulation aims to ensure a high level of food safety and public health. It complements Regulation (EC) No 852/2004 on the hygiene of foodstuffs, whose rules mainly cover the approval of operators in the sector. Its rules apply to unprocessed and processed products of animal origin. They generally do not apply to food that contains both products of plant origin and processed products of animal origin. EU countries must register and, where necessary, approve establishments handling products of animal origin.

2073/2005 [5] – This regulation concerns the microbiological criteria applied to foodstuff.

1881/2006 [6] – This regulation lays down the maximum limits for certain contaminants in food in particular to protect the health of the most sensitive population groups, i.e. children, the elderly and pregnant women.

589/2008 [7] – This regulation lays down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs:

- Describes the characteristics for CAT A and CAT B (shell and cuticle, air space, yolk, albumen, germ, foreign matter and smell)
- Grading Cat A by weight defining the classification in different sizes
- Defines shelf life and timing to grading, marking and packing eggs
- Defines how to handle industrial eggs
- Defines the code to mark the eggs
- Indicates which records to be kept by producers, collectors and packing centres
- Checks
- Non compliances and tolerances

2. Authenticity issues

2.1. Identification of current authenticity issues

This section concerns pasteurised eggs used in food preparations, starting from liquid eggs already shelled, provided by suppliers located in the EU with integrated supply chains for farms, feed mills and transformation plants.

2.1.1. High risk issues

The **highest risk factors** that can impact egg authenticity include:

Different farming systems for hens with an impact on animal welfare

There is an increasing market demand for eggs from barn hens or from cage free farming systems. However, existing facilities need to be converted and it is evident that a number of uncertainties remain as to whether all the volumes are/will be satisfied within the animal welfare requirements.

Currently, there are no available analytical methods able to categorise different farming approaches (barn hens or cage free farming system) and this fact increases the opportunity for fraud. In addition, intermixing of eggs is possible at the farm level, during transportation and at the transformation steps.

Fresh eggs

Eggs can be declared as fresh eggs within 28 days shelf life. However, eggs over 28 days shelf life can be found on the market still declared as “fresh” in order to fraudulently exploit a higher price compared to the others.

Albumen and yolk contain enzymes, and if eggs are not stored at a sufficiently low temperature, the proteins can be altered. The optimal temperature for correct egg storage is normally about 6-8 °C. Enzymatic alteration of the albumen modifies its viscosity, which can be used to recognise the freshness of the egg: in fact, when the egg is not fresh the albumen tends to liquefy and the yolk breaks easily.

Immediately after the laying phase, the contents of the egg with its entire shell are practically sterile and can be contaminated from environmental microorganisms only if the shell is broken.

Egg categories

Over the last few years, there has been an increasing demand for cat A eggs, with quality parameters as described by the regulation currently in force. There are insufficient farms able to keep up with the demand, and Cat B eggs are cheaper than Cat A ones.

This intermixing of categories is possible at farm level, during transportation and at the transformation facility.

Dilution with incubated eggs

The eggs that come from the incubation process must be sent for destruction or use as animal feed. It is not possible to use them for human consumption. The price of these eggs is very low and can lead to the illegal use in some periods (when the egg offer on the market is low or when availability from the incubators is high). There are parameters regulated by law in order to avoid the use of these eggs for human consumption.

Artificial colorants

Artificial colorants are allowed but some supply chains claim to be free from artificial colorants. Eggs intermixing is possible at farm level, feed mill and at the transformation facility.

2.1.2. Lower risk issues

Lower risk factors, but still possible, include the following:

Use of eggs from different animals

This type of fraud is not always economically viable and there are some technical and mechanical restrictions in the shelling lines.

Dilution with water

The opportunity of this type of fraud is decreased by its detectability with current analytical methods (dry matter analyses).

2.2. Potential threat to public health

The microbial contamination of eggs could be due to: (1) endogenous factors, due to contact with microorganisms present in the cloacae which go up in the oviduct and contaminate the egg during its own formation process, and (2) exogenous factors, that is, microorganisms in certain conditions could enter through the shell which is highly porous. This contamination frequently happens in pasteurised and hulled eggs.

Egg microbial contamination could be due to pathogen and/or alterative microorganisms responsible for organoleptic changes (colour and odour). Among the most well-known pathogens are: *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Salmonella Enteritidis*; the latter is the most frequent and feared. Amongst the most well-known alterative microorganisms are: *Pseudomonas spp*., *Aeromonas spp*., *Alcaligenes spp*., *Escherichia coli*, *Proteus spp*. and *Serratia spp*.

During the last ten years, the sector has faced many different issues regarding fraud (i.e. utilisation of incubated eggs that is forbidden by law) and the Fipronil incident (many different EU countries have knowingly or unknowingly used this pesticide for the treatment of red mites despite the law declaring this molecule illegal for livestock usage). In addition, different cycles of Avian Influenza that, without affecting human health, have led to huge modifications in the sector's infrastructure and the supply and demand equilibrium.

3. Analytical methods used to test for authenticity

An overview of methods for authenticity testing is reported in a table format in section 4. Methods are divided into "officially recognised", "other commonly used" and "future analytical approaches".

3.1. Officially recognised methods

The residual quantity of shells, of egg membranes and other possible particles in the egg products must not exceed 100 mg/kg of egg product.

The significant microbial growth that occurs in a shelled egg, before the pasteurisation process, causes the formation of different microbial metabolites and leads to a significant alteration of the original enzymatic properties.

Egg products that have been restored through thermal pasteurization must respect in particular these two microbial parameters: total viable mesophilic bacteria, max 5 log CFU g^{-1}; *Enterobacteriaceae* count, max 2 log CFU g^{-1}.

Microbiological analysis can be performed following specifications reported in ISO and AOAC official documents [8–10].

In general, total viable mesophilic bacteria are enumerated using spread plates of plate count agar incubated at 30 °C for 72 h; Enterobacteriaceae counts are determined by using violet red bile glucose agar with a double layer, incubated at 37 °C for 24 h.

The egg contains a series of organic acids such as succinic and lactic acids, the presence of which is directly correlated to microbial quality and which cannot be altered through thermal restoration actions [11].

3-hydroxybutyric acid is a specific indicator of fertilised, incubated eggs. Succinic acid is used to evaluate microbial spoilage. Lactic acid is increased in both cases, and can be used to screen egg products for suitability for human consumption.

The amounts of lactic and succinic acids in high quality liquid fresh egg products are usually not higher than 200 and 5 mg kg^{-1} dry egg, respectively [12]. Currently, the legal European Union limit is: lactic acid ≤ 1000 mg kg^{-1} dry egg [4,13–15].

The level of 3-hydroxybutyric acid, again following European legislation, must not be higher than 10 mg kg^{-1} dry egg.

A gas chromatographic approach can be used as analytical method for routine testing of these egg carboxylic acids to indicate pre-pasteurisation spoilage of egg products: NaOMe is used for methylation; the carboxylate esters are separated by gas chromatography on a 5% dimethyl siloxane column under gradient temperature [16].

Also available on the market are several enzymatic tests designed to carry out the quantitative determination of lactic, succinic and 3-hydroxybutyric acids in egg products. Sample preparation and analysis can be done using an UV–vis spectrophotometer and following kit instructions / recommendations and other corresponding studies reported in literature [17].

3.2. Other commonly used methods

Alternative analytical methods have been presented in the literature for different type of egg derivatives: egg products, shelled eggs and egg powder.

Simple but effective tests such as solids content and dry matter can be used to detect illegal water addition to liquid egg products.

In any case one of the most critical issues is eggs freshness: this parameter makes a major contribution to the value of the product, for obvious safety reasons and also because consumers may perceive variability in freshness as a lack of quality [18].

Non-destructive methods to determine egg freshness, including optical and spectroscopic measurements on shell or yolk colours, have been proposed in the past [19]. Scientific literature

presents several rapid non-destructive methods able to assess this parameter: both NIR [18,20,21] and Vis-IR [22,23] spectroscopies coupled with chemometric data treatment are able to detect this fraud directly on the shell egg.

At the same time, researchers have attempted to identify volatile components that contribute to the egg's unique flavours and aromas, working with different extraction and analytical techniques (steam-distillation, solvent extraction, purge and trap, etc.): several aldehydes, aromatic compounds and sulphur compounds have been identified in greatest concentrations [24]. In particular, methyl-sulphide compounds are strictly related to deterioration and the perception of unacceptable odours in whole eggs [25]. In most cases these methods are interesting to demonstrate all the potential compounds that can be emitted from eggs, but often the corresponding necessary heating procedures may produce an excess of volatiles which is not representative of the real situation of an egg product which is refrigerated and evaluated by a sensory panel at room temperature or after a short treatment at 30–40 °C.

An alternative strategy to sensing the global profile of organic volatiles emitted by eggs can potentially be achieved by using artificial olfactory systems (AOS), also called "electronic noses". Currently, the application of AOS has been encouraged thanks to the outstanding developments which sensor technology and data processing systems have undergone over the last 20 years [26,27].

Furthermore, in the last years other tests based on hyperspectral imaging are presented as other non-destructive ways to solve the same problem [28].

Recently, a fast-GC electronic nose was presented as a rapid way to collect the volatile fingerprint of hen eggs; subsequently, thanks to chemometric data treatment, eggs were clearly separated according to their storage time, and a prediction of this factor was calculated and validated with a PLS model [29].

Other interesting approaches for freshness evaluation use the intrinsic fluorescence of thick albumen and egg yolk [30] or the quantification of S-Ovalbumin [31]; in addition, a rapid colorimetric test based on the reaction between albumen and 3,3',5,5'-tetramethylbenzidine is mentioned in the literature [32].

Different confirmatory techniques are presented, for instance the evaluation of albumen freshness combining the results obtained with NIR and NMR spectroscopies [33].

Another important issue is the discrimination between organic and conventional eggs and the common approach reported in literature is the evaluation of the carotenoids fingerprint with HPLC analysis, usually followed by chemometric elaboration [34].

Stable isotope analysis has been explored in the past and it seems that the isotopic composition of egg components depends on the diet consumed by the laying hen [35] and on the farming conditions [36]; on the contrary, ratios are not influenced by the pasteurisation process [37]. However, further studies are required before considering this technique a robust tool for egg traceability.

Incubated eggs fraudulently added to fresh egg products are usually detected by exploiting enzymatic assays with 3-hydroxybutyric acid as specific molecular marker [38], as previously indicated. However, it seems that the combination of this legislated marker with the presence of uracil (generated as a consequence of high microbial contamination) could provide a more robust evaluation of the hygienic quality of the products [39].

The analytical method used up to now for uracil determination in eggs is the one presented by Morris [40].

Another possible adulteration is the undeclared addition of dyes to eggs or derivatives which can be detected using liquid chromatography coupled to mass spectrometry [41].

A growing issue of the last years is the introduction of melamine (that results in an apparent increase of the protein content) in eggs. The literature presents portable instruments that, thanks to an approach based on surface-enhanced Raman spectroscopy [42], are able to detect this fraud; however, chromatographic techniques are also widely used [43–45].

3.3. Looking to future analytical perspectives

Future analytical methods for fraud detection in eggs and egg-derived products will continue to explore both rapid and confirmatory approaches. Industries require fast and robust methods for the acceptance or rejection of a batch before its introduction in the production chain but, in parallel, confirmatory methods are required for quality and authenticity certifications, ensuring an improved value to the raw material itself and to the food products.

The creation of predictive models with electronic noses or similar tools able to collect the global fingerprint of the products is the emerging approach for a rapid detection of specific frauds. These methods are fast, easy to use and cheap; instruments are “trained” with pure and adulterated samples and a predictive chemometric model specific for the target fraud is created. Subsequently, unknown samples are analysed and their “authenticity” is predicted by the model. On this topic, a recent study for the assessment of egg product freshness with a GC-IMS instrument was presented in the literature [46].

As regards confirmatory approaches, the novel frontiers for fraud detection will be probably explored with HRMS metabolomic studies: the identification of markers responsible for specific frauds could represent the first step that can lead to the development of specific target methods able to certify the authenticity of the eggs. At the moment the European legislation requires that only a few chemical molecules have to be monitored [4] and the increasing of this “target list” could lead to a more robust evaluation of a specific issue. The first scientific papers are starting to appear on this topic, such as a recent study on a metabolomic approach for the identification of some freshness markers in egg products [47].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Fraud	Short method description	Reference
False Freshness and quality declarations	Tests based on hyperspectral imaging with a combination of analytical techniques to determine the internal quality of eggs	[28]
	NIR spectroscopy with different chemometric techniques for non-destructive freshness assessment on shell eggs	[18,20,21]
	A MOS-artificial olfactory system is described. A correlation with the legal freshness parameters is demonstrated	[27]
	Eggs freshness is evaluated using the intrinsic fluorophores of thick albumen and yolk	[30]
	A Vis or VIS-IR wavelengths range (400-1100 nm) transmittance method allows the evaluation of intact chicken eggs quality	[22,23]
	S-Ovalbumin is presented as a reference index to express commercial shell egg freshness as equivalent egg age	[31]
	A colorimetric test based on the reaction between albumen and 3,3',5,5'-tetramethylbenzidine is used for freshness evaluation	[32]
	Albumen freshness is evaluated combining the results obtained with Vis-NIR and NMR spectroscopy	[33]
	GC-E nose for freshness discrimination and for prediction of storage times in hen eggs	[29]
	Uracil determination with HPLC-UV detector	[40]
False hen farming declaration	Nitrogen isotope composition of chicken eggs, measured with IRMS techniques, is able to differentiate eggs laid in a caged regimen and eggs laid by free range hens	[36]
Melamine contamination	Portable surface-enhanced Raman Spectroscopy is used for fast detection of Melamine contamination, also at trace levels	[42]
	HPLC-MS/MS methods with specific sample pre-treatments for the simultaneous detection of Melamine and Cyanuric Acid	[43,44]
	GC-MS coupled with UPLC-MS/MS methods	[45]
Conventional eggs declared as organic	Carotenoids fingerprint obtained with HPLC-PDA and KNN elaboration	[34]
Dyes addition	Simple sample extraction procedure and UHPLC-MS/MS analysis	[41]

5. Conclusion

Eggs and egg-derived products are widely used in the food industry. In addition, there is a clear and growing need for a more transparent supply chain in order to reduce the risks involving product authenticity and traceability: in fact, especially during the last ten years the sector has faced many different issues linked to food fraud.

The food industry requires fast and robust methods for the acceptance or rejection of a batch before its introduction in the production chain but, in parallel, confirmatory methods are required for quality and authenticity certifications.

Eggs contain a series of metabolites, such as some specific organic acids, whose presence is directly correlated to the freshness and microbial quality and which cannot be altered through thermal restoration actions: gas chromatographic and enzymatic methods are available for their quantitative evaluation.

Future directions will involve finding and validating new analytical solutions to detect non-declared addition of dyes/additives, to categorise different farming approaches and to discriminate between organic and conventional eggs (through LC-MS, IRMS, etc.) and enlarging the range of non-destructive methods (mainly based on optical and spectroscopic measurements).

Alternative emerging strategies deal with HRMS metabolomics and sensing organic volatiles patterns by using electronic noses/ion mobility or similar instruments, then creating predictive models able to collect the global fingerprint of the products in relation to potential fraud issues.

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Honey

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General overview of the products

Honey is a truly natural product traded and consumed all over the world [1]. For thousands of years honey has been produced by bees in the same way and offers a wide spectrum of versatility. Already our ancestors used honey and not only as a sweet food; honey was known as a universal remedy, a valued beauty care product, an effective preservative and was even accepted as a means of payment.

Today honey is used mainly for human consumption either as pure honey or as an ingredient in other food products as a sweetener for juices and cereals. The pure honey available in the market varies from blends favoured for their consistency of colour and flavour to specialist honeys from particular floral, geographical or topological sources. Honey is also commonly used as an additive in beauty care products.

China is the world's largest exporter of honey, with total exports of 128 330 tons in 2016. They are followed by Argentina (81 183 tons), Ukraine (54 442 tons), Vietnam (42 224 tons), India (35 793 tons), Mexico (29 098), Spain (26 874 tons), Germany (25 325 tons), Brazil (24 203 tons), and Belgium (20 816 tons) [2].

The USA is the major importer of honey (166 477 tons in 2016), followed by Germany (81 959 tons), Japan (48 445 tons), UK (41 135 tons), France (35 433 tons) and Spain (27 988 tons). In fact, considered all together, the European Union (EU) of 28 Member States imports more honey than the USA (with a total 283 299 tons) and is a major net importer [3]. The majority of imported honey is blended and labelled as honey from the EU, from non-EU countries or a blend of both, EU and non-EU. These blends are generally sold through supermarkets. Local production offers unifloral and special honeys for the local market.

Whereas bee products royal jelly and propolis are known and accepted as beneficial for human health, pollen is combined and consumed with other food. Wax is less important and used to produce candles or as an ingredient for cosmetic and pharmaceutical products.

Honey received special attention in the US in 2013 when U.S. Immigration and Customs Enforcement and Homeland Security investigations charged five people and two honey-processing

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companies with dumping honey imports from China, including some that were adulterated with unauthorised residues of antibiotics. This incident was considered to be just “the tip of the iceberg” in honey fraud [4].

In the same year in Europe, in the aftermath of the horsemeat scandal, the EU included honey in a top ten list of food products most at risk of food fraud, putting further focus on honey adulteration. However prior to these events, the honey industry sector had been well aware of the concerns of economically motivated adulteration of honey, particularly given the ease with which sugar syrups can be added and premium honey diluted with cheaper types. This has led to considerable efforts being undertaken by various trade bodies such as Apimondia (the International Federation of Beekeepers’ Association), the IHC (International Honey Commission) and the IHEO (International Honey Exporters Organisation) to control the presence of fraudulent product in the market place.

In addition to the diversity of countries exporting honey, often from remote regions with little or no transparency of supply, the practice of beekeeping itself is also under threat. During the last few decades, intensive agriculture and the use of pesticides resulting in a reduction and/or contamination of available areas for bee foraging and the emergence of new bee diseases have all led to a decline in traditional beekeeping activities. The availability of cheap, often fraudulent products in the market resulting in lower prices for domestic honey, has also pushed in the same direction. The ensuing decline in bees, which pollinate a large portion of global food production, poses a serious threat to the food chain. In the EU, it is estimated that pollinators, including honey bees, bumblebees and wild bees, contribute at least EUR 22 billion each year to the European agriculture industry. They ensure pollination for over 80 % of crops and wild plants in Europe [5]. Alarm bells have sounded particularly in Europe and North and South America. An up-to-date review of the current situation on the international honey market is given in reference [6].

1. Product Identity

1.1. Definition of the product and manufacturing process

Honey is primarily a concentrated solution of sugars, composed mainly of glucose and fructose, together with other components such as organic acids, enzymes, vitamins, acetylcholine, flavonoids, minerals is trace quantities [7]. Honey production itself must be considered at two levels, taking into account both the collection and processing of plant fluids by the bees [8], and the extraction and processing of honey by beekeepers and honey packers. The latter includes a number of processing steps which vary according to the unique characteristics of the honey being processed. In general the production process follows six main steps: extraction, dehumidification, liquefaction and blending, heating, pasteurisation, crystallisation, and final packing. Reference [9] provides a detailed description of each of these steps. The INPhO (Information on Post-harvest Operations) of the United Nations FAO (Food and Agriculture Organisation) have produced a Honey Processing toolkit [10] to help in the setting up of a honey infrastructure.

1.2. Current standards of identity or related legislation

Honey, its composition, its specification and related methods are clearly defined and described in international accepted standards such as CODEX, EU, ISO, DIN and guidelines of different trade and beekeeping associations.

1.2.1. In the European Union

If honey is placed on the market in the EU it must meet the requirements of the Honey Directive 2001/110/EC [11]. The definition of honey is given in the first paragraph of Annex I of the EU honey directive: "Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature." According to origin the main types of honey are blossom or nectar honey, obtained from the nectar of plants, and honey dew honey, obtained mainly from excretions of plant sucking insects on the living part of plants or secretions of living parts or plants.

According to mode of production and/or presentation the following types of honey are defined: comb honey, chunk honey or cut comb in honey, drained honey, extracted honey, pressed honey and filtered honey. These definitions apply to honey which is placed directly on the market. If the honey has a foreign taste or odour, or is beginning to ferment or has fermented, or has been overheated, it is only suitable for industrial use or as an ingredient in other foodstuffs, where it is known as baker's honey.

The processing of honey is limited under the terms of the EU honey directive and exists solely of filtering and homogenisation under controlled temperature. When placed on the market as honey or used in any product intended for human consumption, honey must meet the compositional criteria given in Table 1.

According to Decision 2011/163/EU [12] it is mandatory for non-EU countries that want to export honey to EU Member States to be listed on a third country list in accordance with article 29 of Council Directive 96/23/EC [13].

The general food-labelling rules laid down in Directive 2000/13/EC [14] also apply to honey but are subject to certain conditions. In particular the country of origin where the honey has been harvested should be included on the label. In addition, the labelling of filtered honeys and baker's honeys is mandatory for every transaction on the bulk market.

1.2.2. In the United States

The Center for Food Safety and Applied Nutrition of the US FDA (Food and Drug Administration) has recently published nonbinding recommendations for the "Proper labelling of honey and honey products" as a guidance for industry [15]. It addresses the labelling of honey whether sold as a single-ingredient food, or as a mixture of honey and other ingredients such as sweeteners or flavourings. The document also highlights the FDA's definition of adulteration under the FD&C Act (Federal Food, Drug, and Cosmetic Act, Section 402(b)) which stipulates that *"a food is adulterated if: (1) a valuable constituent has been omitted in whole or in part from a food; (2) if any substance has been substituted wholly or in part; (3) if damage or inferiority has been concealed in any manner; or (4) if a substance has been added to a food so as to increase its bulk or weight, reduce its quality or strength, or make it appear to be better or of greater value than it is".*

Since honey is a commodity that is known to be subject to economic adulteration through addition of cane or corn sweeteners, the FDA regularly monitor imported products labelled as honey to ensure that they contain only honey as the sole ingredient. Results of this surveillance exercise are publicly available at Import Alert 36 [16].

Table 1: Compositional criteria for honey [11]

1. Sugar content	
1.1. Fructose and glucose content (sum of both)	
● blossom honey	not less than 60 g/100 g
● honeydew honey, blends of honeydew honey with blossom honey	not less than 45 g/100 g
1.2. Sucrose content	
● in general	not more than 5 g/100 g
● false acacia (*Robinia pseudoacacia*), alfalfa (*Medicago sativa*), Menzies Banksia (*Banksia menziesii*), French honeysuckle (*Hedysarum*), red gum (*Eucalyptus camadulensis*), leatherwood (*Eucryphia lucida*, *Eucryphia milliganii*), *Citrus spp*.	not more than 10 g/100 g
● lavender (*Lavandula spp*.), borage (*Borago officinalis*)	not more than 15 g/100 g
2. Moisture content	
● in general	not more than 20 %
● heather (*Calluna*) and baker's honey in general	not more than 23 %
● baker's honey from heather (*Calluna*)	not more than 25 %
3. Water-insoluble content	
● in general	not more than 0.1 g/100 g
● pressed honey	not more than 0.5 g/100 g
4. Electrical conductivity	
● honey not listed below, and blends of these honeys	not more than 0.8 mS/cm
● honeydew and chestnut honey and blends of these except with those listed below	not less than 0.8 mS/cm
● Exceptions: strawberry tree (*Arbutus unedo*), bell heather (*Erica*), eucalyptus, lime (*Tilia* spp.), ling heather (*Calluna vulgaris*), manuka or jelly bush (*leptospermum*), tea tree (*Melaleuca* spp.)	no limit defined
5. Free acid	
● in general	not more than 50 milli-equivalents acid per 1000 grams
● baker's honey	not more than 80 milli-equivalents
6. Diastase activity and hydroxymethylfurfural content (HMF) determined after processing and blending	
(a) Diastase activity (Schade scale)	
● in general, except baker's honey	not less than 8
● honeys with low natural enzyme content (e.g. citrus honeys) and an HMF content of not more than 15 mg/kg	not less than 3
(b) HMF	
● in general, except baker's honey	not more than 40 mg/kg (subject to the provisions of (a), second indent)
● honeys of declared origin from regions with tropical climate and blends of these honeys	not more than 80 mg/kg

1.2.3. Codex Alimentarius

Codex Standard 12-1981 [17] was adopted in 1981 with revisions in 1987 and 2001. The Codex definition is not worded identically to the Honey Directive but there is very little, if any, difference in meaning. Additionally it defines Blossom or Nectar Honey as the honey from nectar of the plants, and Honeydew honey as the honey coming mainly from excretions of plant sucking insects (*Hemiptera*) on the living parts of plants or secretions of living parts of plants.

Under its requirements for essential composition and quality factors, the Codex Standard also stipulates that honey sold as such should not have added to it any food ingredient, including food additives, and should not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. And chemical or biochemicals treatments to influence honey crystallisation are not permitted.

The Codex Standard also provides acceptable ranges for moisture, sugars and water insoluble solids contents. It provides guidelines for sampling and analysis, as well as for labelling, with clear recommendations for how the honey should be designated.

1.2.4. Further legislation on honey production and quality

It is important to know that non-EU countries have developed definitions and specifications for honey which are slightly different and can differ from the EU honey directive and as a consequence do not meet EU regulation. A review of the differences that exist in international legislation is given in reference [18].

1.2.5. Other bee products

Recently royal jelly was defined in the standard ISO 12824:2016 [19] which specifies the production and sanitary requirements for royal jelly and establishes a series of organoleptic and chemical test methods to control royal jelly quality. It applies to the production of royal jelly (collecting, preliminary processing and packaging) and trade links but not to royal jelly products in which other foods are mixed.

The definition of the other bee products is more diverse and follows more often industry specifications or guidelines of industry associations. The extraction or production of other bee products is not strictly regulated and follows individual procedures.

2. Authenticity issues

2.1. Identification of current authenticity issues

In general honey needs to meet the given definitions and fixed specifications. Questions of authentication occur on two levels. Firstly, 'pure' honey may have been extended by addition of sugar, syrup and/or water. Secondly, if the honey has a more detailed description indicating botanical, geographical or topological origin, the description may be false even though the product is pure honey. There are other possible incorrect descriptions and information such as health claims, if it is 'organic', has 'antibacterial activity' and so on which are difficult to evaluate.

2.1.1. Intentional addition of cheap sugars and sugar syrups

The main focus regarding honey authenticity is on economically motivated adulteration by the addition of foreign sugars. As honey is more highly priced than sweet substances such as sugar and industrial syrups, extension by addition of these at some stage during processing could be an attractive route to adulteration. Existing and regulated methods to analyse the sugar spectrum of honey can show that honey meets its specification in both qualitative and quantitative sugar

composition. However these methods are limited when required to identify sugar addition by different types of syrups from different botanical sources.

Most bulk sweetening materials are derived from cane sugar, beet sugar or by the hydrolysis of starch. The starch is often derived from maize but new sources such as rice are now easily available on the market. Some forms of rice syrup have even been bio-chemically engineered to make them more difficult to detect.

2.1.2. Feeding hives during a nectar flow

Normal beekeeping practice is to ensure that sugar syrup is not laid down in the combs as if it was honey. Providing syrup at the same time as the honey flow constitutes an easy means of adulteration at the earliest stage of production.

2.1.3. Honey moisture content

Mature honey typically contains 13 to 23 % water [20]. If it is over 18 %, there is a risk of fermentation, roughly related to the level of contamination by yeasts and the water content. The Codex Alimentarius sets an acceptable moisture content of not more than 20 % for all honeys with the exception of heather honey (*Calluna*) for which it is set at not more than 23 %. A certain amount of water is lost during processing prior to final bottling into retail packs and this water is usually replaced.

2.1.3.1. Harvesting of immature honey

In some countries such as China, beekeepers harvest the honey early before the comb is capped resulting in a product that has a moisture content around 30 – 40 %. The product is then dehumidified using a vacuum-activated honey dryer to reach an acceptable moisture content. There are ongoing discussions whether such “water honey”, which has a different compositional profile to mature honey, can be considered as pure honey.

2.1.3.2. Illegal use of resin technology

Some honey producers use resin technology to remove unpalatable tastes and aromas linked to certain floral sources. This process involves bringing moisture level of honey up to 40 % and then reducing it to 18-19 %. Although resin technology is regularly used for a number of food products to remove contaminants, its use in honey production is controversial. In particular the process can remove pollen and thus disguise country of origin or floral source. It also removes certain colour components of the honey, transforming dark honey into lighter, more acceptable product.

The current position of the US FDA as regards the use of resin technology is that “the product should be labelled with a name that sufficiently describes its characterizing properties in a way that distinguishes it from honey which has not been treated with resin technology.” Further scientific efforts are underway to establish analytical methodologies and global databases to better assess the use of this technology.

2.1.4. Mislabelling of botanical source

Individual bees forage from a single species of plant as long as that source lasts but honey, even from a single comb, will not often be entirely from a single source. A judgement has to be made, therefore, about a particular honey crop as to whether it can justifiably be called unifloral and thus command a premium price.

2.1.4.1. Incorrect description of blossom and honeydew honey

Blossom honey is derived from the nectaries of flowers. Honeydew honey is derived from non-floral plant secretions. As a particular crop is unlikely to be entirely from one source or the other, it is necessary to judge the designation of the honey on its physical, chemical and microscopical characteristics. Honeydew honey tends to have a higher pollen count, electrical conductivity and ash, to be darker and to contain soot and mould spores [21].

2.1.4.2. Incorrect description of floral source

Some sources such as orange blossom and acacia (*Robinia pseudoacacia*) command a premium price. Such a description will not be justified if the sample contains too much honey from other floral sources.

Authenticity and quality issues cannot always be separated, as exemplified by the case of acacia honey. This may be collected in association with rapeseed cultivation. The characteristic on which the acacia premium is based is the fact that it remains liquid for a long time due to its high fructose/glucose ratio. The relationship between the rate of crystallisation (resulting in an unpleasant gritty taste) and the fructose/glucose ratio is not well understood and research on honey crystallisation is underway. Rapeseed honey crystallises very readily.

Acacia is considered 'pure acacia' if the pollen consists of 20 % or more *Robinia* pollen. If the other pollen present is from many sources, the honey will probably stay liquid and be of the quality associated with acacia. If, on the other hand, the other 80 % is rapeseed, the honey will crystallise quickly and be of unacceptable quality. In this sense, authenticity and quality issues cannot be completely separated.

Specific case of Manuka honey from New Zealand

Manuka honey is recognised as particularly beneficial to human health due to its exceptional antiseptic properties. Manuka (*Leptospermum scoparium*) is a scrub-type tree that grows only in New Zealand and some parts of Australia. Its nectar contains a specific molecule, dihydroxyacetone (DHA), that converts into methylglyoxal (MGO) during maturation and aging of the honey [22]. It is this latter compound that is primarily responsible for the strong antimicrobial activity of the honey.

To deal with the increasing risk of fraud, the New Zealand Ministry for Primary Industries recently published a science definition of monofloral and multifloral manuka honey [23]. This details a combination of five attributes (4 chemicals and 1 DNA marker from manuka pollen) which are required to authenticate monofloral and multifloral manuka honey. These attributes can be identified using 2 laboratory tests. A chemical test for the 4 marker molecules 3-phenyllactic acid, 2'-methoxyacetophenone, 2-methoxybenzoic acid and 4-hydroxyphenyllactic acid which are determined by liquid chromatography and a DNA test for very specific manuka DNA *leptospermum scoparium* performed by quantitative or real-time PCR (polymerase chain reaction).

2.1.4.3. Mislabelling of geographical origin

A honey from a particular geographical origin may also command a premium compared with the bulk blend. For example, Greek Hymettus honey and certain 'forest' honeys fetch a higher price.

A number of specific honey origins have been recognised under the EU quality labels (PDOs and PGIs) that guarantee that the product is from a specific region and follow a particular traditional production process [24]. These include honeys from France: miel des Cévennes, de Provence, d'Alsace (PGI); miel de sapin des Vosges, de Corse (PDO); from Italy: miel Varesino, delle Dolomiti

Bellunesi, della Lunigiana (PDO); from Spain (Mel de Galicia (PGI); Mel Villuercas-Ibores, de Liébana, de Tenerife, de Granada, de la Alcarria (PDO). These special labels are recognised by the consumer and command a premium price.

2.2. Potential threat to public health

Honey is recognised as a healthy product. For people with a pollen allergy nectar honey can have a low potential threat to their health. Also a contamination by bee feedings like milk proteins can potentially cause an allergic reaction. In any case such allergic reactions are very seldom.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

There are numerous publications devoted to analytical methods to test for honey quality and authenticity, including physical parameters (electrical conductivity, rheological properties, specific rotation, colour and water activity) and chemical components (moisture, sugars, enzymes, HMF, acidity and pH, formol index, insoluble solids, organic acids, proteins, amino acids, vitamins, minerals, volatile and semi-volatile compounds and polyphenols). A comprehensive and recent review of these analytical methods is provided in reference [25].

3.1.1. AOAC methods

The AOAC International Compendium of methods provides details of AOAC methods for the main physical and chemical parameters of honey. These have also been included in Codex Standard 12-1981 and its subsequent revisions. Some of the main parameters and related methods are:

- AOAC 969.38B Determination of moisture content
- AOAC 980.23 Hydroxymethylfurfural (HMF)
- AOAC 958.09 Diastase activity
- AOAC 998.12 Detection of C_4 sugar in honey (more details provided below)

3.1.2. IHC methods

In addition to officially-recognised methods, the International Honey Commission (IHC) has collaboratively tested a wide range of different methods to test for honey authenticity [26]. Harmonised methods for which precision criteria are available include:

- Moisture (refractometric method), electrical conductivity, ash content, pH and free acidity (titration),
- Hydroxymethylfurfural (HPLC, or White/Winkler methods),
- Diastase (Schade method, Phadebas α-amylase assay),
- Sugars (by HPLC or GC),
- Insoluble matter, invertase activity, proline and specific rotation.

3.1.3. Focus on specific methods

3.1.3.1. Melissopalynology

Melissopalynology, or pollen analysis, is an essential part of honey authenticity testing. Pollens grains from different types of plants have a distinctive morphology that can be identified by microscopic examination [7]. The technique, which requires expert judgement, is used to determine the country of origin by linking pollen type to the characteristic flora of the geographical source, or to verify authenticity when a particular botanical origin is claimed.

The pollen count can be used to estimate the proportions of nectar present. However the method does have some limitations, mainly due to the natural variability of amounts of pollen from botanical sources. For example, in some cases the specific pollen may be 'under-represented' such as for citrus and lavender, whereas for others, such as forget-me-not, the pollen is 'over-represented'.

Pollen is also available as a product and the determined adulterator could filter out all the pollen and add back pollen of choice.

Despite its limitations however, pollen analysis is still a useful method to control country of origin. A review of harmonised methods of melissopalynology is given in reference [27].

3.1.3.2. HMF as an indicator of freshness or excessive heating

All honeys contain some amount of hydroxymethylfurfural (HMF) which is formed by from the action of the acidity in honey on reducing sugars through the Maillard reaction. Excess heating during processing or unsuitable storage conditions can HMF content, making it a useful indicator of honey quality. Both the EU Directive and Codex Standard 12-1981 and its subsequent revisions have fixed a limit of 40 mg/kg for HMF in honey after processing and/or blending, with a higher limit of 80 mg/kg in the case of honey of declared origin from countries or regions with tropical ambient temperatures, and blends of these honeys. Reference [28] provides an overview levels of HMF in honey and its effect on bee and human health.

Reversed phase HPLC with UV detection is the most commonly used method for the determination of HMF in honey. The sample should be a clear, filtered aqueous solution of honey. Details on sample preparation are available under AOAC 920.180 or in the IHC description of methods of analyses.

3.1.3.3. Determination of C_4 sugars in honey

Maize and sugar cane metabolise by the Hatch-Slack or C_4 metabolic pathway. As a result, syrups derived from them exhibit a $^{13}C/^{12}C$ ratio, expressed as a δ-value close to -10 ‰ compared with a value for honey which on average is around -25.4 ‰. This difference has been used very successfully to detect adulteration in honey [29,30], and is an official AOAC Method 998-12. In this method the $^{13}C/^{12}C$ ratio, expressed as $\delta^{13}C$ of the whole honey is measured by SIRA (stable isotope ratio analysis) and compared to the $\delta^{13}C$ value of the protein isolated from the honey. The difference between these values is a measure of the C_4 sugar content of the honey, provided that both honey and protein have been analysed on the same instrument [31,32].

Addition of syrups derived from beet and other plants utilising the Calvin or C_3 metabolic pathway remains a considerable analytical challenge. Further solutions are described in the following sections.

3.2. Other commonly used methods

3.2.1. Chromatographic techniques

A variety of chromatographic methods have been developed in order to authenticate honey from different floral origins and to detect added sugar and sugar syrups. This section will provide a few examples of methods available in the literature.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are commonly used to quantify the major carbohydrates, glucose, fructose, sucrose [33]. A method using anion-exchange chromatography in conjunction with pulsed amperometric detection (HPAEC-PAD) has been used to provide a qualitative and quantitative analysis of the minor oligosaccharides present in honey [34]. This method has also been successfully used to detect the addition of certain starch-derived sweeteners in fruit juices.

Carbohydrate profiles quantified by HPAEC-PAD and chemometrics have been used to characterise the botanical origin of honey from a single geographical area [35]. The same technique together with an integrated chemometric approach has been described as an improved COFRAC (COmité FRançais d'ACréditation) method for the evaluation of honey quality and the characterisation of floral source [36].

Chromatographic methods have also been extensively used to analyse chemical components other than sugars as markers of honeys from specific floral sources. Examples the content of phenolic acids, including caffeic, chlorogenic, p-coumaric, ferulic, homogentisic, p-hydroxybenzoic and vanillic acids, and flavonoids, such as apigenin, genistein, hesperetin, kaempferol, luteolin, rhamnetin, rutin, tricetin and quercetin [37]. Amino acid analysis of honey by HPLC together with statistical treatment of the resulting data has also been used to discriminate different botanical origins and to detect the addition of sugar syrup [38].

Methyl anthranilate is a good indicator for orange blossom honey [39–41], which contains very little citrus pollen. Synephrine has been described as another biomarker for orange honey authenticity and can be determined following a LC-MS/MS method [42]. Numerous other biomarkers have been described in the literature.

3.2.2. Stable isotope analysis

3.2.2.1. Detection of sugar and sugar syrups

As described above, stable isotope analysis is the official AOAC method for the detection of sugar addition in honey. The method cannot, however, detect all sugar sources. A new technique developed by Elflein and Raezke [43] combines Liquid Chromatography with Isotope Ratio Mass Spectrometry. This method enables the separation of the individual sugars including their individual $^{13}C/^{12}C$ ratios and is a considerable improvement in the detection of honey adulteration [44,45].

3.2.2.2. Verification of geographical origin

The stable isotope ratios of the light bio-elements have been successfully used to verify country of origin of a number of foodstuffs. An investigation was undertaken as part of the European product

TRACE[1] in which the stable isotope ratios of the elements carbon, nitrogen, sulphur and hydrogen were measured in the protein fraction extracted from honey produced in 20 European areas [46]. The honey protein fraction was specifically chosen since it is part of the preparation in the method to detect added C_4 sugar (as described above) and less easy to manipulate. The study demonstrated that both hydrogen and carbon isotopes in honey protein are correlated to precipitation and climate. The sulphur stable isotope composition of the honey protein is clearly influenced by the geology of the rock underlying the soil in which the flora grew, and from which the bees foraged nectar and pollen. Despite the natural variability of the product and the similarity of geological and climatic conditions across the countries investigated, the study concluded that the four stable isotope ratios considered here, measured on honey protein can be applied to verify the origin of honey.

3.2.3. DNA-based techniques

Biomolecular methods are becoming more frequently used in the authentication of honey since it contains intrinsic DNA markers that can be used to identify origin. One of the most important examples is the use of a DNA marker for manuka honey, required under the New Zealand government's definition for authentic manuka honey, as described above.

Several studies applying DNA-based techniques have been proposed in the literature. Sobrino-Gregorio et al. [47] use both conventional and real-time PCR DNA amplification techniques to the detection and quantification of rice molasses in honey. In another study, PCR primers have been used to amplify specific fragments from the informative mitochondrial DNA region of *Apis mellifera* [48]. Soares et al. [49] have exploited DNA barcoding combined with high resolution melting (HRM) analysis to establish the botanical origin of honey, using lavender honey as a case study.

Honey can be produced by different species of honeybees, of which there are two main species of economic importance. These are *Apis mellifera* (known as the European honeybee) and *Apis cerana* (known as the Asian honeybee). Due to the decline of the wild populations of the Asian honeybee, this honey generally attains much higher market value, being prone to adulteration. A novel real-time PCR method with high resolution melting analysis has been developed to target the 16S rRNA gene of both bee species, which was then further successfully applied to the authentication of Asian and European honey samples [50].

3.2.4. Spectroscopic methods

3.2.4.1. FT-MIR – NIR

FT-NIR (Fourier Transform Near Infrared) and FT-MIR (Fourier Transform Mid-Infrared) spectroscopies have been proposed as rapid methods for honey authentication. They provide simultaneous determination of sugars and other physicochemical parameters and can be used in routine quality control of honey.

Pita-Calvo et al. [51] have used FT-NIR and FT-MIR spectroscopy to distinguish between honeydew and blossom honey. Two characteristic markers of honeydew honey, the trisaccharide melezitose and a diacylglycerilether, made it possible to classify honeydew and blossom honeys correctly.

[1] TRACE Project. Tracing the origin of food. 2005-2009. Funded by the European Commission under the 6th Framework Programme.

NIR spectroscopy is particularly useful as a rapid and non-destructive method for the detection of honey adulteration. Combined with chemometric data treatment the technique has been used to discriminate honey adulterated with high fructose corn syrup (HFCS) demonstrating its potential as a screening method for quality monitoring [52].

3.2.4.2. ^{1}H NMR screening

An innovative analytical approach using proton-NMR (Nuclear Magnetic Resonance) profiling coupled to suitable quantification procedures and statistical models has been developed to tackle the most common adulterations and quality deviations in honey [53]. The NMR technique has a number of advantages: it is highly reproducible and requires a very simple sample preparation. In addition the NMR spectra can be used as "fingerprints" to compare, discriminate or classify samples while its structural elucidation power can be used to characterise novel or unknown biomarkers.

Having a wide screening potential, based on a global observation of all soluble components of honey, NMR profiling is now also widely used for authenticity checks. Since it is independent from potential manipulations of pollen, it also provides a complementary tool to check the declared botanical and geographical origin, beyond the detection of sugar addition and the fast monitoring of many honey quality parameters. This spectroscopic technique also produces a unique fingerprint for each sample, which can be used to check the traceability along the supply chain.

The NMR method has been used to characterise known manuka honey markers, methylglyoxal and dihydroxyacetone. Together with a newly identified NMR marker, leptosperin. The technique can be used to discriminate manuka honey from other floral honey types from Oceania [54].

The NMR technique is now considered as one of the most powerful methods to detect the various forms of adulteration that were described earlier [55].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data, analyte or parameter	Authenticity issue or information
Microscopy	Pollen analysis	Botanical and geographical source
Refractometry	Moisture content	Compliance with regulated limits
Conductimetry	Electrical Conductivity	Distinguishes blossom honey & honeydew
Colorimetry	Diastase	Detects heat abuse
HPLC, Colorimetry	Hydroxymethylfurfural (HMF)	Detects heat abuse
Photometry	Heat-stable α-amylase (diastase surrogate)	Foreign enzyme as marker for foreign syrup additions
Photometry	Foreign α-amylases (diastase surrogates)	Foreign enzyme as marker for foreign syrup additions
HPLC, GC	Sugars	Sugar profile, detects abnormal sugar profile
IRMS	^{13}C ratios of whole honey and extracted protein	Detects C_4 sugar addition
IRMS	Isotope ratios of H, C, N and S	Geographical origin
^{1}H-NMR	Untargeted and targeted screening against reference data base, sugars	Syrup additions, quality deviations, mannose, botanical/geographical origin
IRMS	Isotope ratios of H, C, N and S	Geographical origin
LC-UV	Foreign ß-/-y-amylases (diastase surrogates)	Foreign enzyme as marker for foreign syrup additions
LC-ELSD	ß-fructofuranosidase (invertase surrogate)	Foreign enzyme as marker for foreign syrup additions
LC-ELSD	Syrup-specific oligosaccharides	Marker molecule for foreign syrup additions
ICP-MS	Arsenic	Trace marker for rice syrup additions (TMR)
LC-MS	Marker molecule	Specific marker for rice syrup / cassava syrup additions
LC-MS	Colorant E150d	Addition of dyes or colourings
LC-ELSD	Mannose	Marker molecule for foreign syrup additions
LCMS	Psicose	Marker molecule for foreign syrup additions

5. Conclusion

Today honey and bee products are continuously recognised as pure natural products. The importance of these products is confirmed by the extent of current controls on honey authenticity at every stage of the global supply chain. The composition of honey, the treatment with veterinary drugs and contamination by pesticides and other contaminants are closely monitored.

With the daily news reporting repeatedly about food fraud, adulteration of honey and other bee products will still draw significant attention in the future. It is the responsibility of all stakeholders of the honey supply chain to optimise existing control mechanisms.

As mentioned earlier, the European Commission started an action plan to tackle food fraud late in 2013. As a follow-up in 2015, the European Commission launched a coordinated control plan on honey authenticity in which its Joint Research Centre carried out analyses to detect honey adulteration with exogenous sugars (Commission Recommendation C(2015) 1558 [56]). The aim of the control plan was to establish the prevalence on the European Union market of: (a) honey mislabelled with regard to its geographical and/or botanical origin and (b) products declared or presented as honey although containing exogenous sugars or sugar products. 2 264 honey samples were collected at all stages of the supply chain; the majority of the samples came from retailers. More than 10 % of the honey samples checked by EA/LC-IRMS did not conform to published benchmark purity criteria indicating that foreign sugars may have been added. Around 20 % of honey either declared as blends of EU honeys, or unblended honeys bearing a geographical reference related to an EU Member State or a third country were suspected to contain added sugar.

The published report demonstrates the need for further investigation by all stakeholders of the honey industry to ensure authentic honey and to justify the trust of the consumer in this natural product.

An outlook is given in the Meeting Report of the Technical Round Table on Honey Authentication [57]. The participants agreed on:

- A critical review of the current definition of identity and purity criteria of honey is necessary.
- Acceptance / rejection criteria for authenticating honey are needed.
- An appropriate analysis of the vulnerability of the honey supply chain should be done and an improved traceability system implemented.
- Screening methods should be developed to economise testing.
- Analytical methods to detect emerging fraud cases should be developed and already existing methods should be validated.
- A mechanism for providing quality assurance tools should be established.
- Chemical and biological characteristics of genuine honeys (including blends), bee feeding products, and products from inappropriate practices should be generated and stored in a publicly available database.

In addition to those methods that are already regulated, it will be important to regulate methods that are more suitable to tackle food fraud. One idea will be the evaluation of EA/LC-IRMS as an official method for honey authenticity controls in future. If this happens authorities should be able to claim “non-authentic” honey more often which will have a significant impact on the whole production chain of honey.

With its wide screening potential, the NMR profiling technique is now also widely used for authenticity checks. More generally, non-targeted methods will be a major add-on to the existing approaches for anticipating new fraudulent practices in the future.

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Meat and meat products

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General overview of the products

Demand for animal-derived food is increasing because of population growth, rising income and urbanisation, with poultry meat showing the fastest trend over the last decades and becoming, in 2016, the meat with the highest consumption worldwide (Figure 1). Estimates from the FAO show that the global production of meat in 2016 is around 330 million tons. At the global level, the three main types of meat produced are: poultry meat (36.5 %), pork (35.8 %) and bovine (21.1 %). The average annual growth rate for poultry meat over the last 45 years was 2.3 %, while it was only 0.7 % for beef and 1.8 % for pork [1].

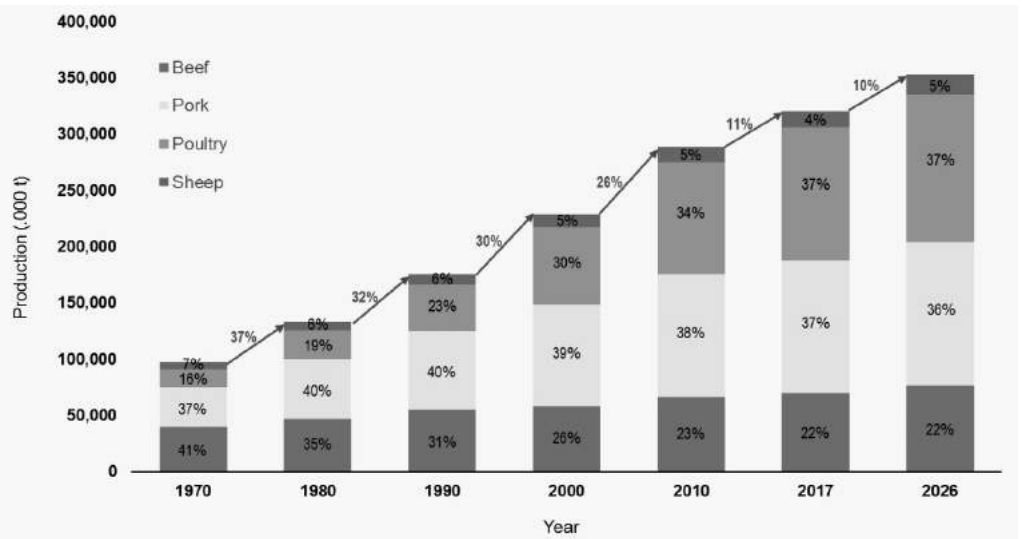


Figure 1: Evolution of global meat production from 1970 to 2016 (Own design, data source: Faostat)

https://doi.org/10.32741/fihb.4.meat

In the last few years, world bovine meat production has been increasing at a modest pace. The United States are the major bovine meat producing country in the world, with 11 million tons (Figure 2). The second producer is Brazil, with 9 million tons, with a herd expansion encouraged by international trade, despite a reduction in domestic demand. The European Union (EU) is the third beef producer (almost 8 million tons), followed by China, India and Argentina. In 2016, China produced about 55 million metric tons of pork which accounted for 47 % of total world production. The EU is the second world producer with almost 24 million tons followed by Vietnam, Brazil and the Russian Federation. The biggest poultry meat producers are the United States, with almost 21 million tons a year, followed by China, with 19 million tons, the EU and Brazil with about 14 million tons (Figure 2).

Global meat production is projected to be 13 % higher in 2026 relative to the base period (2014-16). This compares with an increase of almost 20 % in the previous decade (Figure 1). Developing countries are projected to account for the vast majority of the total increase, with a more intensive use of feed in the production process. Poultry meat is the primary driver of the growth in total meat production in response to expanding global demand for this more affordable animal protein compared to red meats. Low production costs and lower product prices have contributed to making poultry the meat of choice both for producers and consumers in developing countries. In the bovine meat sector, cow herds are being rebuilt in several major producing regions, but the decline in cattle slaughter in these regions is projected to be offset by higher carcass weights. Pork production will also increase after 2017, driven by slow herd expansion in China.

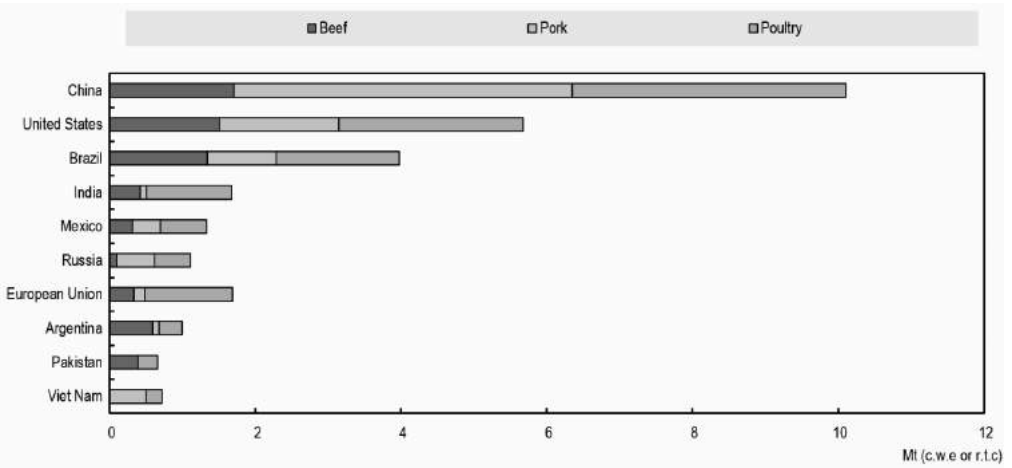


Figure 2: Countries with the greatest share of additional meat production by meat type [1]

In the EU-28, pork is by far the main meat produced, followed by chicken meat and beef (Figure 3). In the EU, beef is mainly produced from cattle breeds grown specifically for their meat, but it can also come from dairy cattle. France (19.0 %), Germany (14.7 %) and the United Kingdom (11.7 %) accounted for almost half (46 %) of the total EU-28 beef production (Figure 4).

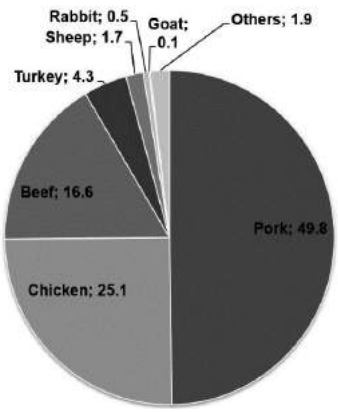


Figure 3: Production share of main meat produced in EU on 2016 (Own design, data source: Eurostat)

As for pork meat production, Germany produced around one quarter (23.9 %) of the EU-28's pig meat in 2016, while Spain produced one sixth (17.9 %) of the EU-28 total, equal to 23 million tons. Finally, Poland, France, the United Kingdom, Spain and Germany each contributed between 10 and 15 % to the EU-28 production of poultry meat in 2016 (Figure 4).

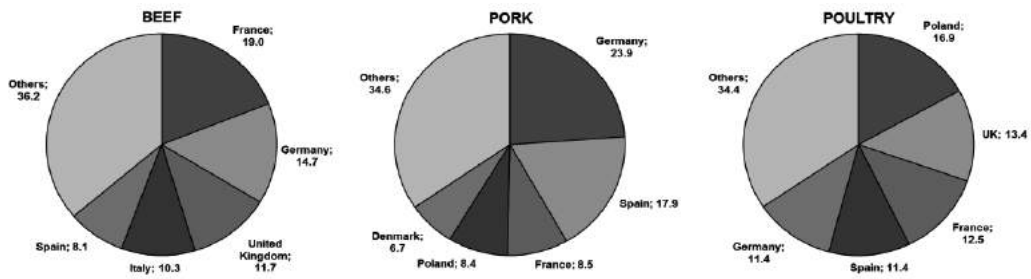


Figure 4: EU Countries with the greatest share of production by meat type on 2016 (Own design, data source: Eurostat)

Global meat apparent consumption per capita is expected to stagnate at 34.6 kg by 2026, an increase of less than half a kilogram compared to the base period (Figure 5). Beef consumption will gradually increase over the next ten years. By 2026, and relative to the base period, it is expected to increase by almost 6 % in developed countries, whereas in developing regions it is expected to increase by approximately 17 %. In per capita terms, beef consumption in the developing world remains low relative to developed countries, at about one-third in volume terms. High population numbers in Asia remain a major driver of growth, combined with the positive perception of Chinese buyers that bovine and ovine meat are healthier and disease-free; the result is an expected 44 % increase in beef consumed in Asia over the next decade [1].

Pork consumption on a per capita basis declines marginally over the outlook period with consumption in most developed countries reaching saturation levels (Figure 5). Among the developing countries, significant regional differences are evident in per capita pork consumption. Growth is sustained in Argentina, Brazil, Mexico, and Uruguay, albeit at a generally slower rate

than the past decade. Pork consumption has grown rapidly over the past few years in Latin America, fuelled by increased domestic production, improved quality, and favourable relative prices that have positioned pork as one of the favoured meats, along with poultry. Conversely, many countries with favourable economic conditions and expanding meat consumption do not traditionally consume high levels of pork relative to other meats, resulting in stagnant and even declining consumption on a per capita basis at the regional level. Population expansion still supports growth in total pork consumption in these regions [1].

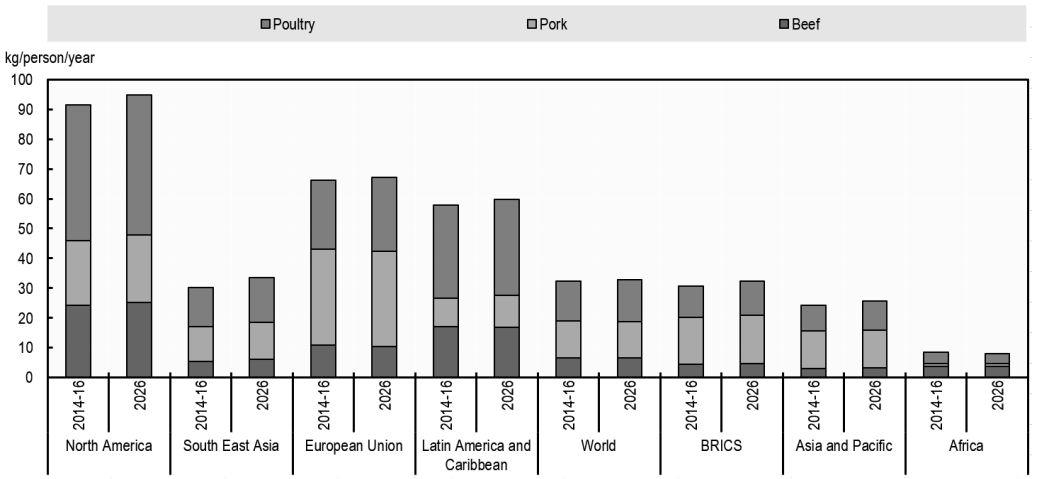


Figure 5: Annual growth in consumption of meat 2007-2016 and 2017-2026 [1].

Consumption of poultry meat increases regardless of region or income level. Per capita consumption will grow, even in the developed world, but growth rates will remain slightly higher in developing regions. Worldwide, poultry grew rapidly and surpassed pork as the preferred animal protein in 2016. This will remain the case during the outlook period and, of all the additional meat consumed over the next decade, poultry is expected to account for almost 45 % (Figure 5) [1].

Per capita consumption of meat is expected to slightly increase in the EU overall from 69.1 to 70.7 kilograms by 2026, whereas the individual big five countries (Italy, UK, Spain, France and Germany) are predicted to experience a decrease in consumption (Figure 5).

1. Product Identity

1.1. Definition of the product and manufacturing process

The first distinction is between fresh and processed meat. Fresh meat is defined as meat having undergone no treatment other than chilling and freezing, while processed meat is a very broad category of many different types of products, all defined by having undergone at least one further processing or preparation step such as, i.e. grinding, adding an ingredient or cooking, which changes the appearance, texture or taste. The main classes of processed meat are described below:

- Minced meat: boneless meat reduced in fragments which contains less than 1 % salt;
- Mechanically separated meat: obtained by removing meat from bones using mechanical devices (high-pressure application machinery) that contribute to the loss or modification of muscle-fibrous meat texture;
- Desinewed meat: obtained by removing sinews, tendons, cartilages and thicker collagen using mechanical devices (low-pressure application machinery) without modification of muscle-fibrous meat texture.
- Meat preparations: fresh meat (including fragments), containing flavourings, additives or subjected to treatments that do not modify the muscle-fibrous texture;
- Meat products: processed products derived from processed meat or further processing of other meat products subjected to treatments that modify the muscle-fibrous texture.

There are many meat products that are produced in different countries, but it is possible to categorise them in six groups, considering the processing technology used:

- Fresh processed meat products: products that are composed of muscle mixed fragments with different amounts of animal fat. They are salted, and small quantities of non-meat ingredients are added to improve taste and binding. All ingredients are added fresh and some of these products are filled in casings. They are cooked or fried immediately prior to consumption (e.g. hamburgers).
- Formed meat: products which may give the impression that they are made of a whole piece of meat, but actually consist of different pieces combined together by other ingredients, including food additives and food enzymes or by other means.
- Cured meat products: products that are submitted to a curing process and treated with small amounts of nitrite. These products are divided in two groups:
    - *Cured raw meat*: products that undergo a process of curing, fermentation and ripening in controlled conditions without any heat treatment (e.g. raw cured beef);
    - *Cured cooked meat:* products that undergo a curing process and then are submitted to heat treatment (e.g. cooked pork ham).
- Raw-cooked meat products: products composed of muscle meat, fat and non-meat ingredients which are reduced in fragments, mixed and portioned before being submitted to heat treatment (e.g. meat loaf);
- Precooked: cooked meat products; products composed of muscle trimmings, fatty tissues, meat from the head of the animal, animal skin, blood, liver and other edible parts, which undergo two different heating processes - precooking of raw materials and cooking of the finished product mix (e.g. corned beef);
- Raw fermented sausages: uncooked meat products obtained by a mixture of lean and fatty tissues combined with salts, nitrite, sugars, spices and other non-meat ingredients filled into casings. They are submitted to a fermentation process (drying and ripening) to obtain the typical flavour and are consumed raw (e.g. salami).
- Dried meat products: lean meat that undergoes a process of drying in natural or artificial conditions to prolong its shelf-life (e.g. dried meat strips or flat pieces).

1.2. Current standards of identity or related legislation

The definitions of "meat", "fresh meat", “carcase”, “offal”, “viscera”, “minced meat”, “meat preparations”, “meat products” and “mechanically separated meat” are laid down in Annex I of Regulation (EC) No 853/2004 (Table 1) [2].

With the Food Information Regulation (EU) No 1169/2011 (FIC) [3], uniform labelling requirements have been applied across the EU and include fresh meat and processed meat products (Table 1).

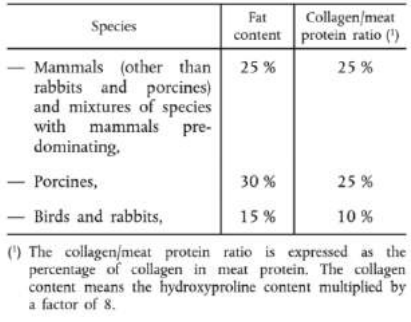
Specific rules for the origin of beef and beef products were introduced after the BSE crisis in 2000. More recently new rules on country of origin information for meat from sheep, pigs, goats and poultry have been issued in the EU (Table 2).

Definitions for meat are also given in the CODEX Standard for Luncheon Meat (Codex Stan 89-1891) and Cooked Cured Chopped Meat (Codex Stan 98-1891), which defines meat as “the edible part, including edible offal, of any mammal slaughtered in an abattoir”; poultry meat as “the edible part of any domesticated birds, including chickens, turkeys, geese, guinea-fowl or pigeons, slaughtered in an abattoir" ; and edible offal as “such offal as have been passed as fit for human consumption but not including lungs, ears, scalp, snout (including lips and muzzle) mucous membrane, sinews, genital system, udders, intestines and urinary bladder. Edible offal does not include poultry skin.” These definitions are used for the raw materials contained in these products.

However, many differences exist in the interpretation of ‘meat’ for use in meat products among different countries such as the EU, the USA, Brazil, and China. Therefore, methods to determine authenticity need to consider the legal requirements specific to each country.

Table 1: Meat definitions according to EU Legislation

EC Regulation	Basic definition of meat	Animal carcass components specifically excluded from the definition	Basis of meat content declaration
Regulation (EC) n. 853/2004	**Meat** Edible parts of the animals (including blood): - Domestic ungulates (domestic bovine including Bubalus and Bison species, porcine, ovine and caprine animals, and domestic solipeds); - Poultry (farmed birds, including birds that are not considered as domestic, but which are farmed as domestic animals, with the exception of ratites); - Lagomorphs (rabbits, hares and rodents); - Wild game (wild ungulates, lagomorphs and birds, as well as other land mammals that are hunted for human consumption); - Farmed game: farmed ratites and other farmed land mammals. Meat can be defined "Fresh meat" if it has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.	Genital organs of either female or male animals, except testicles; urinary organs, except the kidneys and the bladder; the cartilage of the larynx, the trachea and the extra-lobular bronchi; eyes and eyelids; the external auditory meatus; horn tissue; and in poultry, the head – except the comb and the ears, the wattles and caruncles – the oesophagus, the crop, the intestines and the genital organs.	Not appropriate
	Carcass Body of an animal after slaughter and dressing. The definition of 'carcass' for bovine, pigs, sheep, goat and poultry is reported in Regulation (EC) no. 1165/2008.		
	Offal Fresh meat other than that of the carcass, including viscera and blood		
	Viscera Organs of the thoracic, abdominal and pelvic cavities, as well as the trachea and oesophagus and, in birds, the crop		
	Meat preparations Fresh meat, including meat that has been reduced to fragments (minced meat), which has had other foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.	The same for meat and minced meat.	
	Meat products Processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat	The same for meat.	

<table>
<tr><th>EC Regulation</th><th>Basic definition of meat</th><th>Animal carcass components specifically excluded from the definition</th><th>Basis of meat content declaration</th></tr>
<tr><td></td><td>Minced meat
Boned meat that has been minced into fragments and contains less than 1 % salt. Raw material used to prepare minced meat must derive from skeletal muscle, including adherent fatty tissues.</td><td>Raw material used to prepare minced meat must not derive from: scrap cuttings and scrap trimmings (other than whole muscle cuttings); mechanically separated meat; meat containing bone fragments or skin; or meat of the head with the exception of the masseters, the non-muscular part of the linea alba, the region of the carpus and the tarsus, bone scrapings and the muscles of the diaphragm (unless the serosa has been removed).</td><td></td></tr>
<tr><td></td><td>Mechanically separated meat (MSM)
Product obtained by removing meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure</td><td>For poultry, the feet, neck skin and head; and for other animals, the bones of the head, feet, tails, femur, tibia, fibula, humerus, radius and ulna
The use of bones or bone-in cuts of bovine, ovine and caprine animals is prohibited for the production (Reg. EC/999/2001 [4]).</td><td></td></tr>
<tr><td>Regulation (EC) no. 1169/2011</td><td>Meat
For labelling purpose, the term “meat” is referred to: skeletal muscles of mammalian and bird species (*) recognised as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed the values indicated below and where the meat constitutes an ingredient of another food:
 
<table>
<tr><th>Species</th><th>Fat content</th><th>Collagen/meat protein ratio (1)</th></tr>
<tr><td>— Mammals (other than rabbits and porcines) and mixtures of species with mammals predominating,</td><td>25 %</td><td>25 %</td></tr>
<tr><td>— Porcines,</td><td>30 %</td><td>25 %</td></tr>
<tr><td>— Birds and rabbits,</td><td>15 %</td><td>10 %</td></tr>
</table>
(1) The collagen/meat protein ratio is expressed as the percentage of collagen in meat protein. The collagen content means the hydroxyproline content multiplied by a factor of 8.</td><td>Mechanically separated meat</td><td>If maximum limits are exceeded, but all their criteria for the definition of ‘meat’ are satisfied, the ‘… meat’ content must be adjusted downwards accordingly, and the list of ingredients must mention, in addition to the term ‘… meat’, the presence of fat and/or connective tissue.
Meat species is required on the label unless indicated by the product name.</td></tr>
</table>

EC Regulation	Basic definition of meat	Animal carcass components specifically excluded from the definition	Basis of meat content declaration
	Minced meat Specific requirements concerning the designation of minced meat:	Mechanically separated meat	In the case of minced meat and meat preparations made from pre-prepared minced meat, except for sausages and sausage meat, the label must indicate the appropriate % of fat and collagen in meat protein (i.e. "% of fat under.....", " % of collagen in meat under....") Meat species is required on the label unless indicated by the product name.

	Fat content	Collagen/meat protein ratio (1)
lean minced meat,	≤ 7 %	≤ 12 %
minced pure beef,	≤ 20 %	≤ 15 %
minced meat containing pigmeat,	≤ 30 %	≤ 18 %
minced meat of other species,	≤ 25 %	≤ 15 %

	Formed meat Meat products, meat preparations which may give the impression that they are made of a whole piece of meat, but actually consist of different pieces combined together by other ingredients, including food additives and food enzymes or by other means		

Table 2: Rules about the labelling of meat products within European Union

Meat	Compulsory information to be provided on the label
Beef (except mince or trimmings)	As required by Regulation (EC) No 1760/2000 and Commission Regulation (EC) 1825/2000, as amended: - Reference number or reference code ensuring the link between the meat and the animal or animals - Member State or third country of birth - All Member States or third countries where fattening took place - Member State or third country where slaughter took place and where the carcass was cut
Pork, lamb, mutton, goat meat and poultry (except mince or trimmings)	As required by Regulation (EU) No. 1337/2013): - Reference number or batch code identifying the specific animal or group of animals from which the meat was derived - name of member state or non-EU country - Member State or third country where an animal has been reared in more than one country, the table below explains what should appear on the labelling. - Member State or third country where slaughter took place

2. Authenticity issues

2.1. Identification of current authenticity issues

In general beef is the main added-value meat product that is the most widely traded, and therefore where major authenticity problems can occur. This is reflected in the list of authenticity topics below. However, some authenticity issues do concern other meat types such as poultry or lamb. These are mentioned where relevant.

Food fraud is a global issue which damages the reputation of companies, disrupts markets and erodes consumer confidence. Food fraud surfaces more frequently in certain supply chains and that of meat is always present. The importance of studies covering these topics is mainly related to economic issues associated with fraud in high-value foods like beef, with cheaper ingredients added. However other fraudulent practices in the meat industry could occur such as: 1) the origin of meat and the animal feeding regime (as in the case of certified regional products of poultry and lamb, for example); 2) substitution of meat ingredients by other animal species, tissues, fat or proteins; 3) modification of the processing methods of meat products; and 4) addition of non-meat components such as water or additives.

2.1.1. Substitution

2.1.1.1. Species substitution

The correct description of the origin of meat and meat products is a common problem reported worldwide. This type of food fraud is, typically, an intentional act for economic gain, using sources of low-priced meats in high-value meat products. Consequences include economic, religious, but also health concerns: counterfeit components may be toxic and the undeclared addition of some ingredients (e.g. soy, wheat, dairy) can pose health risks for consumers with food allergies or intolerances. The most important authenticity issues are the species of meat, followed by specific cut, breed of the animal and geographical origin of the meat or meat product [5].

The flesh of many meat species differs only subtly in appearance and texture, making it difficult to identify the species just by visual inspection. Once meat is comminuted and incorporated into value-added products, however, identification based on appearance and other sensory parameters becomes virtually impossible.

Many countries have legislation for regulating such practice. The EC Minced Meat Hygiene Directive requires each species of the animal used in the minced meat to be listed. Similarly, the EC Meat Products Hygiene Directive requires naming of species in meat products. The EC Labelling Directive requires each species of meat used in products to be clearly identified in the ingredient list.

It is not possible to be specific about the extent of species substitution that is occurring. However, in the last few years, several meat adulteration scandals have had great repercussions worldwide, such as the recent horsemeat scandal in the EU.

2.1.1.2. Protein substitution

Proteins can be added to meat products in most countries within a prescribe limit, but the types and the amounts prescribed differ greatly. There are often regulations or requirements for a meat content declaration prescribing the minimum content of muscle meat in meat products.

Cheap animal protein might be fraudulently used to substitute more expensive animal protein. Casein is by far the most commonly used milk protein, sometimes in combination with excessive amounts of water and polyphosphates. Whey proteins are also used for this purpose.

Vegetable protein such as cheap and readily available soy is probably one of the most commonly used proteins: in recent years, the addition of soybean protein as a raw material replacing red meat in burgers for example has increased significantly due to its functional characteristics (which include increased water and fat binding capacity, emulsification ability), and improved organoleptic properties, such as appearance, (smooth texture, and cutability), nutritional value, as well as its low price. For these same reasons the addition of vegetable protein can be carried out fraudulently, leading to a potential safety concern due to its allergenic properties.

Another special sanitary issue has been the use of gluten which causes intolerance reactions in some individuals. Microbial proteins have been developed for use in foods but are not widely used in meat products.

Finally, the addition of melamine and urea to meat products is an unlawful method of increasing the apparent protein content [6].

2.1.1.3. Fat substitution

The replacement of animal fat with a cheaper vegetable may occur; however, the incorporation of these vegetable fats, especially in meat products, may be associated with a reduction in quality mainly due to a significant oxidative instability.

Substitution could also be used in order to make a claim about the nutritional quality of meat products in response to consumer demands for natural and healthy foods; in fact, the substitution reduces the level of saturated fatty acids (SFA) and increases the level of polyunsaturated, both essential for the prevention of heart disease. Nonetheless the oxidation of unsaturated lipid fractions along with oxygen presence during meat grinding and the need to add salt during processing could have a negative impact on the quality of these products, misleading the unaware consumer [7].

2.1.1.4. Tissue substitution

Offal represents any of various non-muscular parts of the carcasses of beef and veal, mutton and lamb, and pork, which are either consumed directly as food or used in the production of other foods. In countries where these parts of the animal can be designated as meat, there is a financial incentive to include them in products due to the difficulties in their detection [8].

Mechanically recovered meat (MRM or MRPM for mechanically recovered poultry meat) means removal of any remnant flesh from bone after manual deboning. MRM is used as an inexpensive product, with good nutritional and technological properties which has encouraged most meat processors to substitute meat partially or totally by MRM in most types of meat and poultry. The nutritional value and chemical components of MRM vary with raw materials (necks, backs, frames and skin) used in its production. Crushing of the bones and subsequent mechanical separation leads to changes in the chemical, physical, sensory and functional properties of the meat including the development of undesirable aromas (rancidity), loss of its characteristic red colour because of lipid and pigments oxidation, loss or modification of the muscle fibre structure, reduced stability during storage as well as its functionality and processing ability [9]. Moreover, consumer defence groups are concerned about the inclusion of bone fragments in mechanically recovered meat , where mechanical separation results in some bone particles (Ø<0.5 mm) remaining in the meat mass. Mechanically recovered meat is cheaper than raw meat, thus it has been incorporated into

many meat-derived products, where it is less easily detected, without declaring it on the label. EU regulations exclude mechanically recovered meat from the definition of meat and it should be separately identified in the ingredients list when it is used in meat products.

2.1.1.5. Breed substitution

The increasing demand for higher quality meat and the pursuit of desirable attributes such as tenderness or a low-fat content, make the fraudulent declaration of breed a common practice. Among the most popular breeds Charolais, Jersey and Aberdeen Angus and Piemontese are those most subjected to mislabelling. Similarly, meat from young bulls and steers may be regarded as superior to that from older cows [10].

2.1.1.6. Sex substitution

A current practice of producers, in order to meet the needs of the consumer, sometimes report the sex of the animals on the product label even if it is not required by legislation. Today there is considerable awareness of the part of consumers of differences in meat quality and people prefer to consume less meat but with higher sensory characteristics such as tenderness, flavour and appearance [11]. Many factors are involved in beef sensory quality, and variations can be induced by production factors such as breed, age, and diet as well as technological factors such as slaughter conditions, ageing time and cooking procedures. Sex is also related to meat quality, as it can affect pH, cooking loss, water holding capacity (WHC) and shear force (SF). In addition, meat and fat colour parameters, as well as sensory texture and overall acceptance attributes are related to sex [12].

2.1.2. Addition of substances

2.1.2.1. Additives

The purpose of Regulation 1333/2008 [13] and further amendments is to harmonise the use of specific preservatives in food products and it gives a list of both authorised and prohibited additives for certain foods, including some traditional meat products. The use of colours (Council Directive 94/35/EC [13]), antioxidants, preservatives and flavourings is generally not allowed in fresh unprocessed meat because they mask spoilage. Similarly, many meat products and preparations have restricted the use of these additives for the same reason.

2.1.2.2. Water

Water is the cheapest extender of meat and meat products and the water-holding capacity of meat proteins facilitates the binding of water. While the practice of 'enhancing', 'injecting' or 'plumping' has been around since the 1970s, particularly in the chicken industry, it is becoming a subject of concern in recent years. While many believe injecting meat with salt water helps give the product some added juiciness, there are some unpleasant truths about this practice. Besides the increase in product weight, both salt water or contaminated water represent a safety risk, due in the first instance to an unknown uptake of high quantities of sodium and in the second to the presence of pathogens in case of polluted water. When the amount of water is greater than 5 % of the finished product, the EU Regulation requires water to be declared in the ingredient list. Although the amount of water added to cured meats can be very different, very few countries have a requirement for a quantitative declaration of added water. However the debate continues to make consumers aware of the possible fraudulent addition [14].

2.1.3. Process/production/welfare deception

2.1.3.1. Fresh versus thawed meat

Fresh meat is a sensitive material, which is not able to maintain its desired sensory and microbial qualities for a long time-span. Long-term storage and transport between slaughterhouses, meat processors and consumers may take days or even several weeks, for instance in case of overseas imports. Freezing is an excellent way of extending the storage life of meat and makes transport easier. However, generally the retail price of frozen or thawed meat is lower than the price of its fresh counterparts. During thawing the meat loses moisture which contains components contributing to the characteristic flavour and nutritional value of meat. The texture of meat is also affected by the formation of ice crystals, which damage the muscle structure and increase the water activity on the meat surface [15]. Due to the perceived higher quality, consumers are willing to pay a higher retail price for fresh meat. Additionally, in the case of poultry meat, Council Regulation (EC) No 1047/2009 [13] defines "fresh poultry meat" as poultry meat which has not been stiffened at any time by the cooling process prior to being kept at a temperature not below –2°C and not higher than +4°C and prohibits the sale of previously frozen poultry meat as fresh poultry meat. There is also the question of added water where immersion chilling is used before freezing compared with air chilling for chilled birds. In many cases, there is a significant price differential between the frozen and chilled product giving rise to an incentive to deceive the consumer. The EC Labelling Directive requires a process or treatment of a food to be declared where it is misleading not to do so. Therefore, in most cases, it is a requirement to indicate if the meat has previously been frozen [16].

2.1.3.2. Slaughtering methods

Council Regulation (EC) N° 1099/2009 on the "Protection of animals at the time of killing" [17] requires, as a general rule, that "animals shall be spared any avoidable pain, distress or suffering during their killing and related operations". However, it allows slaughter without stunning for particular methods prescribed by the Jewish (kosher meat) and Muslim (halal meat) rites if it takes place in a slaughterhouse. Therefore, there must be a correct labelling system to avoid that meat obtained through Jewish or Islamic ritual slaughter may be purchased by unwilling consumers who prefer not to eat this meat, while vice versa, to avoid meat derived from stunned animals being sold to Muslim or Jewish consumers [18].

2.1.3.3. Geographic origin

For consumers, foods of animal origin, such as meat products, may have a particular value associated with the geographical origin or production system from which they derive, e.g. "Protected Designation of Origin (PDO)", "Protected Geographical Indication (PGI)". The authentication of regional and traditional foods made from meat poses a significant challenge. It continues to be a very difficult task which requires employment of quite advanced analytical techniques [19]. These products, despite a similar process of manufacturing, differ in taste and aroma. This happens due to the use of special breeds of animals, the application of appropriate feeding regimes as well as the effect of the place and climate. Obvious examples are Parma or Serrano ham, but provenance can also be important for raw meat, for example New Zealand lamb or Scotch beef, although this distinction is often associated with a specific breed such as Aberdeen Angus for Scotch beef, as well as the husbandry of the animal. Confirmation of geographical origin authenticity, as with other origin issues, is achieved by checks and audit trails carried out by the product buyer.

2.1.3.4. Organic versus conventional meat

In the case of animal-derived foods generally, and meat specifically, not only is geographical origin important but so also is the authentication of "biological" or "organic" meat and meat products, as well as those which involve less intensive rearing and husbandry methods. In this case the issue focuses on the dietary background of animals, since diet can be a distinct feature of certain production systems, e.g. "organic" or "grass-fed" and can have profound effects on the composition and quality (nutritional and sensory) of the meat and the sustainability of the production of animal-derived food products. In such cases, animal or birds should not be treated with growth hormones, which are illegal in many countries, nor should there be the prophylactic use of antibiotics and other veterinary compounds to improve growth rates. Furthermore, some consumers restrict their purchases to certain production chain. This has led to producers making claims such as "antibiotics free" to declare that animals have been farmed without the use of antibiotics or advertising specific animal welfare practices, such as free-range or other less intensive housing, as well as insisting on more humane handling of animals during transportation and slaughter even if not required by legislation. In some cases, veterinary drug residues may indicate mislabelling but generally authenticity can only be checked by audit trails [20].

2.1.3.5. Feed intake

It is possible to determine the feed intake by different chemical methods, which can detect in animal blood and fat the metabolised forms of typical feed constituents [21]. The most common procedures are:

- Carotenoids content (higher in pasture than in concentrate and hay) in heifer fat, detected by HPLC;
- Fatty acid composition in meat, detected by GC (higher ratio of polyunsaturated fatty acids than saturated ones and of n-3 polyunsaturated fatty acids than n-6 ones, in grass-fed animals than in concentrate fed animals);
- Vitamin and terpene contents in meat.

2.2. Potential threat to public health

In 2013 mislabelled meat products containing horse meat were discovered in many European countries (Ireland, UK, France, Norway, Austria, Switzerland, Sweden and Germany), inducing Member States to increase their surveillance. At that time, the main threat for consumers was linked to the presence in some samples of the horsemeat of an anti-inflammatory molecule, phenylbutazone, and this raised concerns that any commercial fraud could in effect hide a sanitary one. This issue led to the setting up of a special Anti-fraud Unit in charge of managing emerging risks by the European Commission.

To date other examples of common frauds in which a risk for human health can be present are:

- The presence of undeclared additives such as sulphites in fresh meat preparation, causing allergic reactions in sensitive peoples;
- Addition of prohibited substances, such as melamine, causing neurological deficits, renal failure and death in young children;
- The false declaration of geographical origin of the meat or meat product, in order to cover up a source in which a sanitary risk may be present (i.e. contaminants, hormonal treatment, infectious diseases).

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

The standards described in this section include official analytical methods dealing with the authenticity of meat and meat products; these are approved by the Association of Official Analytical Chemists (AOAC International) or by the International Organization for Standardization (ISO). Some analytical methods are also reported in the Codex Alimentarius: most of them refer to AOAC or ISO.

The official methods described here can be very helpful to find suitable analytical solutions for most of the authenticity issues described in session 2.1. However it is important to point out that recently developed methods have also been shown to be efficient in meat authentication.

3.1.1. Substitution

3.1.1.1. Species substitution

These two official methods are related to species identification:

- Multiplex PCR – ISO/NP 20148, still under development.
- Identification of beef and poultry adulteration of meat products by ORBIT (overnight rapid bovine identification test) and PROFIT (poultry rapid overnight field identification test) kits [22].

3.1.1.2. Protein substitution

These official methods deal with the determination of proteins. Most of these methods consists in digesting a test portion with concentrated acid, to convert organic nitrogen to ammonia ions. This is followed by an alkalisation, distillation of the liberated ammonia, titration using boric acid, and finally a calculation of nitrogen content of the sample from the amount of ammonia produced. Other approaches involve combustion, use of dye binding and enzyme linked immunosorbent assay (ELISA) as follows:

- Determination of nitrogen content (reference method) [23] (this standard was last reviewed and confirmed in 2001);
- Determination of nitrogen in meat by the Kjeldahl method [24];
- Determination of crude protein in meat and meat protein by a combustion method [25];
- Determination of crude protein by a digestion method [26];
- Determination of protein in raw and processed meat by an automated dye binding method [27];
- Determination of protein content in processed meat and poultry products, cooked cured ham and in cooked cured pork shoulder and in luncheon meat by titrimetry and Kjeldahl digestion – Codex Alimentarius [28], different AOAC and ISO methods;
- Determination of soy proteins in raw and heat processed meat by Enzyme Linked Immunosorbent Assay [29].

3.1.1.3. Fat substitution

The official methods dealing with the determination of fat and consist in extracting it under specific operating conditions; the total fat content is expressed as a percentage by mass:

- Determination of total fat content in meat and meat products by a gravimetric method [30];
- Determination of fat (crude) or ether extract in meat by a gravimetric method [31];
- Determination of fat (crude) in meat and meat products by a gravimetric method [32];
- Determination of fat content in processed meat and poultry products, cooked cured chopped meat, cooked cured pork shoulder, cooked cured ham and luncheon meat by a gravimetric method [28,30].

3.1.2. Addition of substances

3.1.2.1. Additives

Nitrites and nitrates

The methods consist in colorimetric and spectrophotometric determinations:

- Determination of nitrites content in cured meats by a colorimetric method [33];
- Determination of nitrites content meat and meat products (reference method) [34];
- Determination of nitrates content in meat and meat products by a colorimetric method [35];
- Determination of nitrates and nitrites content in meat by a spectroscopic method [36];
- Determination of nitrites and nitrate content in meat and meat products by spectrophotometric determination after enzymatic reduction of nitrate to nitrite [37];
- Determination of nitrites content in meat and meat products, processed meat and poultry products, canned corned beef, cooked cured chopped meat, cooked cured pork shoulder, cooked cured ham and luncheon meat by a colorimetric methods [28], different AOAC and ISO methods.

Ascorbic acid

Determination of total vitamin C in food – semiautomated fluorimetric method [38].

Phosphorus and polyphosphates

Different principles are on the basis of these methods, ranging from spectrophotometry to gravimetry:

- Determination of total phosphorus content in meat and meat products (reference method) [39];
- Determination of total phosphorous content in meat and meat products by spectrometric method [40] (this standard was last reviewed and confirmed in 2001);
- Determination of linear condensed phosphates in meat and meat products by thin layer chromatographic separation [41];
- Determination of total phosphorus content by gravimetric method [42];
- Determination of phosphorus in meat and meat products by spectroscopic method [43].

Colouring agents

Detection of synthetic, water-soluble colouring agents in meat and meat products by a thin layer chromatographic method [44].

Sulphur dioxide

Detection of sulphurous acid (free form) in meat by a titrimetric method [45].

Preservatives

Detection of preservatives (sorbates, ascorbates, benzoates, sulphites) in ground meat by a spectroscopic method [46].

3.1.2.2. Water

The official methods for the determination of water content are basically based on the measure of the loss in mass obtained for a sample under specific conditions, such as different kind of heat treatments, divided by the mass of the test portion; moisture content is expressed as a percentage by mass. NMR analysis can be applied as well:

- Determination of moisture content in meat and meat products (reference method) [47];
- Determination of moisture in meat and meat products by air drying [48];
- Determination of moisture and fat by microwave and NMR analysis [49].

3.2. Other commonly used methods

In this section, an overview of commonly used analytical methods for each of the current authenticity issues described in the section 2.1 is provided. A special focus is on the analytical methods used routinely in laboratories, and therefore widely available to industry. In addition to these, R&D methods can also be adopted with satisfactory results for some issues, although not described in this section.

3.2.1. Substitution

3.2.1.1. Species substitution

Species identification is mainly achieved by different kind of analytical methods:

- Chemical determinations, since content in certain components varies among species (e.g. glycogen, fat);
- Genetic methods based on nuclear or mitochondrial DNA, such as end-point PCR, multiplex PCR and nested PCR;
- Immunological methods, such as precipitation test – Overnight Rapid Beef Identification Test (ORBIT), Multispecies Identification Field Test (MULTI-SIFT), ELISA and immunoblotting.

3.2.1.2. Protein substitution

Animal proteins could be replaced with vegetable cheaper ones, such as soy, that can be identified using techniques such as ELISA and PCR. Histochemical analysis and immunohistochemical techniques are also routinely adopted in the laboratories. Analytical methods normally used to

measure total nitrogen content (e.g. Kjeldahl and Dumas) are not able to discriminate between nitrogen atoms derived from proteins or chemical compounds, thus chromatographic techniques are employed (HPLC or GC usually coupled to mass spectrometry).

3.2.1.3. Fat substitution

Vegetable fat contains phytosterols that are absent in animal fat; these compounds needs to be isolated from the fat through preparative steps and are routinely detected by different chromatographic methods, such as HPLC or GC coupled with several kind of detectors, as well as NMR.

3.2.1.4. Tissue substitution

h-caldesmon ELISA can be used as a histological method able to differentiate tissues (it is present in smooth muscles and absent in cardiac and skeletal muscles) to detect this type of fraud.

3.2.1.5. Breed substitution

Several analytical methods can be used to differentiate breeds, even if they are not so widespread and routinely used either in quality control laboratories or in industry:

- Genetic analysis;
- Analysis of the microsatellite DNA markers is used to identify, for example, Italian cattle breeds Chianina, Marchigiana, Romagnola and Piemontese [50];
- SNP array, adopted to detect the cattle breeds Holstein and Japanese Black [51].

3.2.1.6. Sex substitution

It is possible to determine the sex origin of meat by detecting sex-specific hormones using different analytical tools. For example, for pork meat, a routinely used method for detecting uncastrated pigs (boars) is based on indole/skatole quantification by HPLC and enzyme immunoassay (EIA).

Molecular techniques can also be used for sex specific identification of raw meat:

- End-point PCR to distinguish the DNA regions that differ between males and females (zinc fingers genes, sex determining region of the Y-chromosomal gene, tooth enamel amelogenin gene);
- Real time PCR to distinguish the DNA regions that differ between males and females (sex-determining region of Y-chromosomal gene, X-chromosomal proteolipid protein gene, tooth enamel amelogenin gene).

These methods can be applied to beef, chicken, pork, and other types of meat, such as goat and sheep. PCR-capillary electrophoresis (DNA analysis using PCR according to IRMM Guidelines and EC Regulation 765/2002 [47]) can be used as a test with four specific primer systems that amplify two loci on both chromosomes, the X and the Y, respectively. Other tests exist on the market that are able to prove the presence of a Y chromosome by amplification of Y-chromosomal regions only, but this technique may lead to false Y-negatives if the amplification itself fails.

3.2.2. Addition of unauthorised substances

3.2.2.1. Additives

Many additives could be fraudulently added to meat. Among these, colouring agents, flavours and preservatives can be detected using HPLC and GC, while fibrinopeptides A and B from thrombin addition are identified and quantified by HPLC.

3.2.2.2. Water

Water could be added to meat in order to increase its weight; thus, extraneous water in meat can be determined by measuring water and protein content, using several methods that are more or less sophisticated (simple determination in oven, NMR, etc.) and also through the determination of the water/protein ratio.

3.2.3. Process/product/welfare deception

3.2.3.1. Geographic origin

Different methods can be routinely used to determine the geographic origin of meat, such as inductively coupled plasma mass spectrometry (trace elements) and isotope-ratio mass spectrometry (stable isotopes ratios). They are based on the principle that the content of these substances in animal tissues depends on feed intake, drinking water, pollution and soil composition, which are strongly linked with the geographic areas in which the animal lives.

3.2.3.2. Fresh versus thawed meat, organic versus conventional meat and feed intake

Microscopy analysis can be used to differentiate fresh versus thawed meat. The method which is validated for poultry meat, is based on the principle that thawed meat present microscopic alteration of muscles fibres which can be related to freezing temperatures.

There are several analytical strategies in the literature showing the possibility to differentiate between animals bred using organic or conventional farming systems, as well as to determine feed intake, however these are not routinely used in the industry.

4. Overview of methods for authenticity testing

The following tables provide a summary of the official and commonly used methods respectively and the authenticity issues they address.

Table 3: Official methods for authenticity testing of meat and meat products

Analytical technique	Indicative data or analyte	Authenticity issue / information
Multiplex PCR	Molecular biomarker	Species substitution
ORBIT (overnight rapid bovine identification test)	Antibodies and antigens	Species substitution
PROFIT (poultry rapid overnight field identification test)	Antibodies and antigens	Species substitution
Kjeldahl	Nitrogen content	Protein substitution
Automated dye binding	Protein content	Protein substitution
Combustion method	Crude protein	Protein substitution
ELISA	Soy proteins	Protein substitution
Gravimetric method	Total fat content	Fat substitution
Colorimetric method	Nitrites and nitrates	Addition of nitrites and nitrates
Spectroscopic method	Nitrites and nitrates	Addition of nitrites and nitrates
Fluorimetric method	Total vitamin C	Addition of ascorbic acid
Spectrometric method	Total phosphorus content	Addition of phosphorus and polyphosphates
Thin layer chromatographic separation	Linear condensed phosphates	Addition of phosphorus and polyphosphates
Gravimetric method	Total phosphorus content	Addition of phosphorus and polyphosphates
Spectroscopic method	Total phosphorus content	Addition of phosphorus and polyphosphates
Thin layer chromatographic method	Synthetic, water-soluble colouring agents	Addition of colouring agents
Titrimetric method	Sulphurous acid (free form)	Addition of sulphur dioxide
Spectroscopic method	Sorbates, ascorbates, benzoates, sulphites	Addition of preservatives
Gravimetric method	Water	Addition of water
Nuclear magnetic resonance	Water	Addition of colouring agents, aromas and preservatives

Table 4: Non-official commonly used methods for authenticity testing of meat and meat products

Analytical technique	Indicative data or analyte	Authenticity issue / information
Genetic methods	DNA	Species substitution
Immunological methods	Protein	Species substitution
ELISA and PCR	Soy protein	Protein substitution
Chromatographic methods	Melamine and urea	Protein substitution
Chromatographic methods	Phytosterols	Fat substitution
Nuclear magnetic resonance	NMR spectrum	Fat substitution
ELISA	h-caldesmon	Tissue substitution
Genetic methods	DNA	Breed substitution
Genetic methods	Microsatellite DNA markers	Breed substitution
Genetic methods	SNP	Breed substitution
HPLC and enzyme immunoassay (EIA)	Indole/skatole	Sex substitution
End point and real time PCR	DNA	Sex substitution
PCR-capillary electrophoresis	DNA	Sex substitution
Chromatographic methods	Colouring agents, flavours and preservatives, fibrinopeptides A and B	Addition of additives
Gravimetric method	Water	Addition of water
Nuclear magnetic resonance	Water	Addition of water
Inductively coupled plasma mass spectrometry	Trace elements	Geographic origin
Isotope-ratio mass spectrometry	Stable isotopes ratios	Geographic origin
Microscopy	Morphological structure	Fresh/thawed conservation

5. Conclusion

Considering the growing demand for meat, related fraud is expected to represent an ongoing challenge in future years. The analytical tools to detect meat fraud will need to be improved based on a number of different strategies. First, those analytical procedures that are not included in existing standards (determination of additives, use of molecular techniques to determine species substitutions) need to be standardised and validated. Standardised methods need to be revised, such as the EU reference method to determine hydroxyproline content in meat. This is a simple spectrophotometric technique, while other more advanced ones such as LC-MS/MS are available but not recognised as reference techniques. Multi-screening and untargeted methods further development to detect simultaneously different and unknown adulterants. And finally, innovative analytical approaches have to be developed and validated to propose solutions for different old and emerging issues directly linked to fraud such as:

- Characterizing different animal breeds, using a larger data set to build effective models (NIR techniques);
- Determining animal feed intake, since the current analysis based on carotenoid content in fat and blood are influenced by other factors such as breed, gender, lactation and rumen environment;
- Determining the slaughter age of animals;
- Assessing animal welfare condition related to intensive vs traditional farming practices;
- Distinguishing different meat cuts (a possible solution could be the evaluation of collagen content that varies among different meat cuts, considering that visual inspection is useful only to differentiate primary beef cuts);
- Quantifying vegetable fat as adulterant in meat, not only revealing its presence by phytosterols detection;
- Establishing the geographic origin of meat, since the simple identification of breed may not be effective since individual breeds can be raised in different countries despite their origin;
- Detecting animal fat from different undeclared species;
- Developing methods to identify fresh-thawed products that are applicable to ground meat and temperatures higher than -12°C (the HADH method is not applicable to ground meat because the grinding process causes similar alterations to those induced by freezing and it is able to detect frozen-thawed meat only if the freezing temperature has been -12°C or below).
- Setting up reliable methods to detect mechanically deboned meat (MDM) and to distinguish among low pressure *vs*. high pressure MDM in meat products.

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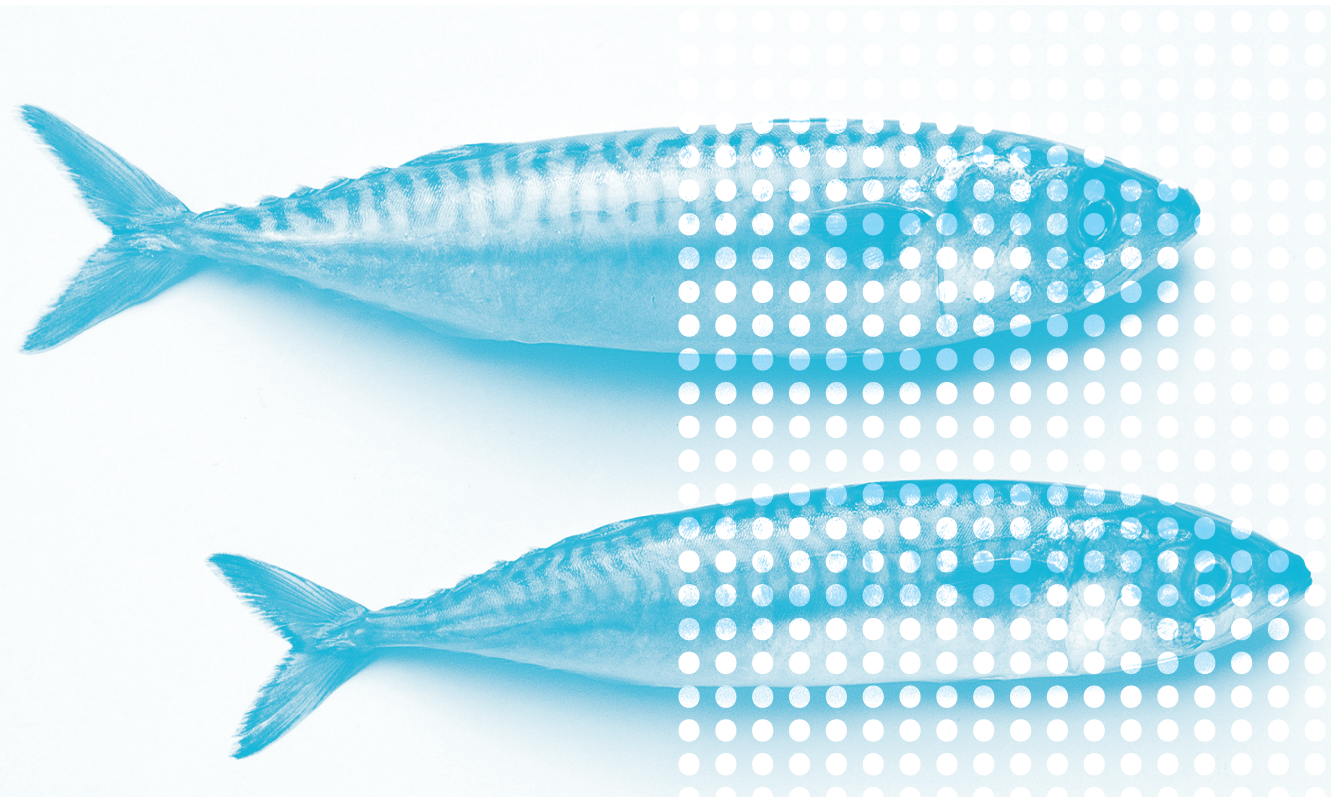
Fish and fish Products



Fish, seafood and related products

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General overview of the product

It is reported that over 171 million tonnes of fish (seafood) are harvested in one year [1], corresponding to the highest ever consumption of 20.3 kg per capita in 2016: production in aquaculture is steadily increasing, but traditional fishery remains the major part of the production process with about 91 million tonnes.

Seafood is currently in a critical situation. On one side, there is an increasing trend towards consumption of fish and seafood because of perceived health benefits, such as their content in omega-3 polyunsaturated fatty acids, and as an alternative source of protein to meat. On the other hand, the sustainability of fishery, coupled with increasing pollution, requires caution. In recent times newspapers reported on mercury pollution, microplastics in seafood and antibiotics in farmed fish. It is clear that these contrasting trends can be conducive to fraud and mislabelling. Different countries in the world have different standards and requirements, complicating the situation. Illegal, unreported and unregulated (IUU) fishing is the other main problem for the market of safe, nutritious and healthy seafood. IUU practices concern many aspects of fishery: species, age of fish, geographic area, amounts of catch, timing, and equipment [2].

Mislabelling is a common problem for fish, and seafood in general (cf. the recent paper on "snapper" identity [3]). This has been evidenced in many studies across the world, particularly using methods based on DNA analysis for identification of species. EUROPOL (European Union Agency for Law Enforcement Cooperation) considers fish the third highest risk category for food fraud [4]. Oceana (an international organisation established by a number of leading foundations to focus on oceans) periodically examines restaurants and stores, finding high percentages (20-30 %) of samples mislabelled [5].

This chapter will deal with fish and invertebrates used as food: molluscs, crustaceans, jellyfish, excluding mammals and reptiles. It will not deal with fish oil.

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1. Product Identity

1.1. Definition of the product and manufacturing process

The commercial designation for seafood products is under the heading 03 in the CN code, Commission Implementing Regulation (EU) 2017/1925 [6].

0302 is for "Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304" and includes all types of fish: Salmonidae, flat fish, tunas, herrings, cod families, tilapias, and also the offal of fish.

0303 is for "Fish, frozen, excluding fish fillets and other fish meat of heading 0304" including again the same types of fish.

0304 is for "Fish fillets and other fish meat (whether or not minced), fresh, chilled or frozen"

0305 is for "Fish, dried, salted or in brine; smoked fish, whether or not cooked before or during the smoking process; flours, meals and pellets of fish, fit for human consumption"

0306 is for "Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked crustaceans, whether in shell or not, whether or not cooked before or during the smoking process; crustaceans, in shell, cooked by steaming or by boiling in water, whether or not chilled, frozen, dried, salted or in brine; flours, meals and pellets of crustaceans, fit for human consumption" and includes lobsters, crabs, shrimps, crayfish

0307 is for "Molluscs, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked molluscs, whether in shell or not, whether or not cooked before or during the smoking process; flours, meals and pellets of molluscs, fit for human consumption" and includes oysters, scallops, mussels, cuttle fish and squid, octopus, snails, abalone and others.

0308 is for "Aquatic invertebrates other than crustaceans and molluscs, live, fresh, chilled, frozen, dried, salted or in brine; smoked aquatic invertebrates other than crustaceans and molluscs, whether or not cooked before or during the smoking process; flours, meals and pellets of aquatic invertebrates other than crustaceans and molluscs, fit for human consumption" like sea cucumbers, sea urchins, jellyfish.

The presence on the market of material which is in the shape of fillets or minced flesh, and material which has been subjected to curing and processing, freezing, smoking, drying, opens possibilities for fraudulent or accidental substitution and mislabelling.

1.2. Current standards of identity or related legislation

Though not a standard, the main reference for scientific names and common names of fish is FishBase [7]. FAO maintains the ASFIS (Aquatic Sciences and Fisheries Information System) database for fishery statistics [8].

The Coordinating Working Party on Fishery Statistics (CWP) has developed a Handbook of Fishery Statistics, published since 1990 [9], which includes the definition of the FAO fishing areas.

Codex Alimentarius has a fairly recent Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003 [10]), incorporating good manufacturing practice (GMP) and Hazard Analysis and Critical Control Point (HACCP) system.

The European Union has a legislation on seafood labelling, Regulation EU 1379/2013, requiring indication of commercial designation, scientific name, method of production (caught, farmed), geographical origin (catch area, body of water, country), fishing-gear category [11]. This is associated to the traceability requirements of the General Food Law Regulation 178/2002 [12]. Other voluntary information is allowed about dates of catching, environmental or social information, and nutritional content.

In the USA, the U.S. Food and Drug Administration has produced and maintains a list of Acceptable Market Names which are allowed for seafood species [13].

2. Authenticity issues

2.1. Identification of current authenticity issues

The main problem for seafood authenticity is mislabelling for the species name, or species substitution [14]. Indication of the species is an obligation in most labelling requirements. However, particularly in processed products where visual recognition is not possible, the identity of the animal can be counterfeited. Usually, there is an economic motivation, substituting expensive and valued material with other species of lesser value or from illegal fishing. A further problem is the fact that many seafood species are marketed under a shared name ("umbrella" term) encompassing different species and/or genera; translation into local languages adds more problems.

A second important issue concerns geographical origin, connected to the FAO fishing zones. When this is declared on the label, it might be a fraudulent declaration to cover for IUU fishery or to mask a species substitution. Similarly, a declaration about the fishing gear may raise the price of the food product and be a fraud.

Processing or treatment can be falsely declared on the label, as in the case of freeze/thaw process to sell fresh fish.

Additives can also be fraudulent, as in the case of tuna added with vegetables extracts, salts or carbon monoxide to change the colour and make it look fresher.

Sustainability is a new issue which generates opportunities for fraud, when declarations about place and way of fishing are untrue.

2.2. Potential threat to public health

Some fish or seafood species are toxic, and mislabelling can cause poisoning: puffer fish, escolar, ciguatoxic species are examples of fish which contain toxins, like tetrodotoxin or histamine. Farmed fish can contain higher levels of contaminants, and organic compounds: also, in this case a fraudulent declaration about the origin of fish or production method can have health effects through exposure to environmental contaminants.

Scombroid syndrome is an allergic reaction caused by some fish species which contain histamine. Substitution and mislabelling can expose allergic consumers to health risks, leading them to consume seafood they would normally avoid.

False declaration about the cold chain, or the freezing and thawing of products, may be hazardous due to development of microbes and possible infections.

The recent Minamata Mercury Convention has highlighted the problem of mercury pollution in fish and seafood. Mercury is transformed into the neurotoxic form methylmercury (MeHg) mainly in aquatic environments, and from animal to animal it accumulates along the food chain. Humans are exposed to MeHg through consumption of predator fish like tuna and swordfish, therefore a correct labelling of the species name is important for an informed choice. The area of origin might also be important in determining the levels of MeHg, but in this case it is hardly expected that consumers might recognize the issue when purchasing fish [15,16].

Mislabelling for the geographical origin could become a health threat in case the seafood comes from polluted areas due to radioactivity, or for the use of veterinary drugs allowed in some countries and not in others.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Chemical analyses can be used to detect addition of: (i) salt or phosphates, used to increase weight by attracting water; (ii) benzoic acid, used to increase shelf life; (iii) citric acid or other compounds as preservatives and to change the colour; (iv) carbon monoxide to increase the red colour; (v) proteins to increase weight; (vi) excess water or brine, overglazing, to increase weight. Standard methods are provided by the Association of Agricultural Chemists (AOAC), European Committee for Standardization and others. A recent review reports about traditional and non-destructive methods for seafood quality analysis [17].

Traditionally, the identification of animal species, also for fish and seafood, was performed through protein analysis, with electrophoresis, chromatography, or immunological methods [18]. The Regulatory Fish Encyclopedia hosted by the U.S. FDA was a repository of information on protein analyses for fish identification, mostly IEF patterns [19]. A possible advantage of protein analytical methods is to address the presence of some specific allergens, which is relevant also for food safety purposes.

However, proteins can be degraded or destroyed by processing, making these methods ineffective. Methods based on analysis of DNA are more effective because of higher specificity and sensitivity, and because DNA can be amplified from few molecules also in degraded samples [20].

A standard method for establishing if the fish has been thawed from frozen is based on microscopy analysis of muscle, by the Italian accreditation body ACCREDIA [21]. Other methods based on physical and chemical parameters are being developed [22–24].

A COMET test on DNA integrity can provide indication to detect foodstuff which has been irradiated, and the method is standardized (EN 13784:2002, [25]).

3.2. Other commonly used methods

3.2.1. DNA-based techniques

DNA-based techniques [2,26] make use of different markers, amplified fragments or restriction profiling: sequencing, AFLP (amplified fragment length polymorphism), FINS (forensically informative nucleotide sequencing), RAPD (random amplified polymorphic DNA), RFLP (restriction length polymorphism), SSCP (single-stranded conformational polymorphism), multiplex PCR and real time PCR for diagnostic fragments [27,28]. An important resource is the Reference Standard Sequence Library for Seafood Identification including over 1000 sequences from seafood vertebrates and invertebrates [29]. The D-loop region in mitochondrial DNA can be a good target for species differentiation because of high polymorphism and mutation rate [30]. A recent survey [31] has singled out the most common methods used by laboratories for identification of species: (i) Forensically Informative Nucleotide Sequencing (FINS), (ii) Restriction Fragment Length Polymorphisms (RFLP) and (iii) Isoelectric Focusing (IEF).

A different approach in DNA-based analyses, the DNA barcoding technique, is a well-known standard to detect species of seafood in food samples, also after extreme processing: for instance, it is used by the Canadian Food Inspection Agency. The initiative Barcode of Life Data System [32] with the FISH-BOL, fish barcode [33], is the main source of data for species identification. The marker of choice is cytochrome b (cyt-b) or cytochrome *c* oxidase I gene (COI) located on the mitochondrial DNA; other markers are 16S or 18S ribosomal DNA (16S-rDNA, 18S-rDNA), the internal transcribed spacer type I-ribosomal DNA or type II (ITS1-rDNA, ITS2-rDNA) [34]. The markers are amplified with PCR from universal primers, and the amplicons are then sequenced for comparison with the data base [35]. A comparison of different DNA methods has been shown to lead to 100 % differentiation in *Merluccius* species [36].

Several research projects funded by the European Commission have produced databases, protocols and standard operating procedures for molecular analyses in seafood identification: recent examples include FishTrace, SEAFOODplus, CHILL-ON, FoodIntegrity, AuthentNet and PrimeFish.

Analyses which can be of use in ascertaining the geographical origin can be based on DNA markers, if the local populations of fish have distinctive features. Otherwise, chemical analyses for elements and trace elements, stable isotopes, fatty acids can be used [37–39].

3.2.2. Stable isotope ratio analyses

Methods for establishing the compliance with declarations about wild or farmed fish have been developed in order to fight frauds connected with provenance and processing which could also impact on health. Following on from early studies that had shown that the content of stable isotopes reflects both the environment in which the fish is grown and the composition of its diet, a major project known as COFAWS[1] was set up to further develop these techniques.

There are several correlations between the content of isotopes and the geo/climatic environment of a food product. The content in ^{13}C and ^{15}N are related to diet; ^{18}O and ^{2}H are influenced by the origin of the water in the product. To differentiate the farmed and wild origin of salmon, isotope ratios $^{18}O/^{16}O$ (expressed as $\delta^{18}O$) and $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) are measured by IRMS (isotope ratio mass spectrometry) on the fish oil and choline from the lipid fraction extracted from the fish

[1] COFAWS – Confirmation of the Origin of Farmed and Wild Salmon and other fish. Part funded by the European Commission under the "Fight against Fraud" action and by the UK Food Standards Agency.

muscle [37]. These parameters successfully separated wild and farmed salmon both from known origins and unknown market samples. The technique has since been used to check mislabelling in the UK market. It has since been extended to other fish such as bream, cod, bass.

Other studies have been reported in the literature including a chemometrics approach addressing the global chemical composition (trace elements, stable isotopes, fatty acids) has been recently suggested [40,41]. Stable isotope ratios for carbon, nitrogen and oxygen have also been suggested as a means for discriminating wild from farmed fish, and organic from intensive production, based on differences in the feed origin [38,42]. A combination of isotope determination and other profiling methods, e.g. trace elements or fatty acids, could be more effective. Isotopes of Strontium could be indicative of geographic provenance, since this element is present together with calcium in bones and calcified materials of seafood [43].

3.3. Future analytical perspectives

New methods or improvements of existing methods should make the analyses for species identification easier to perform, and sufficiently rugged to be executed on board vessels for instance, requiring no DNA extraction and no electrophoresis, for example, lab-on-chips approaches, or ultra-fast Real Time PCR [44]. Multiplexing the amplification [45,46] or using DNA chips could increase analysis throughput. Also developing methods for rapidly detecting gene variants without sequencing could be beneficial, such as the application of High Resolution Melting (HRM) analysis after amplification of marker genes [47]. For the same reason, handheld devices for non-destructive analyses will also be highly appreciated on board vessels and for controls on line [48]. Quantification of species composition could become a necessity in some cases, for example when verifying the fish content of complex foods. Molecular markers can be employed in quantitative PCR for the purpose [49], but the use of mitochondrial gene markers require sophisticated considerations, due to the fact that multiple copies of the mitochondrial DNA exist in cells [50].

More recently, methods based on proteomics or high throughput protein analyses are envisaged, which at times avoid extraction of proteins or digestion [18,51–54]. A new chemotaxonomic approach could add new tools for species identification in a rugged context [55].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Gel electrophoresis, isoelectric focusing, capillary electrophoresis, immunoassay	Proteins	Species identification
Multiplex PCR	Mitochondrial 16S rDNA	Identification of species
PCR-RFLP	Mitochondrial DNA D loop Cytochrome oxidase COI	Identification of snapper species Identification of *Merluccius* species
PCR-RFLP, FINS	16S mitochondrial rDNA	Species identification for sea cucumber
Real Time PCR	Nuclear and mitocohondrial genes	Detection and quantification of *Mytilus* species
DNA mini-barcoding followed by High Resolution Melting (HRM) analysis	COI, cyt b marker genes	Discrimination of species
MALDI-TOF (Matrix-assisted laser desorption/ionization time-of-flight) Mass spectrometry	Proteins and peptides patterns	Species identification Trout species identification
Front face fluorescence spectroscopy (FFFS)	Several compounds with double conjugated bonds (vitamins, amino acids, etc.)	Fresh and frozen fish
NIR spectroscopy, 780-2500 nm, with chemometrics	Whole product	Freshness, frozen/thawed material
Hyperspectral imaging, 380-1100 nm, with chemometrics	Whole product	Freshness of fish, frozen/thawed fillets
Isotope ratio mass spectrometry (IRMS)	Stable isotope ratio	Geographic origin
Multi-element profiling Stable isotope analysis	Different chemical elements	Identification of species, geographic origin and method of production
Stable isotope analysis, gas chromatography/mass spectrometry (GC/MS)	Stable isotopes ratio and fatty acids profiling	Wild and farmed salmon Geographic origin
Tri-step infrared spectroscopy and chemometrics: Fourier Transform Infrared Spectroscopy (FT-IR), Second Derivative Infrared Spectroscopy (SD-IR), Two Dimensional Correlation Spectroscopy 2DCOS-IR)	Nutrients fingerprints	Species discrimination in surimi

5. Conclusion

FAO [14] has identified the main needs to combat food fraud in the seafood sector: (i) reaching agreements on names of products and species; (ii) introducing mandatory labelling; (iii) improving the systems for official control of food; (iv) improving systems for food safety in production; (v) adding new Codex guidelines.

It is widely recognised [1] that seafood is essential for healthy nutrition, providing nutrients, micronutrients, vitamins. The steadily increasing consumption shows how public awareness has grown. For pregnant women and children, particularly in low/middle income countries, seafood contributes to development of the nervous system and is an accessible source of animal protein. This can increase the exposure to methylmercury leading to risks for neurotoxicity [15].

Since fish and seafood are highly perishable, the transportation to consumers, in long supply chains, provides logistic challenges and risks for health. Consumers nowadays require innovative ways for chilling, preserving, delivering seafood, and in this area authenticity or fraud issues might arise. Control of the cold chain and traceability with Universal Identifiers will be an area for development, e.g. by blockchain technology [1].

Pollution will surely become more relevant, particularly considering abandoned, lost, discarded fishing gear (ALDFG) and microplastics, on which knowledge is still missing. Fishery will also be impacted by climate change and extreme weather events, requiring adaptation measures. Aquaculture is included in the strategy for Climate Smart Agriculture, aiming to increase or maintain production and mitigating impacts. Climate change will affect stocks worldwide, opening the possibility for fraudulent behaviour in declarations on species or geographic origin. Sustainability of fishing is also connected to climate change and geographical origin.

Considering the commercialisation of transgenic salmon in Canada, a possible additional requirement for analytical methods will concern the traceability of transgenic material [56].

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Plant Products

Cereals and cereal-based products

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General overview of the products

According to the FAO's definition the term cereals refers only to crops harvested for dry grain. Crops harvested green for forage, silage, or grazing are classified as fodder crops. Cereal products are defined as derived from the processing of grain by mechanical or chemical processes, or from the processing of flour, meal or starch. All together the FAO definitions cover 17 primary cereals, the major ones being wheat, barley, maize (or corn), triticale, rye, oats and rice. In 2014, in Europe (EU-28), all these grains (excluding rice) represented in the food, feed, industry (including fuel) and seeds sectors, 24 %, 61 %, 11 % and 4 % respectively [1].

Cereals are generally from the gramineous or Poaceae family and identified according to their genus (see Figure 1 for the phylogenetic relationships of the cereal species and subspecies mentioned in this chapter). With carbohydrates comprising 65-75 % of their total weight, cereals and cereal-based products constitute the main source of energy for the majority of human populations and are therefore important staple foods. Different cereal species have different uses with a wide range of qualities often linked to specific varieties. These perceived differences in quality in the final consumer product can lead to substantial differences in price, with the potential for cheaper varieties to be passed off as the more expensive kind. Hence the need to establish the authenticity of cereals.

The main authenticity issues for cereals generally involve wheat and wheat-based products and rice, making these the main focus of this chapter, with other cereal types mentioned only where relevant.

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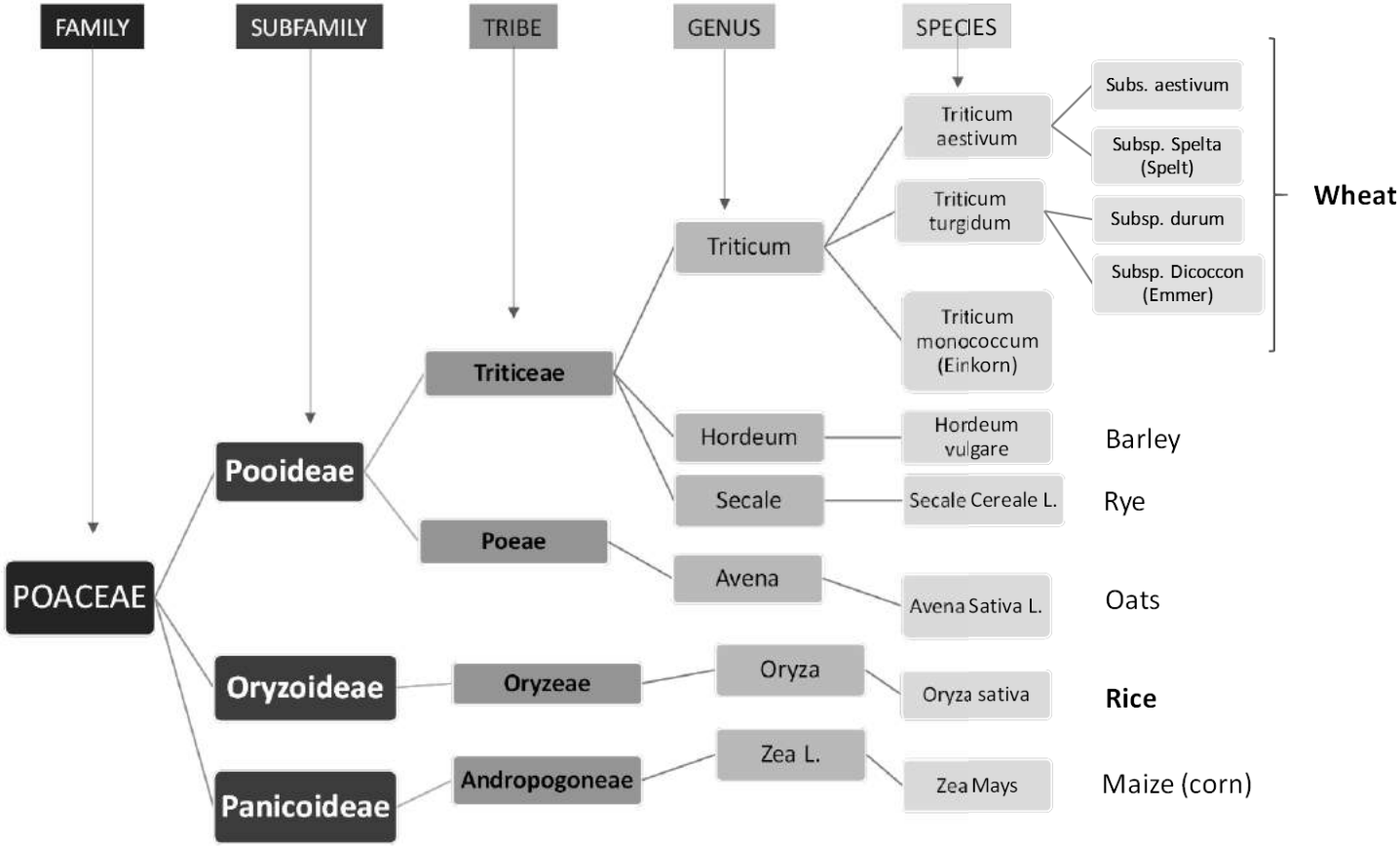

Figure 1: Phylogenetic relationships of the cereal species and subspecies mentioned in this chapter (nomenclature reported according to **http://www.ars-grin.gov**)

Wheat and related products

1. Product identity

1.1. Definition of the product and manufacturing process

Wheat is widely grown around the world under diverse climatic conditions and has been the staple food of the major civilisations in Europe, Asia and North Africa for 8000 years. Of the many species of wheat that make up the genus *Triticum,* the most widely grown is common wheat, *Triticum aestivum*. The second most cultivated species after common wheat is durum wheat, also known as pasta wheat (*Triticum turgidum* subsp. *durum).*

Within each species there are a number of cultivars or varieties that can be placed into a number of groups or types; these may be acceptable botanical groups based on grain or plant characteristics, e.g. red and white grained, hard and soft grain textures, spring and winter types, or groups based on other attributes such as baking performance or gluten characteristics. The harder the wheat, the higher the protein content in the flour. Soft, low protein wheats are used for cakes, pastries, biscuits and oriental noodles, whereas hard, high protein wheats are used in making bread. Durum wheat is used for pasta and noodles.

Wheat production in 2016 accounted for 672.7 million tonnes worldwide [2]. In 2017, production by the 28 EU Member States was 152.6 million tonnes, approximately 22 % of the worldwide production. Of this European production, 93.6 % (142.8 million tonnes) was soft wheat with durum wheat accounting for the remainder (6.3 %; 9.6 million tonnes) [3]. Production of soft wheat was concentrated in France (25.3 %), Germany (17.0 %) and the UK (10.3 %). Poland, Romania and Hungary produced 8.0, 6.9 and 4.4 % respectively. In the case of durum wheat, Italy accounted for 45.4 % of total production; other major producing countries were France (21.7 %), Greece (13.0 %), and Spain (12.6 %).

1.2. Current standards of identity or related legislation

1.2.1. In the European Union

Different European and national regulations apply to cereals depending on whether they fall into the food, feed, or seed sectors.

For the food sector, Regulation (EC) No 742/2010 of 17 August 2010 [4] establishes the eligibility criteria to be met by cereals for public intervention and the methods to be used for carrying out tests to establish such eligibility. For the feed sector, the regulation (EC) No 767/2009 of 13 July 2009 [5] lays down rules on the placing on the market and use of feed for both food-producing and non-food producing animals within the Community, including requirements for labelling, packaging and presentation. Regulation (EC) No 1829/2003 of 22 September 2003 [6] lays down Community procedures for the authorisation and supervision of genetically modified food and feed as well as provisions for their labelling.

There are also specific regulations for cereal products, particularly for those destined for consumption by infants. Commission Directive 2006/125/EC [7] on processed cereal-based foods

and baby foods for infants and young children lays down requirements for the composition of such products, including cereal, protein, carbohydrate, mineral and vitamin contents. Further compositional and labelling rules for processed cereal-based food in the EU Regulation on food for specific groups [8].

At national level, regulations, directives, recommendations are in application in each European country. They concern the production and sale of cereals, milling products, bread and pasta. Some of them, dedicated to authentication, can be found in the regulations section of the FARNHub tool [9]. For example, in Italian regulations, the presidential decree N° 187, dated 9 February 2001 [10], stipulates that durum wheat milling products may contain up to three per cent of soft wheat flour. More general regulations at national level can also be found on the EU-N-Lex website [11].

The upstream cereals sector concerning the seeds is also legislated by regulations defining the production of new varieties, their registration and varietal purity. Council Directive 66/402/EEC of 14 June 1966 on the marketing of cereal seeds [12] establishes rules, amongst others, on the production, packaging, sampling, sealing and marking in order to ensure the identity of the certified seeds. This Directive has been amended several times and in particular by Commission Directive 2009/74/EC of 26 June 2009 [13] as regards certain Annexes to Directive 66/402/EEC in the light of developments of scientific and technical knowledge regarding seed purity.

From the point of view of the general public, consumers are showing increasing interest for different qualities of bread produced from cereals such as spelt (*T. spelta*), emmer (*T. dicoccum*), einkorn (*T. monococcum*). In order to preserve quality food products coming from particular geographical areas and to protect consumers against imitations and false information, the European Commission has defined, via Regulations [14] several quality labels, among which are the Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) labels. Products such as Farro di Monteleone di Spoleto (emmer) produced in Italy-Umbria (PDO), Farro della Garfagnana (emmer) produced in Italy-Tuscany (PGI) and Petit épeautre de Haute Provence (einkorn) produced in France (PGI) are protected by these European labels. Other cereal products such as Epeautre d'Ardennes (spelt) produced in Ardennes in Belgium are protected by regional labels based on specifications defined by the spelt sector [15].

In addition to the legislation, the cereal sector is managed by standards defining the best practices in cereal sampling and quality analytical control. These are described in section 3.1.1. below.

1.2.2. In the United States

The Department of Health and Human Services of the US Food and Drug Administration (FDA) has published requirements for specific standardized cereal flours and related products in the Code of Federal Regulations (CFR) Title 21, Part 137. These regulations provide the definitions and standards of identify of a wide range of cereal-derived flours. Further up-to-date information on FDA regulations are available on the Government Publishing Office's e-CFR at reference [16].

1.2.3. At the international level: Codex Standards for cereals

The Codex Alimentarius [17] has published a comprehensive document that includes all texts adopted by the CA Commission up to 2007 for Cereals, Pulses, Legumes and Vegetable Proteins. These standards provide accepted definitions for each cereal or cereal product, including certain quality attributes such as moisture content, an important parameter used in the trading of cereals. The relevant standards are summarised in Table 1.

Table 1: Codex Standards for cereals and cereal products

Cereal / cereal product	CODEX STAN	Definition	Moisture content
Couscous	202-1995	Product prepared from durum wheat	<13.5 %
Durum wheat semolina and durum wheat flour	178-1991	Products prepared from grain of durum wheat by grinding or milling processes.	<14.5 %
Maize (corn)	153-1985	Shelled grains of *Zea mays indentata L.* or *Zea mays indurata L.*	<15.5 %
Oats	201-1995	Grains of *Avena sativa* and *Avena byzantina*	<14.0 %
Wheat and durum wheat	199-1995	Wheat is obtained from the varieties of the species *Triticum aestivum L.*	Wheat <14.5 % m/m
		Durum wheat is obtained from varieties of the species *Triticum durum Desf.*	Durum wheat <14.5 % m/m
Wheat flour	152-1985	Product for human consumption preparer from grain of common wheat *Triticum aestivum L.* or club wheat *Triticum compactum Host* or mixtures thereof by grinding or milling processes	<15.5 % m/m

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Species substitution, varietal identification

One of the main authenticity issues for cereals and cereal products is the deliberate substitution with cheaper species or varieties. Different varieties or class of varieties have different end-use qualities, some being more suitable than others for certain types of food industrial processing or animal feed, and this may lead to significant differences in food and feed market prices. Effective species/variety discrimination of cereals based on product composition is increasingly vital for the needs of the food processing industry.

2.1.1.1. Case of common wheat in durum wheat

Quantifying the degree of adulteration of durum wheat flour with common bread wheat flour is of particular interest in the Italian, French and Spanish markets, where semolina is the only allowed constituent for pasta, while in the north European countries both bread and durum wheat are permitted. The use of common wheat in durum wheat, is considered as fraud according to current Italian legislation [10] with only a maximum of 3 % common wheat allowed to account for any cross-contamination that may occur during the agricultural process. However, mixtures of both wheats can be found due to delivery problems or to reduce prices. For this reason, efficient methods for the detection of accidental or intentional contamination of durum wheat with common wheat are required at the entrance to food operators' premises.

2.1.1.2. Case of common wheat in spelt

A growing interest in foods delivering high nutritional value and health benefits has encouraged breeders to develop new grain species that meet consumer expectations. The grain of hulled wheats (spelt - *Triticum spelta*; emmer - *T. dicoccon*; and einkorn - *T. monococcum*) and the resulting products meet the requirements set for functional foods. To give added value to the genetic and breeding efforts as well as guaranteeing the differentiated quality of bread obtained from these new grain species, efficient methods are needed to assess quality based on the composition [18].

2.1.2. Geographical origin

Most countries have their own specific grain varieties, suited to their own environmental conditions and agronomic practices. Being able to verify the geographical origin of cereal to ensure full traceability from the food to the production location is important when grain from a specific area commands a higher price, or to ensure that the grain does not originate from a region known to be contaminated.

2.1.3. Certification of organic production

Today's consumers are increasingly concerned by the quality and safety of the food they eat, with more and more of them turning to organically grown products. For a cereal-derived product to be labelled as organic, the producer must follow and comply with specific rules laid down in international regulations. This will inevitably lead to higher costs for producing organic products compared to conventional ones, followed by higher prices in the market. An authenticity issue will arise when cheaper non-organic product is passed off as organic.

2.1.4. Gluten-free products

Coeliac disease is caused by a reaction of the immune system to gluten, a protein found in wheat, barley, rye and oats. It can be a serious disease if undiagnosed and can only be treated by following a gluten free diet for life. Food products labelled "gluten-free" are usually prepared using cereal species which naturally do not contain gluten such as rice, maize, amaranth [19,20]. However, both intentional and unintentional contamination can occur leading to an authenticity issue for such products.

2.2. Potential threat to public health

Probably the most serious potential threat to human health concerns the potential contamination of gluten free cereals as described above. For food companies involved in the processing of several species of cereals, accidental contamination of gluten free cereals with wheat, for example, can occur and such products can cause illness or severe reactions for individuals with wheat allergies or coeliac disease [21]. Separate production lines and good traceability are required to reduce the risk and the impact on the public health.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. General methods for quality control

In order to identify and discriminate varieties, a large number of analytical methods have been developed, including visual examination of kernel morphology (colour, size, shape, texture); simple laboratory tests and measurements (yield, Thousand Kernel Weight [TKW], specific weight, kernel size, germination analyses).

The International Association for Cereal Chemistry (ICC)[1] also provides a number of standard methods for general quality control of cereals. Its compilation of standards includes guidelines for sampling of grain, and methods for the determination of moisture, protein, starch, fat and dietary fibre contents [22].

The American Association of Cereal Chemists International (AACC Intl.) also provide a collection of approved methods for cereals laboratories and companies involved in grain processing, available at [23]. Together AACC Intl. and the ICC have developed a sub-set of harmonized methods for the analysis of key constituents and parameters that are frequently tested on an international basis.

As regards the specific case of wheat varieties, the International Union for the Protection of New Varieties of Plants (UPOV) [24] provides guidelines for the "examination of distinctness, uniformity and stability and the development of harmonized descriptions of new varieties of plants", including specific tests for wheat.

At the European level, the European Committee for Standardization through the technical committee on cereal and cereal products (CEN/TC 338) establishes norms on cereal quality [25]. National agencies of normalisation such as the French agency of normalisation (AFNOR) or the Belgian bureau of normalisation (NBN) define/adapt norms at the national level.

As regards the determination of parameters such as moisture and protein in cereals by NIR spectroscopy, international standards and guidelines have been developed recently by the NIR spectroscopy community [26].

3.1.2. Protein-based methods

Many methods for authenticity studies in cereals use grain storage proteins, which often represent the most important features for the quality of the processed products. In the case of wheat, the bread- and pasta-making properties depend on specific storage proteins, the prolamins [27]. Wheat proteins can be classified into two types: gluten and non-gluten proteins. Gluten protein makes up the bulk of total wheat protein, composed mainly of two fractions: gliadins and glutenins (with high and low molecular weight respectively) which affect the visco-elastic properties of dough. Non-gluten proteins include albumins and globulins [28].

The most established method for the identification of wheat varieties uses polyacrylamide gel electrophoresis (PAGE) to separate the wheat proteins extracted from grain [24]. The high molecular weight glutenin sub units are used for the identification of varieties. ICC Standard 143 [29] specifies a method for the identification of the variety of a given lot of soft or hard wheat, in

[1] The ICC is an international network of cereal scientists and technologists dedicated to the improvement in safety and quality of cereal-based foods, one of its missions in the validation and standardization of suitable test methods.

the form of individual ground kernels, flour, farina or semolina, by the separation of gliadin proteins. The separated protein components are visible from the stained polyacrylamide gels and compared to a variety catalogue established for major wheat varieties. This method is in common use in many countries.

3.2. Other commonly used methods

3.2.1. Biomolecular methods

More elaborate methods such as DNA detection based on the differences in genetic background of the wheat species or varieties are beginning to be more widely used for authenticity purposes. These methods can be used both to discriminate between species and to identify varieties. A thorough description of these methods and their advantages is given in reference [27].

3.2.1.1. Identification of different cereal species in food products

The identification of cereal species in food products can be performed by targeting species-specific genomic information and analysing the nucleic acids extracted from food products. A marker for species identification should be a reference gene showing no allelic variation, with a low number of copies in the genome. The study described in reference [30] has proposed specific marker genes for barley, rice and wheat, respectively: γ-hordein, gos9 and acetyl-CoA carboxylase. A different set of species specific-markers has been proposed [31] for detecting adulteration in chestnut flour by: barley, bread and durum wheat, oat, rye, maize and rice. Amplified fragments were of different dimensions and could be analysed in duplex PCR reactions.

A different approach based on microarrays was proposed for simultaneous detection of several species: wheat, rye, barley, oat, rice and maize [32]. The target, common to all species, was the intron of the chloroplast transfer RNA gene, trnL, which can be amplified with universal primers from all plant species. The application of species-specific probes then allows discrimination among different cereals without cross-hybridization.

To discriminate cereal species within a mixture, another study [33] looked at the same target sequence with a padlock probe approach on microarrays: the trnL target sequence is linked to a unique labelled cZIP-code sequence. It was applied to detection of adulteration in the Italian PGI (Protected Geographical Indication) cereal “Farro della Garfagnana”, emmer wheat.

3.2.1.2. Detection of gluten-containing cereals in “gluten-free” products

Methods based on the Polymerase Chain Reaction (PCR) can detect the presence of traces of material derived from gluten containing cereals [34]. This PCR approach exploited primers specific for wheat, barley and rye. The test showed specificity and sensitivity of 100 %; it recognized all wheat cultivars tested, and it did not recognize all the non-gluten species tested. The sensitivity allowed identification of contamination at 0.1 % (w/w). The test described in [35] based on wheat glutenins, components of gluten: a 135-bp specific fragment of the low molecular weight glutenin gene could be amplified from the Triticum species, but not from barley, rye and other cereals, with a Limit of Detection (LOD) of about 1 copy.

A quantitative competitive PCR system (QC PCR) has also been described as a suitable indicator of contamination of gluten-free food with gluten-containing cereals. This system simultaneously detects Wheat- Barley-Rice-DNA on the basis of a non-coding region of chloroplast trnL gene. The

method has been favourably compared with the more commonly used ELISA method. A positive QC-PCR signal and a negative ELISA result indicates a possible gliadin-free wheat starch addition whereas the opposite situation indicates a possible addition of wheat-free gliadin as a food additive [36].

3.2.1.3. Identification of Triticum aestivum in pasta products

DNA-based methods are used to detect and quantify the presence of common wheat (*Triticum aestivum*) in durum wheat (*Triticum durum*) pasta and other products. DNA is extracted from the sample and four sections of the nuclear genome are amplified using universal primer pairs for both species of wheat. The amplicons are analysed for their species-specific fragment lengths by capillary electrophoresis. Fragment lengths are compared to a previously-established database which enables the identification of durum or common wheat in the sample. By calibrating the system, it is possible to quantify both species in the sample. This method is applicable to pasta, as well as noodles, semolina, couscous, cracked wheat.

3.2.2. Near Infrared (NIR) - Mid Infrared (MIR) spectroscopy

Several studies have also shown the potential of Near Infrared Spectroscopy (NIRS) to identify and discriminate varieties.

In the wheat sector, NIR technology is nowadays considered as an essential analytical tool that greatly contributes to enhancing the quality and safety of agricultural products. Moreover, it has been implemented with success at different stages of the production chain, making it possible to carry out larger numbers of analyses, thus saving time and money. NIR technology is currently used for the quality control of raw materials and end products, for the detection of undesired products and also for the detection of fraud in the both the food and feed chains.

As such, near infrared (NIR) spectroscopy can be considered as a potential powerful tool to detect wheat species such as common wheat in durum wheat [37]. The protein content and the vitreousness of durum wheat is generally higher than that of common wheat. Both criteria can be assessed by NIR spectroscopy. This technique is often used for authentication and traceability of agricultural and food products [38–40]. Mid infrared (MIR) can be also used to discriminate wheat species in particular hulled wheat such as spelt, emmer and einkorn [41]. Differences on cellulose/hemicellulose and lipid contents can be observed between those species.

3.2.3. NIR hyperspectral imaging

To meet the quality product specifications required by the world grain markets and by the agro-food industries, NIR technology has been adapted for the analysis at the kernel level. To achieve this, NIR hyperspectral imaging has been developed in order to detect contamination and fraud in cereals. One particular case-study can be cited to illustrate this kernel by kernel analysis: the detection of common wheat kernels in durum wheat [42]. The macroscopic and microscopic morphological features are important criteria to discriminate wheat species. RGB (red, green and blue model) images can be used to discriminate between durum wheat and common wheat kernels [43]. NIR is also used to assess amongst other protein content and hardness [44]. NIR hyperspectral imaging combines imaging and NIR. It has been used to classify kernels and to simultaneously determine protein content, moisture content, oil content, and hardness, as well as to detect sprouted, insect-damaged, and fungal-infected kernels in wheat [45,46]. NIR hyperspectral imaging has also been assessed as a fast method for the at-line and on-line discrimination between durum wheat and common wheat at the single kernel and bulk sample

level according to the morphological profile, the NIR spectral profile, the protein content and vitreousness [39].

This NIR technology has also been explored on other species such as barley, maize, and rice to identify and discriminate varieties [38,39,44].

3.2.4. Stable isotope ratio analysis

Stable isotope analyses of both heavy (strontium) and light isotopes (C, N, S, O) provide an isotopic signature that can used to verify the geographical origin of a plant. The light isotopes are incorporated into plants during metabolism, linking the plant to specific features of the environment of provenance [47]. The heavy isotopes like Sr also provide geographical information as their content depends on the geology of the plant's growing area.

Building up a database of isotopic signatures from samples taken around the world can be used to verify specific provenance claims. The availability of authentic samples to establish such a database remains a major limitation to the widespread use of this method. Work was undertaken in the FP6 TRACE project[2] to study how geochemical markers and the relationships between these markers could be used to determine the provenance of food products. The study looked at wheat and other cereals from all over Europe and investigated the potential of stable isotope ratio measurements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$) together with strontium isotope ratio measurements ($n(^{87}Sr)/n(^{86}Sr)$), and 5 elements (Na, K, Ca, Cu and Rb). Samples were classified in different categories, comparing cultivation regions in the north and south, and near the Atlantic Ocean or the Mediterranean Sea [48].

Stable isotope ratio analyses ($\delta^{13}C$, $\delta^{15}N$ and δD, alone or with $^{87}Sr/^{86}S$) have also been used to identify the geographical origin of winter wheat in China [49]. A further study by the same authors determine $\delta^{2}H$ values for soil water in three growth periods, and rainwater, groundwater, and defatted wheat in the maturity stage, in order to provide a potential indicator for tracing wheat geographical origin [50].

The geographical origin of Indian wheat has also been studied using isotopic composition ($\delta^{13}C$, $\delta^{15}N$) wheat samples collected from adjacent states of India. Results obtained using $\delta^{13}C$ showed good potential; the difference in the $\delta^{15}N$ values from different states were not significant [51].

[2] FP6 TRACE Project. Tracing the origin of food. 2005-2009. Funded by the European Commission under the 6th Framework Programme.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
PAGE (Polyacryamide gel electrophoresis)	Wheat protein glutenin	Identification of wheat varieties
DNA detection (various techniques)	Species-specific markers	Detection of various species (barley, bread and durum wheat, oat, rye, maize, rice)
Polymerase Chain Reaction (PCR)	Specific primers for wheat, barley, rye	Detection of gluten-containing cereals in "gluten free" products
NIR spectroscopy	Protein content, hardness	Discrimination of species/ varieties
MIR spectroscopy	Lipid, cellulose/hemicellulose content	Discrimination of species/ varieties
NIR hyperspectral imaging	Morphological and spectral information	Discrimination of species/ varieties
Stable isotope ratio analysis	Light element isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$)	Geographical origin
	Heavy element isotopes ($^{87}Sr/^{86}S$)	

5. Conclusion

Potential authenticity issues in the future are likely to come from new products becoming available in the market. The "pseudo-cereals" such as quinoa (*Amaranthaceae*), buckwheat (*Lamiaceae*) and chia (*Lamiaceae*) are becoming increasing popular amongst consumers due to perceived health benefits [52,53]. As these products command a higher price, there is the possibility that adulteration or mislabelling will occur [54].

Fraud on wheat seed coating can also be cited as a potential issue. At the current time, no rapid method exists that is able to assess the coating of cereals seeds. Kernel by kernel analysis by NIR could be a way to address this potential fraud [55].

As regards the future of analytical methods, improvement is likely to be seen in the progress in technology and instrumentation. Biomolecular methods remain the most powerful for differentiating between the different cereals or different varieties of cereals. As the technology surrounding DNA-based methods progresses, moving toward rapid throughput screening and efficient instrumentation at an accessible cost, these techniques will be the methods of choice for unambiguous discrimination. New tools based on proteomics can improve the application of protein-based identification of species, cultivars or genotypes. Proteomic analysis of glutenins can be used to detect allelic variants and quality-related issues in durum wheat flours [56].

Over the last few years, a growing number of handheld instruments based on near-infrared spectroscopy including imaging systems, have appeared on the market. They are particularly characterised by their compact appearance, ease of use, the ability to be controlled using a wireless connection via a tablet or a smartphone. It is expected that innovative technology will be used in order to get integrated NIR systems (spectral information) combined with imaging analysis techniques (morphological information), sampling systems (representative information), and GPS

devices (geolocated information). Some of them include predictive models for the simultaneous determination of different quality parameters of the products. Other are connected through the cloud to a central database and software making remote prediction of these parameters.

Beside the NIR sensors for solid/liquid measurement, new NIR sensors for gas analysis or other sensors based on alternative spectroscopic techniques (mid-infrared, Raman, terahertz, nuclear magnetic resonance etc.) are emerging on the market.

These new, smaller and low-cost instruments compared to conventional infrared devices should answer the forthcoming challenges, in terms of precision agriculture, quality control and fraud detection to improve authenticity and processing issues on food always more sophisticated [57].

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Rice and related products

1. Product Identity

Estimated world production of paddy rice in 2016 was 741.0 million tonnes [1]. Of this, Asia accounted for over 90.1 %, Americas for 4.9 %, Africa for 4.4 % and the 28 EU Member States for about 0.4 %. The three main producers, China, India and Indonesia, produced more than 60 % of the world's rice. Within the EU, Italy, Spain, Greece, Portugal, France, Bulgaria, Romania and Hungary are rice producers. Italy accounts for 52.6 % of the total European production in 2017, followed by Spain with 28.0 %, Greece with 6.5 % and Portugal with 5.6 % [2].

The majority of the world's paddy rice is consumed in Asia where it is produced. In the international rice trade, a relatively small number of exporting countries, notably Thailand, Vietnam and India, interacts with a large number of importing countries in Asia, in Africa and also in Europe [3].

1.1. Definition of the product and manufacturing process

1.1.1. General taxonomy

Rice belongs to the genus *Oryza* and the tribe Oryzeae of the Poaceae family. Of the different species belonging to the *Oryza* genus, *O. sativa* is the most widely grown cultivated species making up the majority of the approximately 100 000 different varieties held by the International Rice Gene Bank (http://knowledgebank.irri.org).

O. sativa includes two main cultivars, Indica, which are grown predominantly in tropical and subtropical regions and Japonica, which are grown in temperate regions. Generally, Indica rice grains are longer and retain their shape after cooking, whereas Japonica grains are shorter, and softer when cooked.

1.1.2. Rice variety classification

In international trade, rice variety classification is primarily based on its grain size and shape. The simplest system groups the varieties into three groups: long, medium and short grain (see examples below), using kernel length and/or width. Other classifications exist, such as that used by the Indian government which provides for 5 groups based on the length/length-width ratio of the kernel.

Three main chemical characteristics are used to measure the quality of rice. These include:

- Starch gelatinisation temperature – this determines the time required for cooking the rice
- Gel consistency – this indicates the tendency of the rice to harden on cooling
- Amylose content. A low amylose content is associated with sticky, moist rice.

In general, these characteristics are available across the different groups of grain types and account for differences in consumer preferences around the world. The IRRI's publication on *Grain quality evaluation of world rices* [4], gives a full review of selected grain quality characteristics of

milled rice from all countries producing more than 0.1 % of the world's rice. However, consumer tastes are changing, and in a highly competitive market with stringent quality requirements and some varieties prized above others, problems of adulteration can occur.

1.1.3. Examples of commonly-encountered rice types

Some commonly encountered rice varieties are described below.

Basmati rice: this is a long-grain, aromatic, non-glutinous rice. It is mainly grown in India and Pakistan. The approved varieties are detailed in section 1.2.1.3 below.

Jasmine rice: this is long-grain variety of fragrant rice. Also known as Thai fragrant rice, it is grown primarily in Thailand. Its fragrance results from the rice plant's natural product of aromatic compounds, of which 2-acetyl-1- pyrroline is the most abundant.

Italian rice: the most commonly used cultivar is **Arborio,** a short grain rice with a high amylopectin content making it ideal as a risotto rice. Other risotto rices include **Carnaroli**, **Vialone Nano**. The latter has been granted a Protected Geographical Indication under the EU, which stipulates that it can only be grown within the 24 municipalities of Verona.

Valencia rice: or "Arroz de Valencia" is a short grain rice is traditionally used in paella. It is grown in the autonomous community of Valencia and protected by a PDO quality label, which includes the rice varieties Senia, Bahia and Bomba. Other areas of rice production in Spain are Delta de Ebro and Calsparra.

Black rice: also known as purple rice is a range of rice types some of which are glutinous. It owes its colour to its high level of anthocyanins. It is used in a number of traditional cakes and desserts particularly in China.

Wild rice: is not in fact a rice but the seed of a type of wild grass (*Zinania aquatica*) which grows in the shallow lake area of North America.

Other rice descriptions that consumers may encounter include **brown rice**, the rice which has not had the bran layers and germ removed. It can apply to all grains whether short, medium or long.

1.1.4. Rice by-products

The main by-products of rice are rice straw, rice husks or hulls, and rice bran. Some of these are used as animal fodder and fuel in power stations. Rice bran, produced from the outer layer of brown rice grain, is used in vitamin mixes and cereals due to its high content in vitamin B6, iron and other minerals. Rice bran oil is also becoming a popular cooking oil.

1.1.5. Other rice-derived products

In addition to direct consumption, rice can also be further milled into rice flour, both brown and white, and is used in many Asian dishes and for making rice noodles. Other components such as starch and protein can also be extracted from the rice. Rice starch has a unique starch granule size [5] and is becoming increasing used as a natural, "gluten-free" ingredient in a number of food products including baby and infant foods. Rice protein or protein concentrate is obtained by separating the protein portion from the starch portion of the rice and used in the formulation of many pet foods. Rice "milk" is considered an alternative to cow milk for vegans or for intolerant people.

1.2. Current standards of identity or related legislation

1.2.1. ISO Standards

According to the ISO Standard 7301 [6], the following definitions apply:

Paddy/Rough rice: freshly harvested rice. The rice is first dried from approximately 20 % moisture content to about 14 %, and then cleaned of foreign material.

Husked rice: paddy rice from which the husk only has been removed. Also known as brown rice, it may be consumed as is or milled into white rice for consumption.

Milled rice: rice obtained after milling which involves removing all or part of the bran and germ from the husked rice. Milled rice is also referred to as polished rice.

Parboiled rice: rice, the starch of which has been fully gelatinized by soaking paddy or husked rice in water followed by a heat treatment and a drying process.

Glutinous rice, waxy rice: special varieties of rice (*Oryza sativa* L. glutinosa) the kernels of which have a white and opaque appearance. The starch of glutinous rice consists almost entirely of amylopectin. It has a tendency to stick together after cooking.

The ISO Standard also provides specification for physical and chemical characteristics, including accepted moisture content and the maximum contents of extraneous matter, defective kernels and other kinds of rice in husked and milled rice.

1.2.2. Codex Alimentarius

Codex Standard 198-1995 [7] applies to husked rice, milled rice and parboiled rice, all for direct human consumption, providing similar definitions to those in ISO Standard 7301 above. It also provides guidance on general quality factors, contaminants, labelling and packaging.

In particular, Codex Stan 198 provides specifications for long, medium and short grain rice, depending on whether the kernel length or kernel length/width ratio is used for the classification.

1.2.3. EU Regulations

1.2.3.1. Common Market Organisation, import tariffs and quotas

European Parliament and Council Regulation (EU) No 1308/2013 [8] provides the Common Market Organisation for rice, including market intervention and trade measures. It applies to the following products:

- Rice in the husk (paddy or rough)
- Husked (brown) rice
- Semi-milled or wholly-milled rice
- Broken rice
- Rice flour
- Rice groats and meal
- Rice pellets
- Flaked rice grains
- Rolled grains of rice
- Rice starch

Commission Regulation (EU) No 1272/2009 [9] lays down common detailed rules for buying-in and selling of agricultural products under public intervention. However, rice is only accepted into intervention if it complies with certain eligibility criteria (quality specifications), related to moisture content, milling yield, defects in the grains, miscellaneous impurities, grains of other rice varieties.

As regards trade with third countries, Commission Regulation (EC) No 1342/2003 [10] lays down specific rules for the system of import and export licences for cereals and rice. Following international agreements under WTO or bilateral negotiations, various Tariff Rate Quotas (TRQs) allow rice imports at low or even zero duty. These are detailed in Commission Regulation (EU) No 1273/2011 [11] which are reopened on 1 January each year and apply specifically to the country of origin of the imported rice.

In addition, for broken rice used in the production of infant foods, a specific tariff quota for 1000 tonnes at zero duty is available through Commission Regulation (EU) No 480/2012 [12].

1.2.3.2. Geographical origin labelling

Although Regulation (EU) 1169/2011 [13] establishes rules relating to the origin of foods in general, the labelling of rice in the EU is currently not mandatory. In 2014, FERM, the European Federation of Rice Millers, undertook a survey of major retailers in six Member States (Belgium, Germany, Netherlands, Portugal, Spain, UK) to assess the level of voluntary country of origin labelling. Of 678 products investigated, 41 % had some form of origin labelling, with 23 % of products specifically mentioning the country of origin [14].

1.2.3.3. Specific case of Basmati Rice from India and Pakistan

Commission Regulation (EC) No 972/2006 (last amended by Commission Regulation (EU) No 706/2014) [14] lays down special rules for imports of Basmati rice and a transitional control system for determining their origin.

A zero rate of import duty is granted to husked Basmati rice of the following 9 varieties originating from India or Pakistan:

- For India (8 varieties): Basmati 370, Basmati 386, Type-3 (Dehradum), Taraori Basmati (HBC-19), Basmati 217, Ranbir Basmati, Pusa Basmati and Super Basmati.
- For Pakistan (4 varieties): Kernel (Basmati), Basmati 370, Pusa Basmati and Super Basmati.

1.2.4. India (Approved Basmati rice varieties)

So far 29 varieties have been notified under the Indian Seeds Act 1966 and subsequent amendments. A detailed list of notified Basmati varieties as of 2017 are available on the APEDA (Agricultural and Processed Food Products Export Development Authority) at reference [15].

1.2.5. Thailand (Thai Hom Mali Rice)

The Thai National Committee on Agricultural Commodity and Food Standards have established a specific quality standard for Hom Mali Rice, the main rice crop grown in Thailand (Thai Agricultural Standard TAS 4000-2003) [16]. Varieties that have been certified by the Department of Agriculture, Ministry of Agriculture and Cooperatives, are the Khao Dawk Mali 105 variety and its derivative Gor Khor 15.

2. Authenticity issues

2.1. Identification of current authenticity issues

Both cultivar and cultivation area are major factors in determining the market price of rice. Hence, the main authenticity issues are the substitution of one variety or cultivar with another, or the mislabelling of the geographical origin of the rice.

2.1.1. Substitution or dilution of premium rice with cheaper varieties

Premium rice varieties such as Basmati and Thai Hom Mali have been the subject of adulteration with cheaper varieties.

The authenticity of Basmati rice depends on both geographical origin and cultivar. Basmati is the name used for a class of rice comprising a few defined varieties grown in the Haryana, Punjab and Uttar Pradesh regions of India and Pakistan. The highly favoured properties of Basmati such as its fragrance and flavour give it the status of one of the premium varieties of rice enabling it to sell for a premium price. Since it is difficult to visually distinguish different types of rice from each other, the adulteration of Basmati rice with other varieties has occurred.

As mentioned in section 1.2.5 above, the Thai government has protected two cultivars Kao Dawk Mali 105 and its derivative Gor Khor 15 of Thai Fragrant rice. A possible adulterant, Pathumthani 1 (another Kao Dawk Mali 105 derivative to be commercialised) and for which cultivation is not restricted to a certain region or season, is sold as a much cheaper price.

2.1.2. Mislabelling of risotto rice

A poor harvest of Arborio rice in Italy in the early 2000, which pushed prices up, led to the adulteration of this premium rice with cheaper varieties [17].

2.1.3. Other authenticity issues

Other issues include the addition of paraffin to rice [18] to give it its desirable translucent appearance and the use of artificial dyes to pass cheaper white rice off as black rice.

2.2. Potential threat to public health

Although passing off cheaper rice varieties as more expensive ones does not pose a particular risk to the consumer, the addition of adulterants such as the paraffin and synthetic dyes described above are obvious potential health hazards.

A particular case of public health concern involved the case of synthetic rice found in China, Indonesia, the Philippines, Singapore, India and Vietnam. The product causes serious disruption to the gastrointestinal tract and is potentially lethal if large quantities are consumed. The counterfeit material looks almost identical to rice grains but is generally made of potato starch mixed with a plastic that is generally found in packaging. In some cases, the plastic rice is mixed with regular grains, making it harder to detect [19]. The use of a handheld Raman spectroscopic device was proposed to the authorities in the Philippines to screen for plastic rice [20].

The most well-known case of adulteration involved rice-derived products and the addition of melamine and melamine-related products in rice protein concentrate. This occurred in 2007, when melamine and cyanuric acid were found in products labelled as rice protein concentrate being used in the production of pet food. These products had been added to increase the apparent protein content. The contaminated pet food led to the sickness and death in some cases of pet dogs and cats in the USA [21].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Standard methods for quality control

The standard methods for the general quality control of cereals published by the International Association for Cereal Chemistry (ICC) are also applicable to rice (see section of wheat and related products above).

AACC International Approved methods for cereals are also applicable. In addition, AACCI has published methods to measure the gelatinisation and paste viscosity characteristics of milled rice flour [22], and to determine the apparent amylose content of milled rice [23], a rapid screening method applicable to milled raw, parboiled and precooked rices.

Rice flour and rice-derived products are often used in the manufacture of “gluten-free” food products. The AACCI also provides a standard method for the detection of gluten in rice flour [24]. The method uses a sandwich enzyme-linked immunosorbent assay (ELISA) kit with proprietary antibodies optimised to determine gluten levels less than 200 mg/kg in samples and is intended for the evaluation of samples with respect to a 20 mg/kg regulatory decision level.

3.1.2. Methods for the detection of melamine and related products

Following the serious incidents in which pet food and some of its ingredients were found to be contaminated with melamine and a related compound, cyanuric acid, a number of analytical methods were developed including both selective quantitative methods and rapid screening techniques. In 2009, the World Health Organisation (WHO) in collaboration with the FAO (Food and Agriculture Organisation, supported by Health Canada published an overview of methods for the analysis of melamine in foods and animal feed [25]. Of the many methods available, the US FDA’s Laboratory Information Bulletin describes an analytical procedure using GC-MS specifically for dry protein materials including rice protein [26].

3.2. Other commonly used methods

3.2.1. Biomolecular methods

One of the most important authenticity issues for rice is the mislabelling of premium varieties, or their substitution or dilution with cheaper ones. Analytical techniques based on DNA based markers are therefore the most suitable techniques for rice variety authentication.

3.2.1.1. Authentication of traditional Basmati rice

Traditionally Basmati adulteration was detected through the analysis of specific aromatic compounds, sometimes by simply smelling the rices after immersion in boiling water [27], or by more sophisticated chromatographic analysis [28]. However, techniques based on molecular markers have been shown to provide a far more accurate discrimination of Basmati, either from other varieties or from other cheap Basmati varieties obtained by crossing with Indica rice.

Of the methods developed for this purpose, work has focused on exploiting DNA based markers ([29] and references therein). Amplified Fragment Length Polymorphisms (AFLPs) have been found to be the most effective, with the maximum discriminatory power. A database of microsatellites for discrimination of different Basmati varieties has also been produced [30]. A more recent development makes use of HRM in Real-Time PCR to allow the analysis of microsatellites without capillary electrophoresis [31]. With this approach, melting curves of the amplified products can be differentiated and identification of heterozygote is possible, and two amplified products of the same length can be distinguished if different in the proportion of GC bases composition.

A recent review of methods for the detection and quantification of adulteration of rice using Basmati as a case study is given in reference [32].

As detailed in section 1.2.1.3. above, EU Regulations specify the Basmati rice varieties from both India and Pakistan that are granted a zero rate of import duty on presentation of an authenticity certificate based on DNA analysis. The UK-based Rice Association published a revised version of their Code of Practice in 2017 which provides an updated list of rice varieties that can be labelled as “Basmati”, with a tolerance not exceeding 7 % of non-Basmati varieties to take into account problems of seed impurity and other segregation issues at origin [33]. The Code of Practice refers to PCR-based methods described on the Food Authenticity Network website [34] designed to detect permitted Basmati varieties.

3.2.1.2. Authentication of Thai Fragrant Rice

As described above Thai Fragrant Rice can be adulterated with cheaper, non-approved varieties. Approved Hom Mali can be distinguished from Pathumthani using DNA Microsatellite fingerprinting, which can determine the quantity of each variety as well as the quantity of any other rice varieties present [35]. Adulteration with non-fragrant rice varieties can be confirmed by testing for a defect in the gene coding for the enzyme betainaldehyde dehydrogenase. Due to this mutation 2-acetyl-1-pyrolline in enriched, which is the characteristic aromatic compound found in Jasmine rice. This technique is also applicable to Basmati rice.

Other molecular markers have also been investigated as a means of authenticating Thai rice. For example, Sequence Characterized Amplified Regions (SCARs) based on previously identified Random Amplified Polymorphic DNA (RAPD) markers, have been shown to discriminate between aromatic and non-aromatic rice varieties [36]. The two SCAR fragments chosen for identification are present in DNA from non-jasmine rice. This can be useful when testing pure jasmine rice samples because the detection of the marker fragments indicates contamination.

3.2.1.3. Authentication of Italian rice varieties

RAPD markers have also been proposed to distinguish between Italian rice varieties [37]. An interesting feature of methods based on molecular markers is the low quantity of DNA required for analysis, which can be extracted even from a single seed. However, in this case the method was not applicable to parboiled rice samples, which undergo thermal treatment and which lacked the amplified fragments of high molecular weight required for the DNA analysis.

3.2.2. Stable isotope ratio analysis

The use of natural stable isotope abundance is becoming increasingly used as a geographical indicator to determine the provenance of food. The main requirement for using these methods to determine geographical origin is the existence of a comprehensive data base of authentic samples from the regions being authenticated. However, the number of studies, and associated data available in the literature, not to mention proprietary databases, have made these methods suitable for determining provenance in routine quality control.

A number of studies have investigated the potential of the stable isotopes of light elements carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$) and sulphur ($\delta^{34}S$), which reflect the plant's metabolism and its environment. Others have included data on heavy elements such as strontium ($\delta^{87}Sr$), which is linked to the geology of the cultivation area. C, N O and S stable isotope ratio measurements have been investigated to discriminate between the same rice cultivars, grown in China, Korea and the Philippines [38]. The same authors also showed that the parameters $\delta^{13}C$ and $\delta^{15}N$ could be used to distinguish between organic and conventionally grown rice in Korea [39].

The potential of stable isotope ratio analyses to verify geographical origin has been shown to be improved, not only by assessing the isotope fingerprint of several elements, but also by measuring isotope ratios in different parts of the plant. This approach has been taken in order to the increase the resolution of light element stable isotopes by investigating the superior spikelets (SS) and inferior spikelets (IS) in the rice panicle. The study involved a rice cultivar Daohuasiang from adjacent sites in Fujin and Wuchang in the Heilongjiang Province in China, with interesting results [40].

3.2.3. Multi-element analysis

Another approach to verify the geographical provenance of rice varieties is multi-element analysis. An example of this is the study to address the geographical traceability of "Arroz de Valencia", a specific rice variety covered by a Protected Denomination of Origin (PDO) label in Europe. The authors looked at thirty-two different elements determined in rice grains from Spain, Brazil, Japan, and India [41]. Linear Discriminant Analysis grouped the Spanish rice samples apart from samples from the other areas with a correct classification of 91.3 %.

A trace element approach together with a data mining technique known as Support Vector Machine (SVM) has been used to authenticate organic rice produced in Brazil [42]. The study looked at 19 different elements, resulting in a correct classification of 98 % of the organic rice samples. Interestingly, a correct classification of 96 % was obtained when only Ca and Cd were used.

3.2.4. Combined multi-element and stable isotope ratio analysis

Best results for geographical origin verification are obtained by combining multi-element and stable ratio analyses. An early study looked at rice samples cultivated in the USA, Europe and Basmati regions using nine key variables (carbon-13, oxygen-18, boron, holmium, gadolinium, magnesium, rubidium, selenium and tungsten) to discriminate geographical origin [43].

A more recent study of geographic authentication of rice has used combinations of elemental/isotopic composition analysis and chemometric techniques to distinguish between rice grown in six Asian countries. The major common variables responsible for differentiation in these models were $\delta^{34}S$, Mn and Mg [44].

Two major targets for geographical authentication are Basmati and Thai rice varieties. For the former, a combination of 10 rare earth elements ((La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Er, Yb) and the isotope ratio of strontium ($^{87}Sr/^{86}Sr$) were used as tracers for differentiating Indian Basmati rice from the other countries of origin[45]. Discrimination of Thai jasmine rice (Khao Dawk Mali 105) cultivated in five different regions was achieved using 9 elements (As, Mg, Cl, Al, Br, Mn, K, Rb and Zn) and stable isotopes $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ with 100 % correct classification [46].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data, analyte or parameter	Authenticity issue / information
Amylograph (AACCI Method 61-01.01)	Gelatinisation and paste viscosity	Characterisation of milled rice flour
Colorimetric determination (AACCI Method 61-03.01)	Apparent amylose content	Cooking and processing qualities
ELISA (Sandwich enzyme-linked immunosorbent assay)	Prolamin and glutelin proteins	Detection of gluten in rice flour
DNA based methods	DNA markers (AFLPs, microsatellites, SCARS, RAPD)	Discrimination of rice varieties
Stable isotope ratio analysis	Light element isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$)	Geographical origin
	Heavy element isotopes ($^{87}Sr/^{86}S$)	
Multi-element analysis	Various elements	Geographical origin

5. Conclusion

The main authenticity challenge in the future will be likely to concern the differentiation of rice varieties. There is currently a huge diversity of rice varieties available, with new varieties being developed all the time. Some of these varieties command premium prices because of their particular cooking characteristics or flavour profile. Others fall under negotiated trade tariff categories granting low or zero duty on imports. GM technology is being investigated for the biofortification (enhanced folate, zinc and iron content). A further example is Golden Rice [47], a variety of *Oryza sativa* that has been genetically engineered to biosynthesis beta-carotene, a precursor of vitamin A, in the edible parts of rice. This variety has been accepted as safe by a number of governments around the world but because of the stiff resistance to GM technology, the product is not yet available.

Today the most complete and comprehensive analytical tool for rice authentication is the combination of DNA-based methods to confirm variety together with stable isotope ratio analysis with multi-element analysis to verify provenance. In the future, technological progress particularly in the instrumentation used will greatly improve the ease-of-use of these techniques. A current

example is the use of modern sequencing, known as Next-Generation Sequencing (NGS), or high-throughput sequencing, which enables the analyst to sequence DNA and RNA much more quickly and cheaply than before.

Today's focus is shifting more and more towards this type of untargeted approach. Other techniques investigated in this respect include ^{1}H NMR, which has been studied as a means of discriminating rice from different regions in China [48], multi-platform MS-based metabolomics and multivariate analysis for the geographical origin [49] and a LC-MS untargeted approach to distinguish between organic and conventional rice [50]. These approaches offer the potential of rapid authentication methods or of a short-cut to identifying suitable markers for use in authenticity testing.

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Nuts, nut products and other seeds

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General overview of the products

Nuts and nut products are a highly heterogeneous category of food, with several applications, consumed roasted, dried, in preparations as ingredients in confectionery, sweets, baking. For the purposes of this handbook, the focus will be on the fruits of plants which are commonly defined as "nuts", which can be sold with or without a hard shell. They are not always nuts in a botanical sense: a nut is in fact defined as a fruit from Angiosperms, dry indehiscent one-seeded, with a hard pericarp, meaning that it does not open spontaneously to release the seed [1]. Considering this, chestnuts and hazelnuts are real nuts, whereas almonds and walnuts are not.

Nuts are highly relevant to some consumers because of the allergy issues linked to their consumption, particularly for almonds, peanuts, hazelnuts. The number of allergic patients worldwide is increasing, and the European Union (EU) has strict requirements for allergen labelling on food products [2].

Recently, nuts have become particularly appreciated in vegan, vegetarian, flexitarian, gluten free diets, and as well as in the paleodiet. They are a source of energy, unsaturated fatty acids and oils, fibre, proteins, vitamins and minerals, including bioactive compounds such as antioxidants, for a healthy diet. As healthy snacks they appeal to people working out of home, in place of sweets and biscuits. They are also marketed as an alternative to meat protein. “Milk” from edible nuts (almond, hazelnut) can replace animal milk. Their production has been increasing in the last years, up to about 4.2 million tons (peanuts excluded), produced mainly in the USA (41 %) followed by China, Turkey, Iran, India. Almonds are the tree nuts with the highest consumption, and Europe is the highest consumer of tree nuts in general, accounting for 40 % of total world imports, with three main importers, Germany, the Netherlands and Italy. Peanut production has reached 41.5 million tons, from China and India. Since many of the nuts are exotic products and healthy snacks, their consumption is expected to grow, if both the market and private means of the consumer increase. The products with rising markets are currently almonds, Brazil nuts, hazelnuts and macadamia nuts.

This chapter will deal neither with oils extracted from nuts, nor with other "seeds" which cannot be classified as nuts: chia, quinoa, amaranth, whose use is more similar to that of cereals.

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1. Product Identity

1.1. Definition of the product and manufacturing process

There is no accepted definition of "nuts" in food commodities. The fruits most commonly considered as nuts include the following (with indication of CN code, cf. Commission Implementing Regulation (EU) 2017/1925 of 12 October 2017 [3]):

Almond (code 0802 11-12): seed of the species *Prunus dulcis*, (or *Prunus amygdalus*) after removal of the fleshy hull (stone fruit). They can be marketed inshell, without shell as kernels, or as blanched kernels after removal of the tegument of the kernel (episperm). There are sweet almond and bitter almonds, containing amygdalin, a glycoside which releases hydrocyanic acid. USA is the major producer followed by Australia and Spain.

Brazil nut (code 0801 21 00-22 00): nuts of the tree *Bertholletia excelsa*, either shelled or after cracking of the shell. The shell is extremely hard and woody, and the kernel is enveloped by a brown seed coat. Bolivia is the major producer, followed by Peru and Brazil; UK is the major importer. The yield is highly dependent on environmental conditions.

Cashew nut (code 0801 31 00-32 00): the nut with the hard shell, also called anacardium, is at the bottom of the fruit (cashew apple) produced by the plant *Anacardium occidentale*. The main producer is Western Africa (Cote d'Ivoire), followed by India and Vietnam.

Chestnut (code 0802 41 00-42 00): these are the fruits of trees from the genus *Castanea,* mainly *C. sativa*. The husk is spiky and breaks open spontaneously, revealing 1-3 fruits; the fruit has a kernel, a thin skin and brown pericarp. The main producer is China, followed by Turkey and Italy.

Hazelnut, or filbert (code 0802 21 00-22 00): nuts of the species *Corylus avellana* and *C. maxima*, free from the husk, sold shelled or after cracking of the shell. Over half of the global production comes from Turkey, followed by Italy, which is also the main importer.

Macadamia nut (code 0802 61 00-62 00): nuts of the species *Macadamia integrifolia*, *M. tetraphylla, M. ternifolia* growing in hot subtropical climates. After drying and shell cracking, the kernels can be dry-roasted or oil-roasted. Used in confectionery, baking, ice cream, snacks. Production is concentrated in Australia, South Africa and Kenya.

Peanut (cod 2008 11): also called groundnuts, they are leguminous fruits of the plant *Arachis hypogaea*, growing underground. They have a thin shell, which is in fact the pod, containing generally two kernels. China is the world major producer, followed by India and Nigeria.

Pecan nut (code 0802 90 10): the seed comes from the tree *Carya illinoensis* and is encased in a husk. It can be consumed fresh or used in cooking. The main producers are Mexico and USA.

Pine nut (code 0802 90 50): decorticated kernels of different species of Gymnosperm, *Pinus*: e.g. *pinea*, *koraiensis*, *sibirica*, *yunnanensis*, *wallichiana*, *gerardiana*, *pumila.* The main producers are in Asia, China, North Korea, Russian Federation. Italy leads the production of Mediterranean pine nut.

Pistachio nut (code 0802 51 00, 52 00): the kernels are in the single-seeded stone fruit of the tree *Pistacia vera*, with a brown seed coat and brilliant green kernel. They are marketed in shell, raw or salted, sugared, flavoured. The major producer is the USA, followed by Iran and Turkey.

Walnut (code 0802 31 00-32 00): nuts of the tree *Juglans regia*, enclosed in a shell made of two halves, free from the outer green and fleshy husk, sold in the shell or after cracking of the shell. China and USA are the major producers.

Nuts are generally harvested by shaking trees, in processes which can be mechanised or performed manually. The fruits are then washed and dried. Some nuts are marketed as such, some undergo bleaching of the shell to improve the appearance, and others are taken out from the shell by cracking. Storage varies, with some nuts being more durable than others. New technologies are actively improving drying and processing, except in cases where natural production requires sun-drying.

They are commonly sold to the food manufacturing industry, as ingredients, to be processed, and repackaged. The chocolate industry is the largest user of many edible nuts, also playing on their health benefits. Breakfast cereals and energy bars involve also edible nuts in their formulations. The snack industry uses large quantities of edible nuts, particularly peanuts. Other industries involved concern bakery, ice creams, nut butter, nut milk, and even pet food. The increase in the use of edible nuts is due to new information about health attributes and claims, the availability of new types of nuts, and new processing and flavouring possibilities.

Increasingly there are requirements for sustainable products in appealing to consumers, particularly when edible nuts come from developing countries.

1.2. Current standards of identity or related legislation

The International Nut & Dried Fruit Council (www.nutfruit.org) is the body of reference for the trade with edible nuts. Statistics on the production and commerce of main nuts are available on the web site.

The standards from the United Nations Economic Commission for Europe (UNECE), Working Party on Agricultural Quality Standards [4] describe the products and the quality requirements at the export control stage, concerning appearance, moisture content, sizing, presence of defects, blemishes and infestation. They also describe packaging requirements. They are available for almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pine nuts, pistachio nuts, walnuts.

The International Standards for Fruit and Vegetables of the OECD also describe nuts in a similar way [5].

Concerning EU legislation, Regulation (EU) 1169/2011 lists nuts in the fourteen groups of allergens that must be declared on the label in a prominent way [6]. Besides allergens, the main concern in EU legislation regarding nuts is the presence of aflatoxins; additionally, the absence of insects and parasites, and of foreign objects. Nuts imported into the EU need a certificate ensuring that they have been sampled for analysis. Aflatoxins of concern, produced by moulds *Aspergillus flavus* and *A. parasiticus*, are B1, B2, G1 and G2 as listed in Regulation (EC) No 1881/2006 [7] amended by the Commission Regulation (EU) No 165/2010 [8]. The main attention is on peanuts from Bolivia, Gambia, Madagascar, Sudan and Senegal, on hazelnuts from Georgia, and on pistachios from the USA. The Rapid Alert System for Food and Feed (RASFF) system periodically informs about instances of aflatoxin contamination in nuts [9].

The standard ISO 1990-1:1982 provides the botanical names of common fruits and vegetables, including nuts [10]. Standard ISO 4125:1991 lists all dry fruits with a low moisture content, and nuts are also included [11].

CODEX has produced a comprehensive document on the Code of hygienic practice for tree nuts, CAC/RCP 6-1972, on the cultivation, processing, shelling etc. A separate document covers practices for peanuts, CAC/RCP 22-1979 [12]. A more recent document concerns the Code of practice for the prevention and reduction of aflatoxin contamination in tree nuts, CAC/RCP 59-2005 [13].

The Transport Information Service [14] provides relevant information and specifications about the transport of nuts and the possible problems which may arise to damage the products. The website Standards Map [15] provides a tool for recovering the information on sustainable trade, linking to the different standards on this subject.

2. Authenticity issues

2.1. Identification of current authenticity issues

The main authenticity issue for tree nuts is probably the country of origin, since this indication is usually mandatory on the label. Indication of the species is also obligatory and relevant, particularly for problems linked to allergies: substitution of nuts from different species can negatively affect allergy patients, if undeclared. The indications on the label can also include the crop year and the variety. The year of harvest is important because older nuts are more prone to infestation and rancidity.

A possibility for fraud exists in the declaration for organic products, which should be produced and processed with specific techniques, including the use of crop rotation, specific crop protection and fertilisation substances.

Additionally, nuts are used in Jewish cuisine and the Kosher certification is another possible source for fraudulent declarations.

The increase in demand, combined with problematic harvests, can lead to the shortage of some edible nuts, and this could open the way to counterfeiting. In the case of Brazil nuts, which are collected in the Amazonian forest, their availability from year to year is not predictable.

Protected denominations (PGI or PDO) for cultivars of some edible nuts are particularly appreciated and can be subject to fraud. One such example is the hazelnut "Tonda Gentile delle Langhe" (Nocciola Piemonte, PGI since 1996) from Italy. Other relevant PDO examples in Europe concern: chestnuts (Portugal and Italy), almonds (Portugal), walnuts (France, Italy).

Some PDO or PGI productions requiring nuts as ingredients are also subjected to fraud. One relevant example is Ligurian pesto, a sauce made with basil containing pine nuts as a highly relevant ingredient. In this case, *Pinus pinea* is the species of origin, but other pine species could be used since recognition by visual inspection is impossible. Mislabelling of pine nuts is relevant for the taste, but also for health effects (see below).

Hazelnut paste is an important ingredient in confectionery, and also the ingredient for appreciated hazelnut spreads for direct consumption (e.g. Nutella). The percentage of hazelnuts in the paste is a critical quality issue and dilution or substitution with artificial compounds or with other ingredients are fraudulent practices.

2.2. Potential threat to public health

In the case of nuts, the main risk to health is represented by allergies. Mislabelling can be very dangerous to allergic patients. On the other hand, precautionary labelling can deprive consumers from enjoying some food products.

The presence of aflatoxins is a relevant threat to health for nuts (cf. Safenut project [16]). The EU RASFF system in 2017 issued a total of 364 notifications for aflatoxins in edible nuts, particularly for peanuts from China, pistachios from Iran and hazelnuts from Turkey. The geographic provenance of nuts is therefore an important component of authenticity issues, since some countries are more susceptible to contamination with aflatoxins.

Pine Nut Syndrome (PNS), also called metallogeusia, dysgeusia, was first described as a disturbance of taste perception, starting with a metallic or bitter taste appearing after consumption of pine nuts and lasting for several days [17]. The cause of the syndrome is yet unknown, with possibilities concerning fatty acids, rancidity, toxins. The prevalence, which is higher in women than in men, has led to considerations concerning genetic polymorphisms and metabolism of bioactive compounds. In particular, the species *Pinus armandii*, from China, has been associated to PNS occurrence; this species is used in industrial applications and not for food production. The uncertainty about the cause of the syndrome and the difficulties in labelling and tracing the provenance of pine nuts are negatively affecting the market.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Most standards on nuts require visual inspection and morphological evaluation. The U.S. Food and Drug Administration (FDA) has a list of methods for the analysis of nuts and nut products addressing mainly defects, infestation, and presence of foreign material [18].

Other methods for nuts based on analytical techniques are applied mostly in cases where the nuts are not easily recognisable visually, for instance in hazelnut spreads or peanut butter. Identifying the nut species in these cases is essential to protect patients suffering from allergies. These methods can be based on detection of proteins/peptides, DNA markers, or specific metabolites and compounds.

The presence of specific nuts in food products is usually checked with ELISA and lateral flow devices for rapid testing. Several companies have placed on the market tests to detect different species of nuts by recognising specific allergenic proteins as target, with the aim of protecting consumers. However, in some processing conditions proteins can be denatured or degraded, making their detection more difficult. In these cases, analysis of DNA markers can be effective, since DNA as a molecule is more stable than protein.

For the recognition of specific cultivars, for instance to check for PGI or PDO varieties, visual inspection or imaging techniques are often applied [19]. However, the aspect of the nut and of the shell can be affected by several environmental factors. In these cases, DNA typing provides a better way for identification, even if DNA analysis is made difficult by the high lipid content of nuts [20]. DNA analysis can be applied to whole nuts or to products made with nuts. The markers of choice are often the genes encoding for allergens, which are considered to be highly specific for each type of nut.

The presence of lipids and fatty acids in nuts and nut products can be a problem in some analytical techniques, but it is also a distinctive feature of nuts. Methods based on analysis of fatty acids or other metabolites have been developed to check for the authenticity of the species or of the cultivar, even in highly processed foods [21–23].It is widely considered that these kind of markers can provide indications about the geographic origin of food products, whereas DNA markers cannot be used for this purpose [24,25]. They could also provide indications about the year of harvest, but no method has currently been developed.

3.2. Other commonly used methods

In some cases, DNA analysis requires specific adaptation. One such example is the discrimination between marzipan (containing almonds) and persipan (containing apricot or peach kernels), which is difficult because the *Prunus* species are closely related. DNA analysis with specific markers and barcoding has been shown to be effective [26]. Potential DNA markers applicable to discriminate between cultivars can be found in the chloroplast DNA or in genes encoding for ribosomal DNA (rDNA) as in the case of analysis for recognition of *Pinus armandii* [27,28] or hazelnut [29].

Multiplex methods can be useful when looking for different species of nuts at the same time in a food product [30]. Methods based on DNA extraction and amplification of specific diagnostic fragments can be multiplexed in different formats, endpoint PCR, Real time PCR, microbeads [31], etc.

Also, there will be a development of methods for performing biomolecular analyses in a quick and rough way, for instance with no requirements for lengthy DNA extraction and no need for costly equipment. An example is Loop-Mediated Isothermal Amplification (LAMP) PCR which does not require a thermal cycler [32]

Similarly, screening methods using chemometrics with no extraction of the sample, such as with spectroscopic techniques, could be interesting additions, when coupled with portable instruments for *in situ* quick non-destructive analyses.

Non-targeted analyses performed with spectroscopic techniques are being developed, to detect differences in lipids, proteins, carbohydrates with fast analyses. The chemical profile could discriminate nuts according to the geographic origin [33].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
ELISA	Allergens	Hazelnuts, etc.
Fourier transform Infrared Spectroscopy, portable	No particular analyte (lipids, proteins)	Variety discrimination
Gas chromatography	Fatty acids	Geographical origin
GC/MS, gas chromatography mass spectrometry	Filbertone	Hazelnut spread
HPLC	Tocopherol	Chestnut varieties
Imaging techniques	Identification of cultivars	Hazelnuts cultivars
Loop mediated isothermal amplification (LAMP)	Allergen marker	Peanut in food
Microbead fluorometric PCR	Allergens	Nuts in foods
PCR	DNA markers	Species identification, e.g. *Pinus armandii*
PCR	Chloroplast markers	Species identification, e.g. *Pinus armandii*
TaqMan real time PCR	ITS marker	Hazelnut allergen

5. Conclusion

The worldwide trend in consumption of nuts, because of their health benefits, supports a sustainable growth for this market. New literature reporting on the beneficial effects of unsaturated fatty acids and other components of some nuts are leading to their introduction in diets for patients or people at risk of cardiovascular diseases. Milk substitutes from nuts, and nuts substituting meat, will be appealing to vegans. Savoury snacks are already widely used, but they are constantly increasing because of health effects, new processing ideas, and availability of several nut types. In view of this, the increased consumption could lead to fraud opportunities. Additionally, since the chemical composition of nuts depends on species and geographic origin, fraudulent labelling for provenance could be envisaged. Current methods can effectively recognise species and cultivars, but recognition of provenance is more difficult.

Consumer interest in sustainability of provenance and fair trade are particularly increasing in the EU market, and this also applies to fruit and nuts. Certification of these schemes will probably become more common, creating potential opportunities for frauds. However, methods to establish the correctness of such claims are not yet available.

In the case of nuts, climate change is expected to have highly negative and unpredictable effects. The areas for production, often in tropical countries, will be heavily affected by changes in growing seasons, temperature and rainfall. Additionally, new pathogens and infectious diseases are expected to appear. Currently, pine nuts are defined as the “caviar of plants” because they are becoming increasingly rare, mainly due to an increase in pathogens worldwide, and to deforestation and changes in climate conditions. The yield and safety of the products will

therefore be affected in a negative way, and this will probably require additional methods for traceability and inspection. In Ligurian pesto, for instance, the pine nuts required by the original recipe are sometimes substituted with cashews. Fraud control will therefore require methods for species recognition, but also methods for provenance recognition, which are not completely developed.

The certification of organic cultivation will also become more important. This market is growing continuously, and the production cannot keep up with the pace. This will also be an opportunity for fraud. Unfortunately analytical controls on organic cultivation are not well developed at the moment.

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Cocoa, cocoa preparation, chocolate and chocolate-based confectionery

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General overview of the product

Although there are many products of cocoa, most of the cocoa production is used to manufacture a product that is often associated with many positive feelings, i.e. chocolate [1]. Nowadays, chocolate represents one of the most popular and widely consumed confectionery products. In 2017 its consumption reached 8.8 kg per person and per year in Switzerland, the biggest European consumer [2]. Chocolate is not only a common confectionery product, it also plays an important social role being an inherent part of many celebrations. Currently, an increasing number of various specialties and gourmet products is appearing on the market and they are in high demand. Some of them are even crossing the confectionery industry line by aiming at consumer interest in health. The products benefit from the positive effect of cocoa (e.g. chocolate with 100 % cocoa solids) as well as from various additional ingredients (e.g. dried berries, herbs, seeds, nuts, dietary fibre or probiotics) [3,4]. Another group of products focuses on the ethical and ecological aspects of production (Fairtrade and/or organic products). By introducing various novel products, the cocoa industry is responding promptly to modern trends in order to keep enticing its consumers. However, these dynamic changes may be extremely challenging for producers who are pushed to offer items that are acceptable both in terms of quantity as well as quality [5].

An enormous growth in cocoa production has occurred since the second half of the 20th century, from 1 695 in 1980/1981 to about 4 587 thousand tonnes in 2017/2018. This corresponds to a steady increase in cocoa and chocolate confectionery consumption. Global demand is still growing annually by 2-3 % [6–9].

The cocoa value chain faces a number of challenges in growing and selling this crop. Cocoa production is located in a limited geographical area around the equator, mostly in developing countries, while its consumption is focused almost exclusively in developed countries. For many of the producers, cocoa is a vital part of their economic income. Farmers often have limited knowledge of modern farming techniques and farm management skills as well as limited access to finance that would enable them to purchase input supplies and quality planting material. Other challenges that impede productivity include aging trees, decline in soil fertility and the struggle with pests and diseases that attack cocoa trees [6].

Due to the increasing importance of social, environmental and economic issues, more focus is aimed at the traceability and sustainability of cocoa production [10]. Cocoa is predominantly a smallholder crop, as more than 90 % of world cocoa production originates from small farms. In

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Africa and Asia, a typical smallholder cocoa farm covers only 2 to 5 hectares of land. In a meaningful concept of sustainability, consumption is of equal importance to production. A sustainable world cocoa economy implies an integrated value chain in which all stakeholders develop and promote appropriate policies to achieve levels of production, processing and consumption that are economically viable, environmentally sound and socially responsible for the benefit of present and future generations, with the aim of improving productivity and profitability in the cocoa value chain for all stakeholders concerned, in particular for the smallholder producers. Basic principles are given in the Cocoa Agreement by the International Cocoa Organisation describing arrangements between producing and consuming countries to safeguard markets and raise average prices to stabilise trade, supplies and prices of cocoa [11]. CAOBISCO (Association of Chocolate, Biscuit and Confectionery Industries of Europe), the European Cocoa Association (ECA) and the Federation of Cocoa Commerce (FCC) are committed to working towards more sustainable cocoa which complies with such requirements for benefit of the consumer, the manufacturer and the farmer [10].

To support the sustainability of cocoa production, independent certification schemes have been established to provide increased transparency and responsibility in cocoa supply chains, providing farmers with the resources they need and helping them to manage their farms professionally, and in turn be rewarded for sustainable production and for providing consumers with products they can enjoy and trust. Examples of these certifications are Fairtrade [12], UTZ Certified [13] and Rainforest Alliance Certified [14].

1. Product Identity

1.1. Definition of the product and manufacturing process

A wide range of cocoa products originates from the seeds of the cocoa tree, *Theobroma cacao* L. Cocoa trees are grown in a narrow band around the equator (approximately 20° north and 20° south), which goes through four continents: Africa, Asia, Australia and Oceania, and South America. Africa produces 73 % of the world production, followed by America with less than 17 %, and Asia and Oceania at about 10 % [15]. For a long time, most cocoa production has been concentrated in 7 countries: Ivory Coast, Ghana, Indonesia, Cameroon, Nigeria, Brazil and Ecuador.

The cocoa tree has four main varieties (some of which are bound to a particular geographical region) and several hybrids, each of which possesses a unique potential for flavour development. In terms of world trade, the quality of cocoa beans is divided into two categories (i) 'fine' or 'flavour' and (ii) 'bulk' or 'ordinary'. The difference between 'fine' or 'flavour' cocoa and 'bulk' cocoa is in the flavour rather than in other quality factors [16]. Forastero accounts for the most of 'bulk' cocoa production and is referred to as a basic variety. Criollo, Trinitario and a rare variety Nacional (last producing well-known Arriba beans) are considered 'fine or flavour' cocoas and are used for gourmet chocolates [1,5,17]. However, it is not only variety that influences flavour development, which is also affected by other factors such as a growing locality and conditions during growth and harvesting. Moreover, the final flavour and taste of cocoa products are highly dependent on individual processing stages and conditions. The processing is thus very important for final product quality, though it may not be necessarily related to its authenticity. While the term quality has different associations, authenticity is always strictly related to true product identity. Especially in the case of chocolate, consumers may have different preferences and expectations, often in relation to their geographical regions. When assessing chocolate

authenticity, tracing the initial ingredients may be much harder due to these differences in manufacturing processes and their complexity [5].

To understand the identity of individual cocoa products, it is important to firstly explain the cocoa manufacturing process. This can be divided into two stages, firstly cocoa processing, and, secondly, chocolate manufacturing. However, both of them are often directly connected since most cocoa is used to make chocolate. After harvest, **cocoa beans** are released from **cocoa pods** (fruit) and then cleaned of any extraneous matter by blowers and sieves. The fresh cocoa beans are then left to ferment under the action of naturally present yeasts. During this process, which is especially important for the development of main flavour precursors, the beans change colour from purple to brown. To prevent the growth of moulds and reduce microbial contamination, the fermented beans are dried and then roasted. During roasting, the beans also gain additional flavour. Loosened hard shells are then removed from the beans (winnowing) to reveal **cocoa nibs** (or, alternatively, deshelling may be performed prior to the roasting). The nibs are ground to a homogenous paste called **cocoa liquor** (or by a number of other terms, such as cocoa mass, cocoa paste, chocolate paste). This paste can be used directly in products such as chocolate or pressed to separate **cocoa butter** (fat) from cocoa solids (**cocoa cake**). Crushing the cocoa cake will result in natural **cocoa powder**. An optional process, typical for Dutch cocoa powder, contains an alkalising step using potassium or sodium carbonate, which leads to lower acidity, a darker colour, more intense flavour, milder taste and better dispersibility in water.

Cocoa is traded at different stages of this process and intermediates/products may differ significantly in composition; thus, the process of authentication is a complex procedure involving various steps for different products.

In chocolate manufacturing, the first step is to mix all its ingredients together while applying moderate heat to melt the cocoa butter. Additional steps, such as refining and conching, are carried out to achieve a smooth texture and intense flavour. Finally, a tempering step occurs which is important to obtain good surface gloss, a snap and a stable structure resistant to fat bloom. All manufacturing processes have a strong influence on the final product quality and, due to their complexity, can make authentication very difficult [5,17,18]. Regarding the ingredients used within the manufacturing process, there are three basic types of chocolate: dark, milk, and white. Dark chocolate is a complex food product in which sugar crystals and non-fat cocoa particles are surrounded by a continuous phase of crystalline and liquid cocoa butter. Milk chocolate is a complex rheological system having solid particles (non-fat cocoa, milk and sugar particles) dispersed in cocoa butter, which represents the fat phase [19]. White chocolate has a similar composition to that of milk chocolate, but the cocoa is represented exclusively by cocoa butter.

1.2. Current standards of identity or related legislation

Various standards of identity and legislation are related to cocoa and cocoa products with some minor differences, often due to different habits in various geographical regions [20]. Most of them define compositional requirements, whereas product processing is not specified in detail.

1.2.1. In the European Union

In the EU, the main legal document related to cocoa and chocolate products is Directive 2000/36/EC [21]. It specifies the categories of the products (sales names) and requirements for their composition and labelling. This latest Directive is much simpler than the earlier Directive 73/241/EEC and its main role has been to harmonise legislation all over the EU. The main

difference, though, is that it has authorised the use of other vegetable fats in chocolate, up to a level of 5 %, which previously had only been acceptable in seven Member states, such as the United Kingdom or Austria. However, only six specified fats, the so-called cocoa butter equivalents (CBEs), without any enzymatic modifications, can be used (illipe, palm-oil, sal, shea, kokum gurgi and mango kernel), together with the mention "contains vegetable fats in addition to cocoa butter" on the product label. The list of specified products has also been reduced together with their detailed descriptions, such as "cocoa beans", "cocoa nibs", "cocoa mass" or "cocoa press cake". Products specified in the new Directive are "cocoa butter", "cocoa powder", "drinking chocolate", "milk chocolate", "family milk chocolate", "white chocolate" among others. According to the definitions, for instance, cocoa butter is described as the fat obtained from cocoa beans or parts of cocoa beans with the specified content of free fatty acids and unsaponifiable matter; and chocolate is defined simply as the product obtained from cocoa products and sugars which contains not less than 35 % total dry cocoa solids, including not less than 18 % cocoa butter and not less than 14 % dry non-fat cocoa solids. There are slight differences when the name is supplemented by any of the specified words (such as vermicelli, flakes, couverture, gianduja nut). For most cocoa and chocolate products, their labelling must indicate their total dry cocoa solids content. Moreover, the Directive authorises the addition of other edible substances (with the exception of flour, starch or animal fat other than milk fat) up to 40 % of the total weight of finished chocolate products, while the content of cocoa butter and cocoa solids still has to be calculated after deducting these substances. Various flavourings, if they do not imitate the taste of chocolate or milk, may also be added to several cocoa/chocolate products. The use and amount of sugar in chocolate products are no longer restricted; any sugars intended for human consumption can be used. Additives that are applicable for cocoa and chocolate products are specified in a separate, general document on food additives, Regulation (EC) No. 1333/2008 [22] as amended. This document lists various additives authorised for certain foods and specifies their maximum levels in the products (or their use according to the principle of *quantum satis (*q.s.*)*, meaning the minimum level for achieving the desired effect). For cocoa and chocolate products, common additives are emulsifiers and acidity regulators. Of emulsifiers, lecithins or mono- and di-glycerides of fatty acids are applicable at q.s. levels, while polyglycerol polyricinoleate has a maximum level of 5 000 mg/kg and ammonium phosphatides of 10 000 mg/kg. Acidity regulators include carbonates, hydroxides, magnesium oxide and citric acid. More attractive surface gloss can be achieved by the use of glazing agents, such as gum arabic, carnauba wax, shellac or pectins. Products with reduced energy or no added sugar can contain various polyols (e.g. sorbitol, mannitol, maltitol) or sweeteners (e.g. aspartame, acesulfame K, saccharin, sucralose and steviol glycosides). No food colour is permitted in cocoa and chocolate products. The EU Directive does not recommend any methods of analysis.

1.2.2. In the Codex Alimentarius

The globally accepted Codex Alimentarius contains four standards related to cocoa and chocolate products: 86-1981 for cocoa butter; 87-1981 for chocolate and chocolate products; 105-1981 for cocoa powders (cocoas) and dry mixtures of cocoa and sugars; and 141-1983 for cocoa mass (cocoa/chocolate liquor) and cocoa cake. This latter standard introduces some more terms compared to the European "Chocolate Directive" 2000/36/EC in force and specifies that cocoa mass (cocoa/chocolate liquor) must not contain more than 5 % of cocoa shell and/or germ. Some other terms are also more specific. According to Codex, chocolate is described as a homogenous product complying with the stated description (cocoa butter content, fat-free cocoa solids, total cocoa solids, milk fat, total milk solids), obtained by an adequate manufacturing process from cocoa materials, which may be combined with milk products, sugars and/or sweeteners, and other

additives listed in the Standard. Following this general description, Codex specifies chocolate types and their composition (chocolate or, alternatively, bitter sweet chocolate, semi-sweet chocolate, dark chocolate, chocolate fondant; sweet chocolate; couverture chocolate; milk chocolate; etc.). The addition of other edible foodstuffs is limited to 40 % and other vegetable fats to 5 %, as in the EU Directive, but the nature of these fats is not further specified. Furthermore, Codex recommends some internationally recognised analytical methods (for example, for the determination of fat content, cocoa shell, free fatty acids or moisture) published by the Association of Official Analytical Chemists (AOAC), the International Union of Pure and Applied Chemistry (IUPAC) or the International Confectionery Association (ICA, formerly International Office of Cocoa, Chocolate and Sugar Confectionery, IOCCC).

1.2.3. In the United States

When compared to the EU Directive and Codex, the US Code of Federal Regulations (CFR) defines cocoa and chocolate products slightly differently. The main parameter for describing the composition of most of the products is cocoa fat content and/or chocolate liquor content. Notice also the difference in this terminology; cocoa fat instead of cocoa butter, and chocolate liquor instead of total cocoa solids. In the CFR, there are no specifications for cocoa fat, in contrast to cocoa butter in the EU Directive or Codex. For the product prepared by finely grinding cacao nibs (and, eventually, the addition of cocoa fat and/or cocoas) containing between 50 and 60 % of cacao fat, there are several appropriate terms listed: “chocolate liquor”, “chocolate”, “unsweetened chocolate”, “bitter chocolate”, “baking chocolate”, “cooking chocolate”, “chocolate coating” or “unsweetened chocolate coating”. In contrast to the EU Directive or Codex, no other vegetable fats may be added to these products, and no limitation for the added amount of edible foodstuffs is specified. Among other product categories listed, some are very similar to those in the EU, such as “white chocolate” and “semisweet/bittersweet chocolate”, whereas “sweet chocolate”, for instance, must contain only at least 15 % chocolate liquor and, thus, could not be labelled as chocolate in the EU. The CFR also recommends methods of analysis, particularly those published by the AOAC, for the determination of shell content and fat content.

1.2.4. Other standards

Many more standards for cocoa, mostly cocoa beans, are recognized by various international organizations. The International Organization for Standardization has published the standard ISO 2451:2017, which specifies the requirements, classification, sampling, test methods, packaging and marking for cocoa beans, as well as recommendations for their storage and disinfestation.

The Federation of Cocoa Commerce (FCC), which aims at promoting, protecting and regulating the trade of cocoa beans and some cocoa products, has developed contract standards and rules for cocoa beans, defining their quality, sampling and weighing, and contract rules for liquid cocoa products and packed cocoa products. In FCC Quality Rules [23], cocoa beans are described according to their size, observed defects, fermentation, dryness, the presence of any foreign matter, contamination, insects, off-flavours and free fatty acid content. Some standards are still being developed, such as International Standards on Cocoa Quality and Flavour Assessments. A draft of this document was prepared in November 2017 under the coordination of the Cocoa of Excellence (CoEx) Programme [24] (Working Group on the Development of International Standards for the Assessment of Cocoa Quality and Flavours, the members of which represent various stakeholders, from associations of cocoa producers to traders, chocolate manufacturers and research organisations). The document aims at standardising the procedures and terminology for

high quality cocoa and chocolate products, and includes also the sampling, assessment of physical quality and flavour of cocoa beans, manufacturing procedures, or storage conditions [25].

For the use by Customs authorities, statistical agencies and other regulatory bodies, all commodities are classified and coded by the Harmonised Commodity Description and Coding System ("Harmonised System") developed by the World Customs Organization [26]. The Harmonised System is applied worldwide to facilitate the international trade and monitor and control the import and export as well as for the purposes of customs tariffs and taxes. The Combined Nomenclature was established by Council Regulation (EEC) No 2658/87 on tariffs and statistical nomenclature and is updated every year; the latest version is now available as EU Regulation No 2017/1925 [27]. In the Combined Nomenclature, the codes of the Harmonised System are used. There are different categories for cocoa and cocoa preparations, beginning with the number 18 (e.g. 18 06 10 for cocoa powder, containing added sugar or other sweetening matter), while white chocolate is classified as sugar confectionery and coded as 17 04 90 30.

At the end of this chapter, it is worth mentioning the famous Swiss and Belgian chocolate. Both these terms are related to the product manufacturing country. The term 'Belgian chocolate' was introduced in 2008 in the 'Belgian Chocolate Code' of The Royal Belgian Association of the Biscuit, Chocolate, Pralines and confectionary (Choprabisco) [28] which is an agreement between Belgium manufacturers and has no legal weight. The only criterion for chocolate to be called Belgian is that the complete process of mixing, refining and conching is carried out in Belgium. 'Switzerland', 'Swiss' or 'Suisse' chocolate are the trademarks registered by the Association of Swiss Chocolate Manufacturers (Chocosuisse) [29] which are used for products manufactured in the Switzerland under specific technical guidelines.

2. Authenticity issues

2.1. Identification of current authenticity issues

In ISO standard 2451:2017 and FCC Quality Rules[23] related to cocoa beans, the term adulteration is defined as the "alteration of the composition of a lot of cocoa by any means whatsoever"; a lot is defined as "quantity of cocoa beans in bags or in bulk established at any point in the cocoa supply chain and from which primary samples and/or incremental samples are to be drawn for quality analysis purposes". For whole beans, the possibilities of adulteration are rather limited, involving the presence or addition of foreign matter or of cocoa beans of poor quality (insufficient deshelling, defective beans). These issues can be quite easily recognized by simple visual inspection or other simple tests. On the other hand, the authentication of different complex products can become a highly demanding task. The most straightforward examples of product adulteration are inappropriate labelling, substitution of valued materials by cheaper ones, and the addition of undeclared or unauthorised materials/substances.

Among various cocoa materials, cocoa butter is considered the most important by-product of cocoa beans due to its unique physical and chemical characteristics and to its specific functional properties compared to other fats, such as brittleness at room temperature, fast melting at body temperature [30]. Since fat, especially its amount, is very important for the sensory properties of a product, cocoa butter may be "diluted" by other fats rather than used in lower amounts than declared. In addition to the economic reasons, such dilution may be motivated by certain technological advantages, such as increased stability [31]. Among various fats, cocoa butter equivalents (CBEs) are most suitable for mixing with cocoa butter in unlimited quantities due to

their similar physical and chemical properties [30]. As mentioned above, in the EU only six CBEs can be added in a limited amount and declared on the product label. Such addition has to be declared by an informative statement on the labelling, in addition to the listing among the ingredients. For other products, any presence of undeclared fat is considered adulteration. This is not limited to vegetable fats, since animal fats, lard or tallow, are used in the adulteration of cocoa butter, mostly in developing countries. This type of adulteration is also of religious concern [32]. Moreover, the quality of cocoa butter intended for the human consumption is strictly defined in various standards; any use of cocoa butter or cocoa fat of poor quality would be categorised as adulteration. For instance, a higher level of free fatty acids in cocoa butter indicates that cocoa beans or cocoa butter have not been handled properly (cocoa beans are diseased or damaged, stored or transported in poor conditions). Such cocoa butter can negatively affect a flavour as well as crystallisation properties (snap, melting properties). In both the EC Directive 2000/36 [21] and the Codex Alimentarius, a level of free fatty acids of 1.75 % is specified as the maximum amount. Unsaponifiable matter is another parameter that is often used to assess fat quality and purity. This includes all compounds that, after saponification, are insoluble in water but soluble in fat. These are those compounds frequently found dissolved in fats and oils that cannot be saponified by the usual caustic treatment but are soluble in ordinary fat and oil solvents. They are mainly various natural components of fat (e.g. sterols, pigments, terpenic alcohols, higher aliphatic alcohols, hydrocarbons), the amount of which is characteristic for particular fat; however, unsaponifiables also include contaminants, such as mineral oil hydrocarbons, which come from transport materials, lubricants, fuels, exhaust fumes or debris from tyres [10,33].

Various cocoa and chocolate products may be adulterated by the use of improperly processed cocoa beans, which contain higher amounts of cocoa shell or germ, or other plant materials than those that occur naturally, or have higher moisture content. Interestingly, not all standards or legislation include the limitation of cocoa shell content; nevertheless, Codex limits the shell content in cocoa mass and cocoa cake to a maximum of 5 % by weigh calculated on the fat-free dry matter. Additional dilution of cocoa and cocoa products may be achieved by the intentional admixture of starch, flour, dextrins or various powdered materials, such as peanut shells, chestnut shells, soybean meal, sesame meal, carob and non-fermented cocoa beans [34].

Since chocolate is a more complex product, possibilities of its adulteration are increasing. Although the presence of some undeclared components (milk, peanuts, and nuts) may be unintentional, other ones may be added to reduce production costs or to increase the palatability of the product. The latter, for instance, may be achieved by the addition of milk. Milk fat not only influences the sensory properties (taste, softer texture) of products, its addition significantly improves resistance to bloom [35]. Improved stability, thus appearance and attractiveness, can be also achieved by the admixture of some foreign vegetable fats into cocoa butter [30,36]. In addition to the alteration of fat content or composition, the content of non-fat cocoa solids may decrease, resulting in a value which is non-compliant either with the legislative limit or, when summed together with cocoa butter, with total dry cocoa solids on product label. The lower cocoa content can then be easily adjusted by increasing the major and cheapest chocolate ingredient, sugar or any of the materials mentioned above.

In the EU [21], the addition of up to 40 % of other edible substances to chocolate brings the possibility of decreasing the mass of cocoa needed for the production of chocolate without affecting the cocoa percentage on the labelling (this value, and also the minimum requirement for cocoa butter and dry non-fat cocoa solids, are calculated after deducting the weight of such substances). If these substances are not clearly visible and do form a homogenous matter with the chocolate, consumers may not be aware of the difference between such a product and a common

chocolate with an identical cocoa percentage on the label. Examples of products which benefit from the rule of calculating cocoa solids for the label are those with the addition of dietary fibre (e.g. inulin, used also as an alternative to sucrose as a sweetener to develop sugar reduced chocolate products) [37].

Particularly vulnerable to adulteration are the various specialty (or premium) cocoa and chocolate products, which are characterised by a higher market price. Recently, the rapid increase in consumer demand for such products has opened a new, very lucrative area for fraudulent practices. Consumers have become especially interested in premium chocolates with a variety of exotic ingredients, chocolates made from single-origin cocoa beans, such as those from Ghana, Ecuador or Venezuela, products that have declaration of 'fine and flavour' cocoa content, or products with a certain type of certification (e.g. Fairtrade, UTZ, organic), or production (e.g. raw, with reduced sugar, glycaemic index or cariogenicity) [38]. Geographical Indications (GIs), first introduced in the EU, are also increasingly used as a marketing tool to differentiate agri-food products, including cocoa beans, in the globalised marketplace. While the majority of origin-specific products are produced in EU countries, some of the developing countries where the cocoa beans are produced have also successfully implemented GIs and origin-based and quality differentiation strategies. Examples from the international cocoa markets for 'fine and flavour' cocoa, vulnerable to fraudulent practices, include Arriba from Ecuador, and Chuao from Venezuela [39,40].

2.2. Potential threat to public health

The adulteration of cocoa products is generally not a serious threat to public health. Most of the fraudulent practices involve the addition of edible ingredients that are safe for human consumption. However, for a small part of the population, the undeclared presence of allergens, such as nuts, peanuts, soya or milk, might be highly dangerous.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

As for all raw materials and foods, sampling is the first critical step to obtain the results that reflect the composition of a whole product. Representative sampling of cocoa and chocolate products is described in several standard operating procedures as well as in ISO 2292:2017, ISO 2451:2017, FCC Sampling Rules or the Cocoa Merchants' Association of America (CMAA) guidelines (for cocoa beans), ISO 5555 (for animal and vegetable fats and oils), and AOAC 970.20 or International Confectionery Association (ICA, formerly International Office of Cocoa, Chocolate and Sugar Confectionery, IOCCC) Analytical Method 3 (for cocoa and chocolate products).

For raw cocoa beans, various procedures are described in FCC Quality Rules, ISO 2451 or the FDA Macroanalytical Procedures Manual V-4. Physical testing is fundamental involving the assessment of bean count (size), sievings (extraneous matter), cut test (slaty and defective beans) and visual examination for the presence of defective beans (e.g. mouldy, slaty, insect-damaged, germinated), insects or any extraneous material. Additionally, beans are assessed for the presence of off-flavours by a panel of assessors (ICA Analytical Method 44) as well as for free fatty acids (alkalimetry, ICA Analytical Method 44) and moisture (oven drying, ICA Analytical Method 43).

Other attributes considered for the authenticity testing of cocoa and chocolate products are related to their essential composition and quality factors [41]. For most cocoa and chocolate products, analytical methods are then related to the two main components of cocoa beans: cocoa butter, and non-fat cocoa solids.

3.1.1. Cocoa butter content

To determine cocoa butter content, the first step is the analysis of total fat content (acid hydrolysis followed by Soxhlet extraction and gravimetry of the obtained fat). For this task, standard procedures have been adopted by AOAC (963.15-1973) or ICA (14-1972). However, fats other than cocoa butter can be present, thereby overestimating such results. It is therefore necessary to investigate the actual composition of the fat. Such methods have moved on since the EU permitted the use of CBEs in chocolate in 2003. The most reliable approach is to investigate triacylglycerol (TAG) profiles. In this chapter, TAGs are labeled according to their fatty acid composition, e.g. POS being 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol. Fatty acid abbreviations are: P – palmitic acid, O – oleic acid, S – stearic acid, B – butyric acid.

The pioneering work in this field was carried out, independently by Padley and Timms [42] and by Fincke [43], back in 1980 [44]. They reported that cocoa butter has a characteristic TAG composition with a linear relationship between the content of C50 and C54 TAGs ('CB-line'), and any deviation from that line caused by a higher C50 content means that the tested sample contains other fats. However, since 2003, the need for the reliable quantification of CBEs has led to more comprehensive studies. Based on the work of Buchgraber et al. [45,46], a method for the detection and quantification of CBEs in chocolate was introduced in international standards ISO 23275:2006, and ISO 11053:2009 for milk chocolate. Similar procedures are described in American Oil Chemists' Society (AOCS) Official Methods Ce 11-05 (2005) and Ce 11a-07 (2007). According to these standards, the TAG profiles (POP, POS, SOS, POO, SOO) determined by gas chromatography with a flame ionisation detector (GC-FID) provide the basis for calculating the CBE content. In milk chocolate, in addition to the main cocoa TAGs, PSB has to be determined (by the same method) to correct for any interferences caused by the considerable POP content of milk fat.

3.1.2. Non-fat cocoa solids

The most widely accepted approach for estimating dry non-fat cocoa solids content is to calculate it from the content of the cocoa alkaloids, theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine). Although the natural levels of these compounds in cocoa can vary slightly according to cocoa variety, geographical origin, soil factors, climatic conditions, cultivation or post-harvest technology including processing [47], this approach has been routinely used for decades and has been standardized in AOAC 980.14. Since the alkaloid content is influenced by the factors listed above, the main issue has been the inconsistent value of the conversion factor to be used in the calculation for the content of dry non-fat cocoa solids. In 2012, Richards and Wailes presented the results of a large project analysing almost 200 cocoa liquor samples collected from various geographical areas over a two-year period [48]. Based on their data, they suggested new conversion factors of 40.7 and 36.1 for calculations using theobromine and the sum of theobromine and caffeine, respectively, with confidence intervals of ±1.7 % (p=0.95). Caffeine and theobromine content can be determined by various methods. In the most common approach, extraction by hot water (or by hot water with the addition of an acid/alkali) is followed by liquid chromatography (LC) separation and UV detection [47,48]. Different approaches, such as titrimetric, spectrophotometric or gravimetric, are mostly no longer applied, although the gravimetric method AOAC 931.05 (non-fat cacao mass of chocolate liquor) is still listed in Codex.

For chocolate, another basic parameter is total dry cocoa solids content, which is then calculated as a sum of cocoa butter content and non-fat cocoa solids content.

3.1.3. Free fatty acids content and unsaponifiable matter

For the analysis of pure cocoa butter, there are two additional parameters to be analysed: free fatty acids (acidity) and unsaponifiable matter. Acidity is determined by titrimetry (alkalimetry) and expressed as the content of oleic acid in the sample (in %). A closely related and frequently used term is acid value, for which the results of the identical procedure are just expressed differently, as milligrams of KOH necessary to neutralize 1 g of sample. Standard procedures to determine the acidity and acid value are described in IUPAC 2.201 (1987), ISO 660:2009, ICA Analytical Method 42, AOCS Ca 5a-40 (acidity) and AOCS Cd 3d-63 (acid value). Another parameter is unsaponifiable matter, whose quantification method is standardised in ICA Analytical Method 23, IUPAC 2.401 (1987), AOAC 933.08 and AOCS Ca 6b-53.

3.1.4. Other authenticity parameters

Other common methods are the same as those being performed for many foods with slight modifications. For moisture content, titration using Karl Fischer method is standardised in ICA Analytical Method 26, AOAC 977.04 and AOAC 977.10 (cacao bean and its products/confectionary coatings, cocoa bean and its products/milk chocolate); and a gravimetric method is described in AOAC 931.04 (cacao products) or ICA Analytical Method 1 or 43.

Although the amount of cocoa shells present in cocoa products is not specified by most standards, there are standardised methods for the determination of this 'foreign' material. The so-called "blue value" is analysed according to ICA Analytical Method 29. The approach recommended in Codex is described in AOAC 968.10 and 970.23.

In milk chocolate, the procedure for protein (fat-free milk solids) determination by the Kjeldahl method is described in AOAC 939.02.

Starch in cocoa mass, cocoa and cocoa products, being considered as a fibre-related substance in fibre-rich products, or as a substance enabling a reduction in the mass of cocoa needed for the production of chocolate, can be determined using method AOAC 920.84, Section 12.043.

For chocolate and sugar confectionery products where various syrups (e.g. agave or date) are used as sweeteners or where no sucrose content is declared among the list of ingredients, methods for the determination of glucose, fructose and sucrose by means of enzymes (e.g. IOCCC 33:1989) or by high performance liquid chromatography (e.g. IOCCC 34:1989) can be used to assess a product authenticity.

3.2. Other commonly used methods

For many of the standard methods mentioned in the previous section, various alternative procedures have been proposed.

3.2.1. Substitution with non-cocoa fats or shell content

To assess fat composition (cocoa butter purity), the approach that has been described in chapter 3.1 can be applied for TAG profiles obtained by different techniques. M. Buchgraber et al. [49] and C. Simoneau et al. [50] showed a good suitability of non-aqueous reversed-phase LC (NARP-LC) for

TAG determination by comparing with GC-FID. Other techniques that have been reported as appropriate for this task are MALDI-TOF-MS (Matrix Assisted Laser Desorption/Ionisation coupled with mass spectrometry) [51,52], silver ion LC-MS [53] and Fourier transform infrared spectroscopy (FTIR); the latter for the quantification of lard content [32] or other vegetable fats in cocoa butter [54]. The potential of different analytical approaches for the reliable quantification of foreign fats in real samples in food control laboratories is still being investigated. Indeed, such quantification is complicated due to a high variability in cocoa butter composition (caused for example by differences in geographical origin and processing) as well as by the variability in admixed fats.

To determine cocoa shell content, the standard photometric method has been criticised for its low sensitivity and selectivity. Alternatively, an HPLC-FLD method for the determination of fatty acid tryptamides (behenic acid tryptamide, lignoceric acid tryptamide) as indicators for cocoa shell was first described by Münch and Schieberle in 1999 [55] and in 2000 published as optimised for routine analyses. Using a high number of various cocoa products (cocoa nibs, cocoa shells, cocoa liquors, cocoa powders, cocoa butters, cocoa pods), the use of this method was further evaluated by Janßen and Matissek [56]. Another detection technique, GC-FID, has been shown to be suitable for these indicators [57], thereby making this approach applicable in a wide range of laboratories.

Colorimetry and photoacoustic spectroscopy have been reported as a suitable tool for the determination of non-fat cocoa solids in dark chocolates [58], thermogravimetry for the characterization of milk and dark chocolates [19,59].

3.2.2. Geographical or botanical origin

Other methods focus particularly on geographical or botanical origin of the cocoa beans, and on the influence of processing on product composition and its characteristic markers. Typical features of a product and verification of its authenticity are then influenced by the intrinsic characteristics of cocoa and cocoa-based products and the complex technological process, making the whole analytical strategy challenging. Various analytical approaches can be applied for this purpose using specific markers and appropriate analytical techniques.

To assess botanical origin, geographical origin and brand of dark chocolates, volatile organic compounds composition responsible for characteristic aroma, analysed by GC-MS [60] and HS-PTR-MS (head space-proton transfer reaction-mass spectrometry [61] followed by chemometrics for further discrimination the samples can be used.

Other approaches using stable isotope composition by IRMS (isotope ratio mass spectrometry) [62], ^{1}H-NMR (proton nuclear magnetic spectroscopy) [63] and multielemental composition by ICP-MS (inductively coupled plasma-mass spectrometry) [64] combined with chemometrics can be applied to differentiate production areas or cocoa beans.

NIR (Near Infrared) spectroscopy has been also reported as a potential analytical method to classify different varieties and predict the chemical composition of cocoa [65]. It can be also used to detect cocoa adulterated with carob flour [66].

The analysis of cocoa proteins and oligopeptide profiles in beans from various geographic origins by UHPLC-ESI-QTOF (ultra-high performance liquid chromatography coupled with electrospray ionisation-quadrupole-time of flight-mass spectrometry) [61], GCxGC-FID [67], UPLC-ESI-MS (ultra-performance liquid chromatography-electrospray ionisation-mass spectrometry) [68] or different cocoa hybrids by MALDI-TOF-MS [69] allows the assessment of differences with respect to cocoa origin as well as its fermentation status (non-fermented vs. fermented), respectively.

Finally, a modern analytical approach, metabolomics, is focused on obtaining very comprehensive information about the samples by either non-targeted fingerprinting or targeted profiling [70]. For this purpose, various instruments are used, such as MS or NMR, and a large amount of generated data are processed using sophisticated statistical methods. Metabolomics employing LC-TOF-MS coupled with a partial least squares discriminant analysis model and phenolic compounds as biomarkers for construction of the predictive model can be applied for the discrimination of cocoa beans based on their geographical origins for effective quality assurance [71]. Profiling based on bioactive compounds present in cocoa beans, such as proanthocyanidins, represented by flavanols and procyanidins, and attributed to the antioxidation activity of cocoa, can be also performed by LC-FLD (AOAC 2012.24).

3.2.3. Sensory analyses

Sensory analysis is a possible option for the assessment of cocoa product quality. Although it does not provide information on product authenticity, it is the only method related directly to human experience with the product. When using a well-trained panel, sensory analysis can be applied to assess the quality of premium products (with high cocoa content where non-chocolate substitutes are inadequate) or specialty products (sucrose-free, containing various fillings etc.). In addition, assessing the changes in sensory attributes (e.g. flavour by volatile compounds) [72] during cocoa beans processing and chocolate manufacturing can help in the monitoring of technological processes.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Physical testing	Physical properties of cocoa beans	Substitution of high quality raw material, cocoa beans
Sensory analysis	Off-flavour, aroma profile	Substitution of high quality raw material, cocoa beans
Gravimetry (acid hydrolysis followed by Soxhlet extraction)	Total fat in all cocoa products	Decreased fat content: substitution of cocoa butter with non-fat components
GC-FID	Triacylglycerols	Cocoa butter equivalents in cocoa butter or chocolate
Titrimetry (alkalimetry)	Total free fatty acids	Substitution of cocoa butter
GC-FID	Fatty acids	Substitution of cocoa butter
GC-FID, MALDI-TOF-MS, NARP-LC-MS, silver ion LC-MS, FTIR	Fat composition	Substitution of cocoa butter
LC-UV, LC-MS	Non-fat cocoa solids in cocoa products based on theobromine and caffeine content	Substitution (lower content of cocoa solids)
Gravimetry (saponification followed by extraction)	Unsaponifiable matter	Substitution
Enzymatic (diastase)	Starch	Substitution
LC-FLD, GC-FID	Behenic acid tryptamide, lignoceric acid tryptamide	Substitution (cocoa shell / shell processing contamination in cocoa products)
GC-MS, HS-PTR-MS	Volatile organic compounds	Geographical or botanical origin Substitution (mono-variety/region products)
IRMS, ^{1}H-NMR	Stable isotope composition	Geographical or botanical origin Substitution (mono-variety/region products)
ICP-MS	Trace elements	Geographical or botanical origin Substitution (mono-variety/region products)
LC-ESI-Q-TOF, GCxGC-FID, LC-ESI-MS, MALDI-TOF-MS	Proteins and oligopeptides profiles	Geographical or botanical origin Substitution (mono-variety/region products)
NIR	Spectral information	Geographical or botanical origin Substitution (mono-variety/region products)
LC-MS	Phenolic compounds	Geographical or botanical origin Substitution (mono-variety/region products)

5. Conclusion

Food fraud in the cocoa beans processing sector is influenced by the increasing price of cocoa as a consequence of the production size, the influence of weather conditions, pests or diseases and a higher demand for cocoa. In addition to social and ethical aspects for less developed countries where cocoa beans are primarily produced, government support for the production and certification of premium products (e.g. geographical indication) must be sustained for this sector to continue developing.

The main challenge to the authentication of cocoa beans and cocoa-based products is the inherent compositional variability due to differences in variety, geographical origin, and also processing techniques.

Nowadays, non-targeted analysis (fingerprinting) to assess the authenticity of a suspicious sample by comparing it to an authentic one is a novel approach that is particularly useful in differentiating geographical origin, genotype and production technology by using fingerprints of cocoa and cocoa-based products obtained by various analytical approaches, such as LC-MS, GC-MS, proteomic, peptidomic, elemental, and combined with appropriate chemometric tools. However, the availability of well-designed, specific and extensive compositional databases, reference materials and reliable, validated analytical protocols are needed.

Due to the large number of different fats, especially artificially prepared mixtures, which can be used as alternatives to cocoa butter, it is difficult to assess fat composition based on only a few physical or chemical parameters. Moreover, the use of several analytical procedures to assess the quality of cocoa butter is time consuming. Thus, lipidomic fingerprinting appears to be an interesting approach for the future.

Analytical methods enabling the determination of various parameters according to the legislation within a single analytical run are desirable, with the aim of increasing sample throughput in control labs. Supercritical fluid chromatography (SFC), coupled with UV detection, refractometric detection (RID), evaporative light scattering detection (ELSD), FID or mass spectrometric (MS) detection for fat composition, saccharides profile and purine alkaloids content might be a solution.

Instead of conventional analytical methods, the application of ‘omic’ technologies also represents new trends for the future. Plants produce a considerable amount of chemically diverse metabolites. Differences observed in the composition of metabolites of a particular species or cultivar of the plant are determined by various genetic and environmental factors. They cause small variations, such as within one fruit tree, between fruit from the marginal and inner parts of the crown, as well as medium or larger differences (due to different soil types and other climatic conditions given by the region). Geographic origin has a significant influence on metabolite composition and is an important attribute for determining the quality and price of many foods including cocoa-based products. The natural variability of metabolites thus provides reliable information on the origin and authenticity of food. Another important factor for the quality of food of plant origin are the conditions of harvesting and storage of crops. Even after the harvest, intense metabolic processes are underway, and plant materials can be degraded by a misuse. Other changes can occur during cocoa beans processing. Metabolomics might be a useful tool for finding the conditions that will be optimal for maintaining quality.

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Plant-derived sugars and sweeteners

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General overview of the products

The products covered in this chapter are all plant extracts that contain varying levels of sugars. The sugar contents vary from very low in the tree waters {saps} (*ca* 0.5 to 3 %) to more typical values seen in concentrated sugar syrups like liquid sucrose, invert, glucose or high fructose corn syrups (*ca* 65 to 70 %) that are found in maple, birch, coconut blossom and agave syrups. Bulk sucrose can also be prepared from two sources either cane or beet.

Over the last few years, due to all the bad publicity about consumption of sugary beverages, there has been a drive to produce low sugar beverages using natural extracts. This has led to the increased popularity of vegetable juices, fruit and vegetable juice blends and of course in the increased sales of coconut water. All these beverages typically have sugar contents between *ca* 4 to 7 %, which is still considered high by some consumers and has led to an interest in "tree" waters which have been a recent addition to the beverage market.

These products typically have much lower sugar contents being less than 3 % but are supposed to contain useful levels of amino acids, minerals and vitamins. However, the actual levels quoted on nutritional labels are below the values that would give any significant effect and no formal claims could be made in the EU. It will be interesting to see if there is a prolonged interest in this latter class of products, but one thing is sure if there any issues with authenticity in this area it would have a very detrimental effect on the sector.

As in many aspects of food there has been a consumer drive to what they consider more "natural" sweeteners and away from refined sugar and particularly high fructose corn syrup, due to all of the adverse publicity there has been around this product and its possible link to increased rates of obesity, whether the link is true or not. This has led to more interest in other sugar sources and there has been a steady growth in maple syrup production in Canada over the last five years. There has also been considerable interest in syrups produced from agave as an alternative sweetener.

Canada is the major source of maple syrup with a production of *ca* ten million US gallons (*ca* 38 million litres) in 2014. Quebec province is by far the largest producer within Canada and this province accounts for some 80 to 90 % of the country's production. Figure 1 shows the seasonal changes in production volumes of maple syrup products (syrup and taffy) from Quebec over the period 1997 to 2014 [1]. As would be expected with a natural product yields vary from one year to the next, due to weather conditions in the spring and during the previous year's autumn when the starch is being laid down for next year's early season growth. However, there has been a general increase in production volumes, which has been achieved by an increase in "tapped" acreage and

https://doi.org/10.32741/fihb.9.sugar

also the addition of more “taps” to the recent plantings, which has become possible as these new trees mature and this allows more taps to be added to each tree.

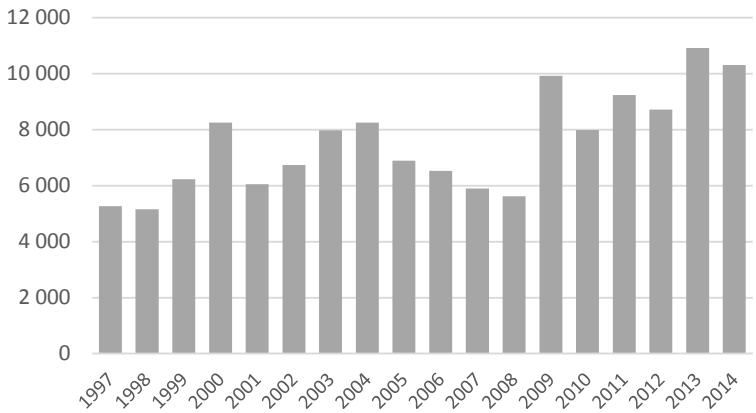

Figure 1: Maple syrup production data for Quebec province in 1 000’s of US gallons from 1997 to 2014

1. Product Identity

1.1. Definition of the product and manufacturing process

The products covered in this chapter are all sugar containing extracts produced from plants. For the purpose of this chapter they will be divided into a number of classes.

“**Tree” waters:** The products discussed here are produced from the sap collected from a tree in the early spring. The liquid is subjected to little additional treatment; apart from processes to limit microbiological changes e.g. ultrafiltration and/or pasteurisation. Two products will be considered here which are extracted from maple or birch trees. There are other “tree” waters like bamboo, but this will not be covered here due to a lack of background information on the product. However, the authenticity issues identified for maple and birch waters may also be applicable to this material.

“Tree” syrups: These products are also produced from tree saps but are concentrated by simple heat or using a pre-concentration step {such as ultrafiltration or freeze concentration} followed by heating to remove most of the water. The most common of these tree syrups is derived from maple sap and the syrup typically has a Brix (sugar) content around 66 %. The former is often used as a topping for pancakes and waffles for instance, whereas birch syrup is often used more in savoury dishes.

Another type of “tree” syrup is extracted from coconut flower spikes. Unlike the true tree syrups, such as maple, this material is actually extracted from the flower spike from which the coconuts themselves will develop later. Once the sap is collected the water is again removed from the sap by heating to give either a syrup (*ca* 65 %) or coconut sugar if the Brix is increased to a higher level that promotes crystallisation of the sugars on cooling.

Agave syrups: These products are syrups derived from a range of succulents of the "agave" family (e.g. *americana*, *tequilana and salmiana*). These syrups are produced by the extraction of the storage carbohydrate (inulin) from the agave pinas and its subsequent hydrolysis to liberate a fructose rich syrup which is then concentrated to *ca* 70 Brix.

Plant derived sugars: Bulk sucrose can be prepared from two sources either cane or beet. The former is produced in hot climates like Brazil and the Caribbean whereas beet sucrose is produced in more temperate climates such as Northern Europe, USA, Canada, Russia and Turkey.

1.2. Current standards of identity or related legislation

Many food products are defined by a set of specifications which may be regulated or may be found in industry sector guidelines. These are often used as the basis for highlighting deviating composition or fraudulent practices.

1.2.1. Tree waters

Although maple syrup has been produced for centuries, originally being exploited by the indigenous inhabitants of the North American continent, the sale of "maple water" is a relatively new product addition.

At present there are no National or International standards for tree waters. Although the defining criteria used in the Canadian standard for maple syrup [2] could be applied to maple water, with the exception of the concentration factor. "Maple water" is the sap extracted from the trees of the Acer family and nothing should be added in the way of colours, acids, antifoam agents or preservatives. There is one industry initiative in Canada (NAPSI) to control the quality and authenticity of maple water, which is run by maple syrup producers of Quebec [3]. This standard defines minimum quality standards and involves both inspections of production facilities to ensure that they comply with the standard and analysis of the final product to ensure that production batches meets the industry's quality criteria. At present there are no industry initiatives to define birch water.

1.2.2. Maple syrup

This is a product that most people will be aware of; it is a syrupy brown product with a pleasant sweet and a rather unique taste.

Unlike the tree waters, there are both Canadian [2] and US [4] standards that define "maple syrup". There are also some regional standards that have been developed by the producer provinces in Canada and in US States such as Vermont [5]. Unlike the US standard, that allows the addition of certain additives that may enhance the taste (salt), microbiological stability (preservatives) or processability (anti-foaming agents) of the product, the Canadian and Vermont standards prohibit the addition of these additives and the syrup has to be 100 % derived from maple sap.

As would be expected the two National standards are basically very similar but the US standard allows more flexibility [4], as described above, where some additives are allowed but would have to be detailed in the ingredients list, when added. There are no worldwide (Codex) or European (EU) standards for maple syrup.

By convention, US and Canadian Standards, the minimum Brix that is acceptable for this product is 66 %. Unlike Birch syrup the majority of the Brix in maple syrup is sucrose (98 to 99 %) with only very low levels (< 1 %) of glucose and fructose being detected.

1.2.3. Coconut blossom (flower) syrup and sugar

As with maple syrup, to produce coconut blossom (flower) syrup the tree or this case the palm has to be tapped to allow extraction of the sap. However, unlike maple and birch syrups, the palm (*Cocos nucifera*) itself is not tapped but a cut is made in the flower spike that will eventually carry the coconuts, hence the term blossom or flower syrup/sugar (see Figure 2). It is typically produced in coastal areas of the Indian and Pacific Oceans, with the major suppliers in Thailand, Indonesia, and the Philippines.

Although there is no International standard (Codex) for coconut flower syrup/sugar, the Philippine Government has prepared a standard for coconut flower sugar [6], but not the syrup. The standard details how the product is produced and contains a few physiochemical properties of the sugar like: typical sugar levels (sucrose, glucose and fructose), water activity, ash content and some visual, taste and odour characteristics. It also lays down maximum levels for a range of micro-organisms including the pathogens: *Salmonella*, *E. coli* and coliforms. It also provides the criteria by which the quality of the product can be judged (premium, class I and class II), from the colour of the sugar and the product's water activity. Although this is a Philippine standard, it seems to be fairly well accepted as a quality standard in a number of countries in Asia.

Figure 2: Palm flower spike being cut prior to collection of sap for coconut sugar production

1.2.4. Palm syrup/sugar

Similar products can also be extracted from palms around the Indian and Pacific oceans. In areas of Africa, Asia and New Guinea the palmyra palm (*Borassus* spp.) is generally used. In the Middle East and the Mediterranean region the date palm (*Phoenix dactylifera*) is generally used, whereas *P. sylvestris* is the more common source of date sugar in parts of Asia like Pakistan and India. Date

sugar is an additional product from this plant as most of these palms are grown for date production. The nipa palm (*Nypa fruticans*) is also commonly used for syrup production on the coastlines and tropical regions of the Indian and Pacific Oceans. It is the only palm that will tolerate high water levels, as seen in mangrove swamps, where often only its leaves and flowers are seen above the water level. Finally, the sugar palm (*Arenga pinnata*) can be used in the coastal and tropical regions of Asia, around China and Indonesia for syrup/sugar production. The sugars are all derived from a tap on the flower spikes of these plants and are analogous to coconut blossom syrup/sugar. There is no standard for these products.

1.2.5. Agave syrup

Although the syrup from *Agave salmiana* can be produced in a similar manner to that employed in palm saps, it can also be processed as given for *Agave tequilana.* In the former case as the agave plant matures it producers a stork in the centre of the plant and if this is truncated the sap will flow slowly into the remaining hole and can be collected daily. The liquid is heated to breakdown the complex carbohydrates to form simple sugars fructose, glucose and small amounts of sucrose.

To produce premium tequilas, *Agave tequilana* should be used as the only sugar source, likewise for premium Mezcal products, only sugars extracted from *Agave salmiana* should be employed.

However, when it comes to agave syrup production there is more flexibility allowed in the selection of permitted species that may be used to produce the syrup. Here it is much less defined and in fact many related species are used here due to:

a) the high price of *Agave tequilana* and *Agave salmiana* pinas,

b) lack of supply for these two types of pinas due to competition from spirit producers

Pinas, as shown in Figure 3, from many different agave species are used in syrup production to reduce costs.

Figure 3: Agave "pinas" after leaf stripping showing relative size (ca 40 to 60 kg)

The finalised agave syrup will be a clear pale yellow/yellow product with a Brix of *ca* 70 %. The majority of the soluble solids of these products are the simple sugars, fructose and glucose, which are the major components of inulin. Typically, the fructose to glucose ratio is around 10.0 in these materials. Sucrose and other disaccharides are also present in the syrup, but at low concentrations, with variable levels of the polyols mannitol and inositol are also seen.

Although there is no International standard for agave syrup, Mexico published a non-binding standard (NMX) for this material in 2008 [7] as a preliminary step towards the introduction of a binding/mandatory norm (NOM), which was adopted in 2016 [8]. Since then, any agave syrup exported from Mexico has to comply with this standard.

1.2.6. Bulk sucrose production

Although there is a Codex [9] international standard for different qualities of sugar, e.g. white, soft white, soft brown, dextrose, glucose syrup and raw cane sugar and one for the EU [10], these only define some basic physiochemical parameters that allows the product to be placed in the correct category. These standards do not differentiate between white sugar, sucrose, prepared from beet or cane.

2. Authenticity issues

2.1. Identification of current authenticity issues

These products, along with most fruit juices and honey, are sold on the basis of their sugar content (Brix). This presents an opportunity for an unscrupulous supplier to adulterate their products as there are always other cheaper sugar sources than can be used as a substitute for the sugar materials extracted from the plant (tree, palm or succulent). This substitution has been found to occur from time to time and some examples of these will be detailed here. Presented in Figure 4 are typical prices for the adulterants that have been used to extend these types of products together with the costs for the authentic materials (where prices for bulk purchases are available).

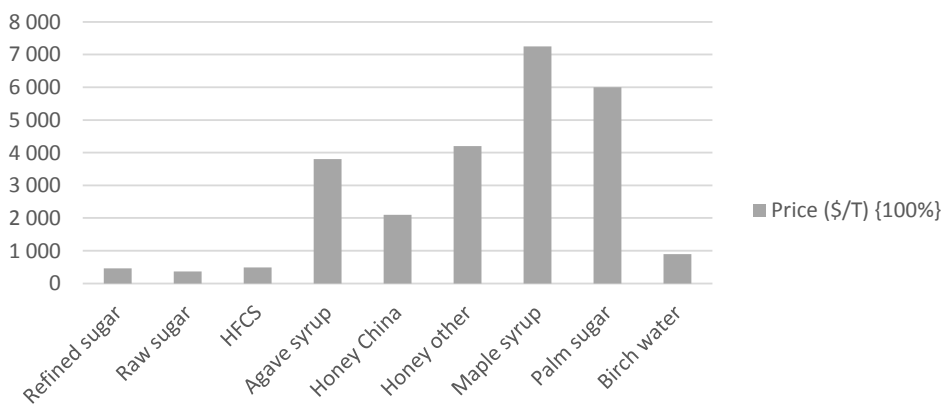

Figure 4: Nominal prices for "real" products and potential adulterants in 2016

Due to the high difference in price between the potential adulterant and the authentic vegetable sugars (a ratio of 1 to 10), the relative additional profit that an unscrupulous supplier can make by extending the authentic material by 20 % with an appropriate type of sugar is clear so there is a significant driving force if someone wants to cheat.

The typical materials that have been used to extend these products are cane and/or beet sucrose, high fructose syrups from starch (corn and/or rice), invert syrups from cane or beet and unrefined sugar.

The material selected to carry out the adulteration has depended on:

a) availability of another cheaper sugar source,

b) the knowledge and sophistication of the adulterator,

c) surveillance operations that have taken place on this product.

The substitution of part or all of maple syrup with cheaper materials is an issue that occurs from time to time. Recently the maple syrup producers of nine US producer states have petitioned the FDA to look at a number of products that appear to them as misrepresenting their contents [7,8]. Under US law only products containing a minimum of 10 % maple syrup can use the term “Maple” in their name. The FDA subsequently published a “note to consumers” to advise them to study the label of products carefully so they were not misled [11].

There have also been other cases in the US where products containing very little maple syrup present themselves in the manner in which they are marketed as if they contain a higher level of maple syrup [12]. In a study published in 2010 the authors [13] looked at reported cases of adulterations of food from 1980 to 2010 and found that there were 16 cases of the adulteration of maple syrup over that period, which represented *ca* 2 % of all of the examples they had examined.

Around a similar time, there was a small-scale study carried out in the UK on palm sugar. This identified that there were samples of this sugar on sale in the UK which were misrepresented as they were blends of palm sugar and cane/corn sugars. Here $\delta^{13}C$ values were shown to be in the region of -13 to -15 ‰ [14], which clearly indicated that the majority of the sugar was actually derived from cane/corn rather than being isolated from the palm.

One reason for the publication of the Mexican standard for agave syrup was the relatively high number of cases of syrup detected in both Europe and the US that clearly showed the presence of sugars derived from other sources (cane and corn) rather than being fully derived from agave [15].

The authors are unaware of any published examples of the sale of adulterated maple or birch waters but there is certainly a risk that these products could be extended by unscrupulous suppliers.

2.2. Potential threat to public health

So far the extension of any of the products covered in this chapter have not presented a risk to health, as their adulteration has involved the substitution of the soluble solids extracted from the appropriate plant with other “food grade” sugar materials extracted from another plant, but which had a lower cost. However, this does not preclude that the adulteration of these products in the future will always present no risk to health.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Major sugars composition

One way to assess if these products look "normal" is to determine the levels of the simple sugars (sucrose, glucose and fructose) they contain. In some cases, this can be very useful, for instance if high levels of glucose and fructose relative to sucrose are detected in a maple syrup/sap or palm flower sugar/syrup. This should instantly arouse suspicion that either invert syrup or a high fructose corn syrup has been used to extend (dilute) the product.

There are many ways that the sugars can be determined either using an ICUMSA method (International Commission for Uniform Methods of Sugar Analysis) [16] or an AOAC procedure [17]. Most of the methods are based on some form of chromatography using either refractive index or electrochemical detection procedures. The chromatography is either based on an amino bonded column using water/acetonitrile as a solvent or a mixed bed resin, such as a Dionex PA-1, using aqueous sodium hydroxide as an eluent. Some of the papers mentioned above for the analysis of these materials have involved the analysis of sugars using gas chromatography and flame ionisation detection (GC-FID). This procedure involves freeze drying and derivatisation of the sugars, which is time consuming but is still a possibility, prior to the separation of the derivatives on a GC column (typically a non-polar e.g. DB5 or similar) [18].

However, if an unscrupulous producer "knows" what they are doing they will carefully select the correct "type" of sugar to extend their products, which means that using a simple sugars analysis will not allow the detection of the adulteration. Therefore other methods have to be employed.

3.1.2. Isotopic analysis

One topic that is of significance to help control the authenticity of these products is the use of isotopic methods. These methods rely on the measurement of the small differences in the levels of the stable isotopes ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$) seen in a product. This allows the detection of the economic adulteration of these products by the addition of exogenous sugars.

In nature there are three different pathways that plants fix carbon dioxide from the atmosphere and these give rise to slightly different levels of the carbon isotopes seen in the sugars and other components of the plant.

1) **C_3 (photosynthetic) pathway:** Most plants use this pathway to fix carbon dioxide from the atmosphere. Here there is a large difference in the rate of reaction of $^{13}CO_2$ and $^{12}CO_2$ during fixation, which means less of the heavy form of carbon, is incorporated into the plant. This gives rise to carbon isotope ratios ($\delta^{13}C$) in the region of -25 ‰ (parts per thousand) for the sugars and acids extracted from these plants. Most fruits and all tree saps fit in this class.
2) **C_4 (Hatch Slack) pathway:** Plants that use this pathway show a smaller isotope effect as the rate limiting step is not the actual fixation of the gas from the atmosphere, which it is in the C_3 route. This means that the sugar and acids in these plants show a higher level of the heavy carbon isotope and so show less negative values for their isotope ratio ($\delta^{13}C$). Products in this category have $\delta^{13}C$ values around -10 to -11 ‰. Plants of interest here are cane and corn, which are often one of the major adulterants used to extend sugar rich products like juices and the products covered in this chapter.

3) **CAM (Crassulacean Acid Metabolism) pathway:** The final route is used by a range of specialist plants that have to limit water loss during the day. This means that they shut their stomata, holes in the underside of their leaves, through which they absorb carbon dioxide during the day and so it is fixed at night. In these plants the isotope ratio ($\delta^{13}C$) is typically between -12 and -15 ‰. Plants in this category are succulents mainly, e.g. pineapples and agave.

Plants also accumulate different levels of hydrogen ($^{2}H/^{1}H$) and oxygen isotopes ($^{18}O/^{16}O$) dependant on climatic and geographic conditions. The use of ($^{2}H/^{1}H$) sometimes allows the differentiation of natural sugars from exogenous sugars, derived from a C_3 source (beet/rice), that have been added to extend a product. The hydrogen isotopic pair together with oxygen isotopes has also been used to help differentiate between not from concentrate (NFC) and from concentrate fruit juices (FC).

All tree and palm saps are extracted from plants that use the C_3 pathway and so show $\delta^{13}C$ *ca* -25 ‰. Therefore the use of carbon isotopic analysis allows the addition of cane sucrose, cane invert and corn-based syrups (glucose and HFCS) to be detected when it is added to maple syrup/sap/sugar and coconut flower sugar/syrup at a level of *ca* 10 % and higher. This sort of detection level is lower than would be possible when using just conventional sugar analysis which has to allow for the natural variation in the glucose and fructose levels seen in these products. However, as agave is a succulent it uses the CAM pathway to fix carbon dioxide and means that standard carbon isotopic analysis is not sensitive enough to differentiate between 100 % agave syrups and materials adulterated with corn syrups.

There are two methods that can be applied here that have been validated on maple syrup. The first of these, AOAC 984.23 [19] {"whole sample method"}. This uses simple combustion of the sample to carbon dioxide which is then analysed by isotope ratio mass spectrometry. Although the AOAC method uses an off-line combustion of the sample and then introduction of the carbon dioxide produced into the mass spectrometer, a continuous flow system is now normally used where each sample is combusted in turn and the liberated carbon dioxide is directly fed into an isotope ratio mass spectrometer (IRMS) by a stream of carrier gas (N_2) (EA-IRMS).

The second method {"ethanol method"}, AOAC 2004.01, uses a similar detection method but involves the initial fermentation of the sugars into ethanol (EtOH), which is then recovered by careful distillation using a spinning band column. The ethanol is then subjected to ^{13}C isotopic analysis using an IRMS machine [20]. The two methods have similar sensitivities, with a detection limit in the region of a 10 % addition of a C_4-derived sugar but give rise to very different $\delta^{13}C$ values. This difference arises from the loss of some carbon atoms, as carbon dioxide, during the fermentation step, which means that it is critical that the correct "judgement criteria" are used to assess a sample e.g. the judgement criteria must be ones developed using the same analysis procedure e.g. "whole sample" or "ethanol" procedure.

The sensitivity of the ^{13}C IRMS method for the detection of added C_4 sugar to maple syrup/sugar can be improved by using malic acid as an internal standard [21]. Here the $\delta^{13}C$ values detected for the sugars and the malic acid isolated from the sample are compared. A similar improvement in sensitivity of the detection of added C_4 sugars to palm sugar has been published by Kelly [22]. This uses a similar approach to that given in the "internal standard" method published by AOAC (998.12) [23]. Here the protein contained in the sample is precipitated and washed to remove any bound sugars. After drying this material is then subjected to combustion and the $\delta^{13}C$ ratio is determined using IRMS. This value is then compared with the $\delta^{13}C$ value obtained on the "sugar" portion or on the whole sample, with no pre-treatment. These two values should be close to each.

If the percentage of C_4 sugars calculated using equation (1), as defined in the AOAC method, is larger than 7 % then the sample can be considered as adulterated with a C_4 sugar source.

$$C_4\ sugars\ \% = \frac{\delta^{13}C_p - \delta^{13}C_s}{\delta^{13}C_p - (-9.7)} \times\ 100 \qquad (1)$$

$\delta^{13}C_P$ = ^{13}C ratio seen in "protein" fraction, $\delta^{13}C_S$ = ^{13}C ratio seen in the sugar

The addition of cane sugar or HFCS to agave syrup (CAM) cannot easily be detected by ^{13}C-IRMS as the three plants share similar global δ^{13}C values around -11 to -12 ‰. This means that other routes must be applied to detect this type of adulteration, which will be discussed in Section 3.2.

If a C_4 sugar (sucrose or cane invert), is added to a tree sap derived material, the C_4 derived sugars are concentrated in one of the components (either sucrose or glucose and fructose respectively) and this causes a disturbance in the carbon isotope ratios and means that the detection level is roughly halved over the global EA-IRMS approach. In work carried out using liquid chromatography linked with elemental analysis and isotope ratio mass spectrometry LC-EA-IRMS on honey [24] and agave syrups it has been reported that the carbon isotope ratios for glucose and fructose were much closer, within 0.5 ‰ for agave syrups, than seen in fruit juices, suggesting that this route offers a method to detect the adulteration of agave syrups with C_4 derived sugars. However due to the complexity and the low implementation of this hyphenated technique in food control laboratories, even if the intra-lab uncertainty of this technique is similar to the current EA-IRMS technique, the inter-lab uncertainty is still higher at the date of this publication.

The methods discussed above only address the addition of C_4 sugars to products derived from tree saps (maple, birch and palm products {syrup/sugar/sap}). However, if the unscrupulous supplier uses beet sucrose, which is derived from a C_3 plant, there will be no derivation in the δ^{13}C isotope ratio as seen with cane sucrose. Here detecting this type of adulteration requires a different method.

The level of deuterium at the methyl site $(D/H)_1$ of the EtOH, produced during the fermentation of the sugars in the maple syrup, has been found to be very useful. The analysis is carried out using Deuterium-Nuclear Magnetic Resonance (^{2}H-NMR) spectroscopy. The EtOH is again recovered from the fermentation broth by careful distillation using a spinning band column as mentioned above for ^{13}C measurements. In fact, if when employing this SNIF-NMR® method the EtOH-IRMS method is also used, it is possible to detect added exogenous sugars derived from both C_3 and C_4 sources. This is described for maple syrup in the AOAC Official method 2000.19 [25]. By combining ^{2}H-SNIF-NMR® and IRMS on the ethanol probe, it is therefore possible to detect addition of both beet and cane sugars/syrup in C_3 syrups such as maple as illustrated in Figure 5 below.

Although not normally used in this sense, the ^{13}C-IRMS and SNIF-NMR® methods can be used to determine whether sucrose is correctly described as "cane" or "beet". In the former case for pure cane sucrose the δ^{13}C value would be *ca* -11 ‰ and the $(D/H)_1$ value would be around 110 ppm, whereas if sucrose derived from beet was added much lower δ^{13}C and $(D/H)_1$ values would be detected. Conversely with beet sucrose, if cane sucrose had been added to the product it would show a less negative δ^{13}C value and the $(D/H)_1$ value would be higher. These two isotopic methods are the only two procedures that can be used to differentiate these two products. Similarly with starch derived syrups for which the corn or rice source can be differentiated from their δ^{13}C values.

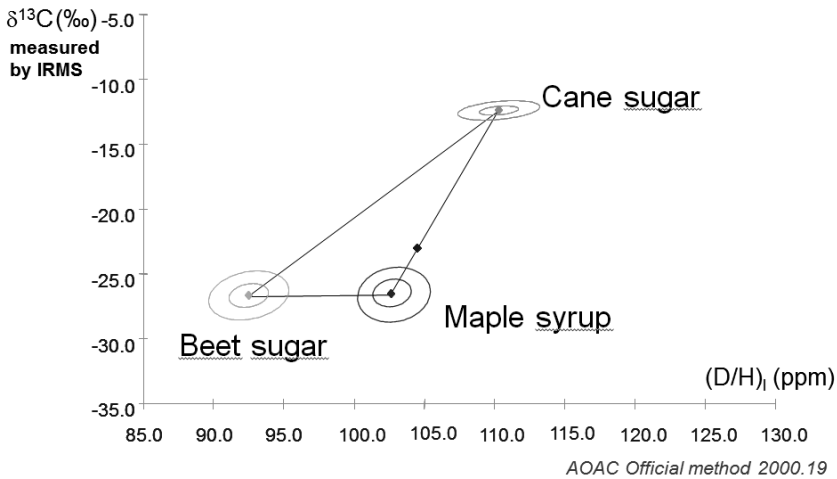

Figure 5: SNIF-NMR® plot for detection of C_3 and C_4 sugars to maple syrup

3.1.3. Mineral profile

Other components in the products can be measured such as minerals (K, Mg, Ca and Na) but the natural variation for these components can be rather large so unless there is a gross adulteration it will probably not be picked up using these components. Meanwhile it can be an additional analytical tool when comparing a suspect sample to a "witness" sample.

3.1.4. Minor sugars and sugar-alcohol profile

The Mexican standard for agave [7,8] defines a maximum level for sucrose/difructose and ranges for fructose, glucose and mannitol which are measured using HPLC. High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD) can be used to measure the levels of mannitol and inositol in agave syrups to detect dilution from the addition of other sugar materials [15,26] using a Dionex MA-1 column.

Ideally a range of methods should be applied to a sample to ensure that there is the best chance of detecting the addition of any exogenous sugars.

3.2. Other commonly used methods

3.2.1. Sugar profile by Cap-GC

One approach that was not mentioned above but has proved very useful at detecting the addition of high fructose syrups derived from starch and cane/beet invert syrups is the use of Cap-GC. Here the syrups show marker peaks that are not seen in the sugars/syrups/saps and allow their detection at relatively low levels of addition (2 to 5 %). This is often more sensitive than any other procedure. The method was originally developed by Pr. Low of the University of Saskatchewan [27] to detect the addition of these same adulterants to fruit juices. It was subsequently extended to detect the addition of high fructose syrups derived from inulin to apple juice [28]. The procedure is detailed, with judgement criteria, in the International Fruit Juice Union's recommendation # 4 [29].

Here the sugar/syrup/sap is freeze-dried to remove the water. The sugars are then derivatised using pyridine/trimethylsilylimidazole mixture (4:1) with heating. The samples are then injected on to a non-polar Capillary-GC column (DB 5) and the peaks are detected using a flame ionisation detector (FID).

Although high fructose syrups from starch (e.g. corn and rice) do not make good adulterants for maple or coconut flower syrups/sugar — as their addition will reduce the sucrose level while also increasing the levels of glucose and fructose — they have been used to extend these types of products because of their low price and large availability. If this type of material is added to maple syrup it is detected by the presence of maltose and isomaltose, as shown in Figure 6.

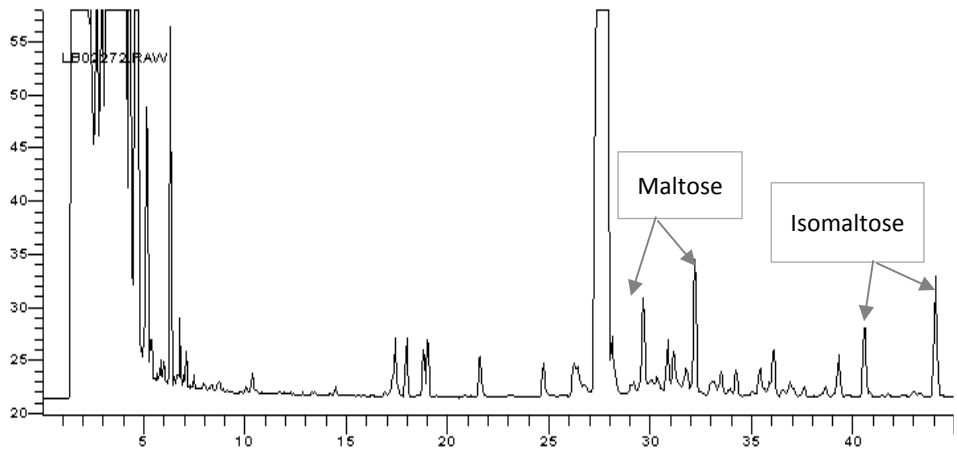

Figure 6: Cap-GC profile for maple syrup adulterated with HFCS

Here an addition of HFCS at a level of around 2 % is often detectable, which is much better than can be achieved using HPLC, to look at the simple sugar levels, or by ^{13}C-IRMS. The Cap-GC procedure can also detect the presence of invert syrup when added to maple syrup/sugar and coconut flower syrup/sugar. Again there are two peaks that show the presence of the exogenous sugars in the product.

The Cap-GC method can also be used to analyse agave syrups for the addition of HFCS. Unlike the case of maple syrup, HFCS makes a “better” extender for agave syrup as they:

a) Are rich in fructose, similar to agave syrups,

b) Share a similar global δ^{13}C value to that of agave as they are produced from a CAM plant.

Both features make them hard to detect by other analytical procedures.

When adulterated with HFCS, two signals corresponding to isomaltose appear in a flat zone, which are not present in typical Cap-GC profile for agave syrup (see Figure 7).

HPAEC-PAD can also be used to look for the presence of exogenous gluco-oligosaccharides that maybe present if a starch derived syrup is added to these syrups [26]. This uses a different column, with a lower retentivity (Dionex PA-100), to that used for the mannitol/inositol quantification discussed above. This method can be complementary to the Cap-GC method discussed above but looks at a different set of compounds.

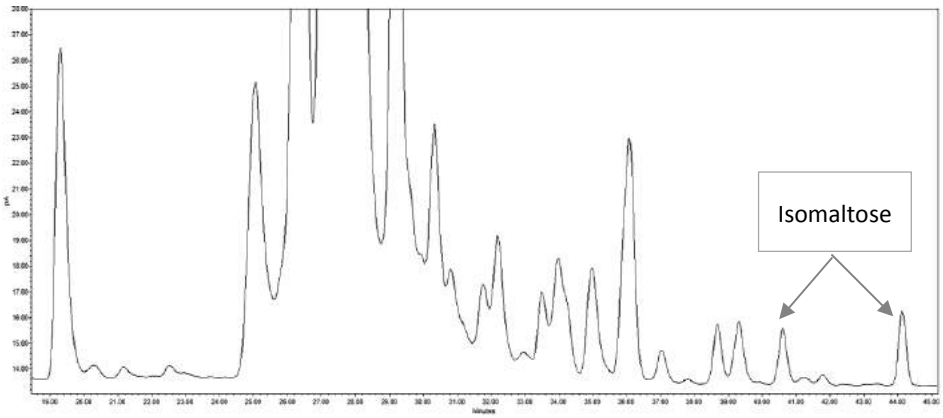

Figure 7: Portion of a Cap-GC profile for agave syrup adulterated with HFCS

Although the Cap-GC method offers one of the fastest and most sensitive routes to detect sugar syrup addition to these types of products, it will not detect the addition of either cane or beet sucrose to maple or coconut flower syrup. The former maybe detectable using ^{13}C-IRMS, if the addition level is high enough, whereas beet sucrose addition can similarly be detected using SNIF-NMR®. This reinforces the need to use more than one method in screening samples to ensure any adulterations are detected.

3.2.2. Carbon 13 site specific natural isotopic fractionation

Another approach that has proved very useful to detect extension of agave syrups with cane and/or corn derived sugars is quantitative ^{13}C-NMR (^{13}C-SNIF-NMR) [30]. Once again, the sugars are fermented into alcohol and recovered by careful distillation as per the SNIF-NMR® procedure. However, instead of using deuterium as a nucleus, carbon is used here, and the relative proportions of ^{13}C at the CH_3 and CH_2 sites allows cane or corn syrups to be detected in agave. Although the global ^{13}C-IRMS method does not show a significant difference between cane/corn & agave derived sugars, it has been found that the relative levels of ^{13}C at the methyl and methylene sites of the EtOH show different levels of the heavy carbon isotope. This means that if ^{13}C-NMR is used on the EtOH from these sources they can be differentiated, which is illustrated in Figure 8.

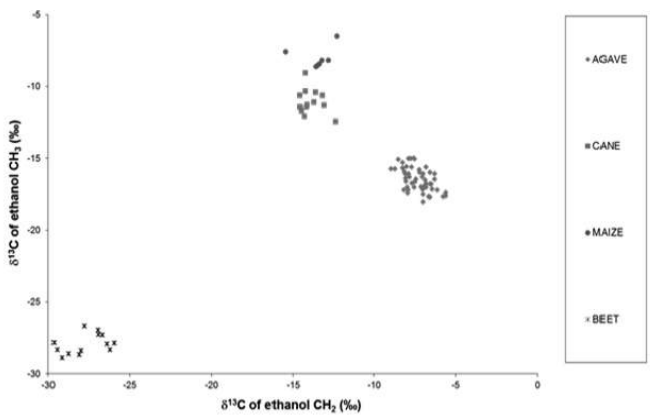

Figure 8: Plot of ^{13}C site-specific values of ethanol detected using ^{13}C-SNIF-NMR for agave and adulterants [30]

The detection limit for this technique to detect the presence of cane/corn sugars in agave syrup is around 15 %. This is much higher than is possible, in some cases, using Cap-GC for HFCS, however, as mentioned above cane invert is only readily detectable using this approach. Cane sucrose addition to agave could be detected by elevated levels of sucrose, which are relatively low in agave syrups. However, some agave syrups naturally contain low, but significant levels of, inulobiose (1-O-β-D-fructofuranosyl-D-fructose) which may be seen to elute very close to sucrose on many chromatographic systems and caution needs to be taken in interpretation these results.

3.2.3. Organic acids screening

Some problems have been encountered with the addition of both citric and ascorbic acids to coconut water, which is another low acid product. Therefore some screening for organic acids in these products should be considered. Malic acid is the major acid seen in many of these products and there is an old validated procedure for this acid at AOAC (# 959.13) for checking the levels of malic in maple syrup, which would be better detected using a more modern HPLC method. This can be used to check for malic and citric acid in one run. Citric acid is often added to low acid products to “improve” their microbiological stability, by lowering the pH, and thus making it less susceptible to the growth of pathogenic micro-organisms. Although not validated on any of the matrices in this chapter, the HPLC-UV method (AOAC # 986.13) [31], would be suitable for malic and citric acids. However, different dilutions might be required to measure concentrations of both acids in these products.

3.2.4. Enzymatic methods

Although not commonly used in North America, there are several enzymatic methods for the quantification of both sugars and acids, which have been validated in Europe [32–39] for the analysis of fruit juices. These procedures would also be suitable for the analysis of the matrices covered in this chapter although they are not officially validated on any of these materials. The enzymatic methods offer one advantage in the analysis of malic acid as there are two different procedures for the determination of the two optical forms (L and D) of the acid. It was found in the past that often racemic D, L-malic acid was added to fruit juices, which could be detected using the D-malic assay [39]. If significant levels of this D-malic acid are detected the product is clearly adulterated.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
HPLC profile	Relative concentrations of simple sugars and sugar-alcohol (sucrose, glucose, fructose and mannitol*)	Detection of added sugar by distortion of sugar proportions & ratios
Atomic absorption spectroscopy or inductively coupled spectroscopy {MS or OES}	Concentrations of minerals (K, Mg, Ca, Mn and Zn)	Dilution of sugar syrup
HPLC-UV	Organic acids	Detection of the addition of citric acid and quantification of malic acid
Enzyme assay for D-malic acid	Organic acid, including optical active forms	Detection of the addition of racemic (D, L)-malic acid
Isotope ratio mass spectrometry (EA-IRMS)	Bulk $\delta^{13}C$ value	Addition of C_4 derived sugars to products[$]
Isotope ratio mass spectrometry (EA-IRMS, LC-IRMS)	Sugar and malic acid $\delta^{13}C$ values	Addition of C_4 derived sugars to products (refined procedure)[$]
Isotope ratio mass spectrometry (EA-IRMS, LC-IRMS)	Sugar and protein $\delta^{13}C$ values	Addition of C_4 derived sugars to products (refined procedure)[#]
Quantitative deuterium nuclear magnetic spectroscopy (^{2}H-SNIF-NMR)	Deuterium level at CH_3 site of EtOH (liberated by fermentation of sugars)	Detection of the addition of exogenous C_3 derived sugars
Quantitative carbon 13-nuclear magnetic spectroscopy (^{13}C-SNIF-NMR)®	Relative levels of ^{13}C isotope at CH_3 and CH_2 positions of EtOH (liberated by fermentation of sugars)	Detection of the addition of added cane/corn sugars to agave syrup[£]
High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD)	Presence of gluco-oligosaccharides	Addition of starch derived syrups
High pH anion exchange chromatography linked with electrochemical detection (HPAEC-PAD)	Levels of mannitol and inositol	Detection of dilution of agave syrup
Capillary Gas-Chromatography (Cap-GC)	Presence of marker disaccharides for sugar syrups	Addition of exogenous sugar syrups (from starch (glucose and HFS) & invert syrup)

* Levels are defined in Mexican Standard for agave syrups. $ excludes agave syrup. £ not applicable to maple, birch and coconut flower syrups/sugars/saps. # applicable to coconut flower sugar

5. Conclusion

The main component of all the products covered in this chapter is sugar. Unfortunately as there are always cheaper sources of sugar that can be used by unscrupulous producers to extend these materials there is a sizeable driving force to "cheat" and make an elicit profit. The route chosen by an unscrupulous producer will depend on the product in question, availability of adulterant and their skill/experience. Most of these products are mainly sucrose and so the use of cane or beet sucrose could be expected.

However, this is not always the case and HFCS is a material which is often chosen instead. This addition will be detectable in maple and coconut flower syrups by distortion of the sugar profile, presence of unusual oligosaccharides by Cap-GC and HPAEC-PAD and dilution of other components plus a shift in the $\delta^{13}C$ value.

Birch sap/syrup on the other hand shows low levels of sucrose and roughly equal levels of glucose and fructose, so addition of HFCS to this type of material will show little changes in the sugar levels. However, the addition of this type of material will still be detectable by IRMS & Cap-GC analysis.

Detection of beet and cane sucrose addition to maple sugar/syrup/sap should be detectable by ^{2}H-SNIF-NMR®. However, due to the much wider distribution pattern for the production of birch sap/syrup/sugar this may make the use of SNIF-NMR less sensitive for this product.

As agave is a CAM plant its $\delta^{13}C$ value seen in the sugars of the product are very different from the other products but are similar to sugars derived from cane/corn using the global method. The presence of HFCS in agave is detectable by Cap-GC or by using isotopic methods ^{13}C-SNIF-NMR.

The origin of sucrose (cane/beet) and glucose syrups (corn/rice) can be achieved using ^{13}C-IRMS and/or SNIF-NMR®.

There are many methods that can be applied to these products, but isotopic methods generally provide one of the best opportunities to detect extension of these products with cheaper sugar sources.

About 5 or more years ago a new screening method was introduced for the analysis of fruit juices using ^{1}H-NMR [40]. This method will be discussed in more detail in the chapter on fruit juices. It has also been successfully applied to the analysis of honey to detect sugar additions and it is likely that the same procedure could be applicable to the analysis of agave, maple, birch and coconut flower sugar but this is a "work in progress" for these products.

As unscrupulous suppliers are always looking for new avenues by which they can to extend their products without being detected this will always present a challenge to the analyst. It may be that they will detect new ways to prepare syrups so that they will not carry the markers in use today to detect their addition to these sugar-based products.

As there is significant ecological pressure on suppliers to reduce the new planting of palm trees in Asia which are used for palm oil production, it is highly likely that this may also extend to palm flower sugar/syrup in the future if the popularity of this product grows. In Mexico there is a growing demand for agave pinas for syrup production. However, the supply of pinas is limited at present and there is pressure on prices and availability of the raw material for their production. The availability of these materials must be shared between both syrup and spirit producers (Tequila and Mezcal) and it is likely that raw material prices will remain high while syrup demand remains high/increases.

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Spices

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General overview of the product

Spices, seasonings, herbs are all products that are generally added to both fresh and processed food to enhance flavour. With documents showing trade in spices as early as 3000 BC, the spice trade itself can be considered as one of the earliest drivers of globalisation. More recently, the global spices and seasoning market has been valued at around USD 12.7 billion in 2012 and is expected to grow to about USD 16.6 billion by the end of 2019 (source: www.statista.com). Among the forces pushing up consumption in spices are the perceived health benefits. Spices contain plant-derived chemical compounds that have been shown to help prevent certain diseases. With concern among the general population about side effects of commonly used drugs, interest is shifting towards other forms of medicine such as Ayurvedic and Traditional Chinese medicines, which focus on the use of herbs and spices.

This chapter provides a detailed review of current legislation defining spices, the problems of fraud that the spice industry faces, and potential analytical methods to deal with authenticity issues. Because of its importance as one of the most expensive spices, saffron is dealt both in the general chapter on spices and in its own chapter, where more details on analytical methods for saffron authentication are given.

1. Product Identity

1.1. Definition of the product and manufacturing process

1.1.1. FAO

According to the FAO, spices can be defined as “vegetable products used for flavouring, seasoning and imparting aroma in foods”. Herbs, considered a subset of spices, are leafy spices, and some, like dill and coriander, can provide both spice seeds and leafy herbs [1].

1.1.2. Codex Alimentarius

Spices and Dried Aromatic Herbs are defined as dried components or mixtures of dried plants used in foods for flavouring, colouring, and imparting aroma [2]. This term equally applies to whole, broken, ground and blended forms.

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1.1.3. FDA (Code of Federal Regulations, 21CFR101.22)

The term “spice” is defined in the U.S. Code of Federal Regulations for specific labelling requirements (21 CFR Sec. 101.22 (2)) [3]. The term spice means any aromatic vegetable substance in the whole, broken, or ground form, except for those substances which have been traditionally regarded as foods, such as onions, garlic and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavouring principle has been removed. Spices include the spices listed in 182.10 and part 184 of this chapter, such as the following: allspice, anise, basil, bay leaves, caraway seed, cardamom, celery seed, chervil, cinnamon, cloves, coriander, cumin seed, dill seed, fennel seed, fenugreek, ginger, horseradish, mace, marjoram, mustard flour, nutmeg, oregano, paprika, parsley, pepper, black pepper, white pepper, red rosemary, saffron, sage, savory, star aniseed, tarragon, thyme, turmeric and saffron.

1.2. Current standards of identity or related legislation

In the General Food Law Regulation EC 178/2002 [4], the general principles and requirements of food law and procedures of food safety are outlined. With regard to the consumer’s interest, the General Food law aims to prevent, “fraudulent or deceptive practices, the adulteration of food, and any other practices which may mislead the consumer”.

The European Food Safety Authority (EFSA) was established legally in 2002 under the General Food Law, following a number of food crises in the late 1990s. EFSA provides scientific advice and communicates risks within the food chain.

In the United States, the FDA and the US Department of Agriculture (USDA) are the principle federal agencies working on food safety. Border protection and import authorities, as well as food safety, food defence and food quality authorities broadly look after food fraud across a number of federal agencies [5]. The primary food safety law administered by the FDA is the Federal Food, Drug and Cosmetic Act (FFDCA) [6]. This act tightened control over food, drugs, and consumer protection, and gave the government enforcement ability. The Food Safety Modernization Act [7] was then passed by US congress. This Act amended Section 415 of the FFDCA with the aim to prevent rather than respond to contamination and outbreaks.

Specific organisations have become involved in the protection of the herb and spice industry. The European Spice Association (ESA) is a non-profit organisation made up of national federations of the spice industry from the EU, Turkey and Switzerland. It has an aim to protect the industry and its members with regard to processing, packaging, quality assurance, food safety and marketing in the herb and spice industry. The American Spice Trade Association (ASTA) works similarly in the US, to ensure clean and safe spices, and enhance the industry and the business interests of its members. The ESA has a set maximum level of 2 % w/w extraneous matter in herbs and 1 % w/w maximum level in spices in the Quality Minima Document [8] whereas ASTA [9] has set a level of extraneous matter at 0.5-1 % w/w. One of the difficulties in keeping the herb and spice industry free from fraud is the issue of long industry supply chains that can exist over many countries.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Complex Supply Chains

Supply chains in the herb and spice industry tend to be long, complex and can pass through many countries. Such complexities present many opportunities for criminals to carry out EMA. The stages of the supply chain can include grower, collector, primary processor, local traders, secondary processor, exporter, importer, trader, processor / packager, food manufacturer / retailer / wholesaler, and finally the consumer. At any stage of this supply chain, a number of fraud opportunities can occur including misrepresentation, adulteration and substitution [10].

“Fraud control measures” can be implemented in companies to detect fraud opportunities or motivations that may occur either internally, or externally of the company [11]. The processing and manufacturing need to be carefully monitored to ensure food protection. Cleanliness and protection of the product from contamination and adulteration is vital. The cost of maintaining these standards can be high. The blending and packaging stage provides an early opportunity for adulteration and needs to be carefully monitored. In more modern processing plants, the product is often enclosed during this process. In addition, careful monitoring is required for the preparation of ready meals i.e. precooked meals, and other food products that have herbs and spices added to them towards the end of the supply chain.

The ESA Adulteration Awareness Document [12] advises companies on ways to prevent adulteration: 1. “Evaluation of the supply chain” (knowing the history of the supply chain, adherence to legal requirements, traceability, adherence to HACCP (Hazard Analysis and Critical Control Points) and adherence to accreditation standards), 2. “The nature of the material” (whole or ground, botanical species and commercial grade), 3. “Product testing” (there is a range of methods being developed for the rapid and accurate detection of fraud). It is important to have these precautions in place for both industry and the consumer, however, cases of adulteration continue to occur, and there may be useful lessons in reviewing old examples of adulteration.

2.1.2. Economically Motivated Adulteration

A large global industry such as the herb and spice sector is under constant threat from fraudsters. With valuable condiments such as saffron, oregano, vanilla, turmeric and paprika, substantial amounts of money can be made by carrying out adulteration of these products at the expense of the consumer and potentially the reputation of food businesses. The long, complex supply chains and the increase in crushed and ground herbs and spices provide excellent opportunities for EMA. However, other vulnerabilities that may affect the chances of adulteration include seasonality and availability of the crop, weather events, cultural and geo-political events, economic indicators, food safety laws, prevalence of corruption and advances in technology to mask fraud [10]. The 2016 garlic crop had potential to become vulnerable to adulteration following severe weather events of heavy rain and snow in late 2015, causing a surge in the price of garlic [13]. This surge in the price caused stockpiling of garlic. Circumstances such as these can all provide motivation for adulteration. Preventative measures can include; knowing product specification, supplier assurance, product type (ground and crushed and where did this process take place), knowing the supply market and being aware of vulnerabilities in the supply chain. Verification and testing can be carried out to confirm the preventative measures are effective. This can involve devising representative sampling and inspection programmes for products, a suitable testing strategy that

meets objectives, a test method in an accredited laboratory, and supply chain verification measures which may include pre-delivery of samples prior to purchase for approval, or evidence of authenticity from an accredited laboratory [10]. The prevention of fraud is not in detecting each individual fraud and controlling one type, but reducing the vulnerabilities, as the fraudsters are always evolving and looking for their next crime [14]. The herb and spice industry has been a victim of EMA on numerous occasions. Table 1 focuses on examples where substitution adulteration occurred with various herbs and spices.

Table 1: Examples of Substitution Adulteration in the Herb and Spice Industry

Ingredient	Adulterant	Reference
Chilli	Oil, rice flour, bran	[15]
	Ziziphus nummularia fruits	[16]
	Plant husks, rice powder, sawdust, stone powder	[17]
Oregano	Sumac, olive leaves	[18]
	Olive leaves, myrtle leaves	[19]
	Satureja montana L. and *Origanum majorana* L.	[20]
	Cistus incanus L., *Rubus caesius L*. and *Rhus coriaria* L	[21]
Cumin	Almond, peanut, tree nuts, peach and cherry	[22]
	Fennel seeds	[23]
	Peanut shell	[24]
Black pepper	Chilli	[25]
	Buckwheat or millet	[26]
	Papaya	[27]
Cinnamon	Coffee husk	[26]
Chinese star anise	Japanese star anise	[28]
Nutmeg	Coffee husks	[26]
Paprika	Almond	[29]
	White pepper, curcuma, barium sulphate, brick powder	[30]
	Defatted paprika	[26]
	Paprika of inferior quality substituting paprika from the Protected Designation of Origin (PDO) 'La Vera' region.	[31]
	Falsely declared Szegedi paprika substituted for *Szegedi Füszerpaprika* PDO	[32]
Saffron	Saffron of unknown origin labelled as being cultivated in the PDO region in Spain can be used for substitution.	[33]
	Beet, pomegranate fibres, dyed corn stigmas, red dyed silk fibres, safflower, marigold to red stigma	[34]
	Safflower, gardenia, meat fibres, gelatine fibres, curcuma, sandalwood, campeche wood powder, stigmas of other saffron types, flowers, starch, glucose	[35]
Turmeric	*Curcuma zedoaria, Curcuma malabarica*	[36]
	Chalk powder	[37]

The addition of colour to spices to improve their value is a common occurrence. Colour can influence the perception of food and stimulate appetite, therefore, increase the value of a product [38]. The addition of colourants to foodstuffs dates back to at least 1500 BCE, and up until the middle of the 19th century, ingredients such as the spice saffron was added for a decorative effect in certain foodstuffs [38]. Natural dyes were commonly used in food around this time, however, as the 1900s began, the use of synthetic dyes became the colouring of choice with ease of production, less expense and superior colouring ability [38].

As with other types of food adulteration, there is a likelihood that certain synthetic dyes may be a threat to public health, and historical records show that injuries and even death occurred following ingestion of toxic colourants [38]. Allergic and asthmatic reactions as well as DNA damage have also been reported [39]. Therefore, the use of most synthetic dyes is forbidden in Europe. The two main types of dyes that may be illegally added to food include azo dyes and triphenylmethanes [40]. Examples of these illegal azo dyes include Sudan I, II, III, IV, para red, orange II, methyl yellow and rhodamine B. Malachite green and its metabolite leucomalachite green are examples of triphenylmethane dyes considered genotoxic and/or carcinogenic.

In May 2003, Sudan 1 was found to be illegally present in chilli powder and foods containing chilli powder in the EU [40]. Following this event, in 2005 and 2006, numerous tests were carried out for the presence of illegal dyes by the UK Food Standards Agency (FSA) [41]. Regulatory legislation was put in place following the scandal, and member states were required to monitor high risk products and provide analytical reports for the presence or absence of Sudan dyes as an emergency measure in the European Commission Decision 2005/402/EC [42]. This legislation was later repealed in the European Commission Regulation (EC) No. 669/2009 [43] to a less intensive testing regime due to a reduction in the presence of Sudan dyes.

Legislation varies in different countries, which can cause problems for importers and exporters [41]. In the EU, Regulation (EC) No. 1333/2008 [44] on food additives was developed "…with a view to… ensuring a high level of protection of human health and a high level of consumer protection…." With regard to food colours, there are currently 25 natural, and 15 synthetic dyes on Annex II of this regulation that can be allowed in food [41]. The US FDA regulates food additives in the US. To indicate the variation between countries, three synthetic dyes approved in the US are not approved in the EU, and nine synthetic food colours in the EU are not approved in the US [41]. There is still a continued risk of adulteration with dyes in spices.

Table 2: Adulteration with dyes [45,46]

Spice	Adulteration
Red Pepper Chili powder	Sudan 1, Sudan 4, Metanil Yellow, Sudan 3, Oil Orange SS, Rhodamine B, Auramine O, Orange II, Dimethyl Yellow, Fast Garnet GBC, Malachite Green, Allura Red
Paprika powder	Sudan 1, Sudan 4, Acid Black 1, Orange II, Annatto
Turmeric powder	Sudan 1, Mentanil Yellow, Orange II, Lead Chromate
Sumac	Amaranth Red, Basic Red 46
Curry powder	Auramine O, Chrysoidin (Basic Orange II)
Saffron flower	Acid Orange II, Mentanil Yellow, Sudan I, Ponceau 4R, Ponceau 6R
Cayenne pepper	Crystal Violet
Five spice powder	Auramine O

The results in Table 2 summarises reported cases of adulteration of spices with dyes from 2013 to 2017 in the US. In this work the most common dyes reported were Sudan 1 and Sudan 4. These results indicate that adulteration with dyes is ongoing. Continued surveillance of spices to detect and prevent adulteration with dyes is vital to the herb and spice industry as well as the safety of consumers. Health risks can occur alongside both substitution and addition adulteration. They can cause more than an economic threat to the consumer.

2.2. Potential threat to public health

The main motivation for the addition to, or substitution of the authentic product is for economic reasons, however, with the cases outlined in Table 3, a number of health risks were a detrimental result of this criminal behaviour. There is an increasing concern over the introduction of hazards from food fraud. It is a constant and growing concern in the food industry, with greater actions needed to be put in place to detect it.

There are three types of food fraud risks that pose a threat to the public: 1. Direct: The consumer is put at immediate risk from a short-term exposure leading to acute toxicity or lethality, 2. Indirect: The consumer is put at risk over long-term exposure with potential chronic effects, 3. Technical: Food documentation may not be representative of the food content [47]. A serious example of a technical fraud risk could be an allergic reaction to an unknown product that has not been outlined in the label.

The detection of undeclared nut protein in cumin and paprika in 2015 was one case where adulteration did not result in just economic losses [22]. This crime had serious consequences for public health and strengthened the demand for food protection. With food allergies affecting approximately 3-4 % of the adult population, an estimated 0.6 % are allergic to peanut and 0.5 % allergic to tree nut [48]. All products that come into contact with nut protein need to be labelled accurately as the risk of an unsuspecting sensitive individual coming into contact with this can be fatal. In a study by Bock, Muñoz-Furlong, and Sampson [49], it was found that out of 32 fatal cases of anaphylaxis from 1994-1999, 94 % of the cases were caused by peanut or tree nuts, indicating that the vast majority of food induced anaphylaxis is caused by these foodstuffs. The adulteration of spices with nuts is a serious public health risk for susceptible individuals.

Table 3: Examples of Economically Motivated Adulteration with Possible Health Impact

Herb/Spice	Adulterant	Possible Health Impact	Reference	Food Fraud Risk
Cumin, Paprika	Nut protein	Anaphylaxis	[22,48]	Direct
Chinese star anise	Japanese star anise	Neurological and gastrointestinal problems	[28]	Direct
Black pepper	Papaya seeds	Liver and stomach problems	[27]	Direct
Turmeric	Yellow chalk powder	Face swelling, loss of appetite, nausea, and vomiting	[37]	Direct
	Curcuma zedoaria	Toxicity in rats and chickens	[51]	Direct
	Lead chromate	Delayed mental and physical development	[52]	Indirect
Cumin	Fennel seeds coated with marble dust and dye	Possible health risk from the use of dye and marble dust	[23]	Indirect
Oregano	Olive leaves	Presence of pesticides-Toxicity, carcinogenicity, mutagenicity	[53]	Indirect

Chinese star anise (*Illicium verum*) is infused in teas to relieve the symptoms of colic in children. The adulteration of Chinese star anise with Japanese star anise (*Illicium anisatum*) has in previous years resulted in the intoxication of children. Japanese star anise looks similar to Chinese star anise, and they are often even more difficult to distinguish as they can be sold in broken or ground form. Therefore, chemical analysis is required to distinguish them. Japanese star anise contains neurotoxins and can result in a child having neurological and gastrointestinal problems [28].

Papaya seeds have been used to adulterate and bulk black pepper. However, these papaya seeds can cause liver and stomach problems, and therefore pose a health risk to the unsuspecting consumer [27].

Turmeric can contain various adulterants that threaten public health. Yellow chalk powder has been used to add bulk to turmeric as it is a cheap material [37,50]. This adulterated product however can cause swelling of the face, loss of appetite, nausea and vomiting. *Curcuma zedoaria* can be used to adulterate turmeric [36], and was found to have toxic effects in rats and chickens by Latif et al. if not processed properly [51]. Lead chromate added to turmeric was used as a dye as well as a bulking powder. Over exposure to lead can cause delayed mental and physical development [52].

In a case reported in the Times of India [23], poor grade fennel seeds were coated with waste marble dust and dye, and mixed in with the cumin product. In this case, it was the treatment of the fraudulent product that caused the public health risk rather, than the fennel seeds themselves.

The use of other plant cuttings such as olive leaves in the adulteration of oregano [19] can also pose a health risk to the consumer. As these leaves are not produced for consumption, it is unknown how these cuttings may be treated. In the case of olive leaves in particular, evidence of pesticides can be found (Elliott, C- personal communication). Pesticide residues pose a health risk, and hazards such as toxicity, carcinogenicity and mutagenicity are associated with them [53].

There are many possible risks with food adulteration. Therefore, it is vital that there is adequate policing of the supply chains and the food industry to deter and try to prevent any fraud before it is too late. Illegal dyes are a constant threat to the international food industry and are found intermittently, as indicated by the alerts in Rapid Alert System for Food and Feed (RASFF) [54]. Examples from RASFF and the possible health impacts can be seen in Table 4.

Table 4: The Possible Health Impacts of Common Illegal Dyes

Common Illegal Dyes	Possible Health Impact	Examples of Spices
Sudan 1	Genotoxic and carcinogenic in rats	Cayenne pepper, Turmeric, Chilli, Paprika, Curry
Sudan 4	Potentially genotoxic and possibly carcinogenic	Curry, Turmeric, Chilli, Paprika, Sumac
Para Red	Potentially genotoxic and possibly carcinogenic	Chilli, Cayenne pepper, Paprika
Orange II	Potentially genotoxic, insufficient data on carcinogenicity	Chilli, Safflower, Sumac, Paprika
Methyl Yellow	Possibly carcinogenic to humans	Curry
Rhodamine B	Potentially genotoxic and potentially carcinogenic	Sumac, Chilli, Paprika, Turmeric, Curry

It is vital that authentication testing is carried out to detect cases of economic fraud and to verify that preventative measures are effectively in place [10]. This prevention not only maintains quality and consumer trust, but also helps to prevent the possibility of public health risk [55].

3. Analytical methods used to test for authenticity

Fast, reliable and competent analytical techniques are required to confirm the authenticity of food with this increasing trend of food adulteration [55]. According to the database records collected by Moore, Spink and Lipp [56], from 1980 to 2010, the top two methods used for detecting food adulteration were liquid-chromatography and infrared spectroscopy. Visual inspection and microscopy are common methods used to detect adulteration in herbs and spices as reported by the British Retail Consortium, the Food and Drink Federation, and the Seasoning and Spice Association in 'Guidance on Authenticity of Herbs and Spices' [10]. However, it requires highly trained analysts and analysis can take a long time, therefore research is continuously being carried out to develop new methods for the detection of adulteration in herbs and spices. Fraudsters tend to be one-step ahead of the food safety agencies but also, techniques for food adulteration are becoming more and more advanced [27]. Recent analytical methods for the detection of adulterants are listed in section 4.

3.1. DNA analysis

DNA analysis is increasingly being used in the fight against food fraud as advances in methods provide cheaper, more efficient and accurate means of detection of fraud. It can be seen from section 4 that DNA analysis plays an important role in the detection of substitution adulteration in herbs and spices. In recent years, Sequence Characterised Amplified Region – Polymerase Chain Reaction (SCAR-PCR) and DNA barcoding are becoming desirable methods for the detection of food adulteration.

SCAR-PCR is an advancement on the use of Random Amplified Polymorphic DNA (RAPD) markers in DNA analysis. RAPD analysis is considered a useful starting point as it has low operating cost and can distinguish between botanical varieties [57,58]. Although RAPD markers are a fast and cheap method, their downfall is that repeatability is low and exchanging results between laboratories creates difficulties [59]. This problem with RAPD markers was corrected with the development of SCAR primers and this increased specificity and reliability [60]. The use of SCAR-PCR was observed for the detection of bulking agents in saffron, where, the method screened large batches with a fast, reliable sensitive and low cost screening method [57]. The detection of adulteration of oregano with *Cistus incanus L.*, *Rubus caesius L.*, and *Rhus coriaria L.*, was carried out by Marieschi et al. using RAPD [58] and subsequently with SCAR-PCR [21] to improve the robustness of the method.

Other SCAR-PCR methods include the detection of olive leaves, *Satureja montana L.*, and *Origanum majoranan L.* in oregano [20,60], the presence of *Curcuma zeodoaria* / *Curcuma malabarica* in turmeric [36] and the presence of plant based materials in chilli [16]. The development of a SCAR and Internal Transcriber Spacer (ITS) region multiplex PCR method allowed the detection of both the adulterant safflower and the spice saffron in the one analysis [59]. It is evident that the use of SCAR-PCR has potential for EMA adulteration detection in a number of herbs and spices. SCAR-PCR is a sensitive method with detection limits at 1 % for the adulteration

of oregano with *Cistus incanus L.*, *Rubus caesius L.*, and *Rhus coriaria L.* [21], 1 % for the detection of olive leaves in oregano [61] and a limit of detection (LOD) of 10 g/kg for the presence of *Curcuma zeodoaria* / *Curcuma malabarica* in turmeric [36] indicate this. However, a limitation of SCAR-PCR is the need for sequence data for the PCR primers design [61].

DNA barcoding is a relatively new method that was first developed in 2003 and is based on the variability within a standard region of the genome, the 'DNA barcode' [62]. It has become increasingly used since its development, and there is successful evidence of this method in the detection of adulterants in herbs and spices. This method has been used for the detection of adulterants in saffron [63], and chilli adulteration in black pepper [25]. DNA barcoding is a fast, reliable sensitive method for a wide range of food commodities, and even strongly processed foods and there is also the possibility of building reference databases to improve the chances of it becoming a routine test for food quality, and traceability [64].

DNA purity and integrity are concerning with regard to DNA barcodes, which, can be a limitation of the test. Poor quality DNA may reduce amplification success of DNA barcodes [65]. DNA barcoding also relies on the availability of sequence libraries to reference against [66].

Whole genome sequencing is becoming a possibility and it has potential for the detection of food adulteration with Next Generation Sequencing (NGS). However, so far, little work in this area has been carried out with the complex work flow and high costs associated with this method [67].

The methods for the detection of adulteration in herbs and spices using DNA analysis described are qualitative. Quantitative methods often result in high measurement uncertainty, although advancements in PCR technologies are improving in this way [67]. Overall, the limitations with DNA analysis may include poor integrity and purity of the DNA, poor efficiency of the extraction, and the risk of contamination is a concern with these methods. Also, low level accidental contamination can be misinterpreted as intentional substitution.

3.2. Mass spectrometry

Mass Spectrometry (MS) is a powerful tool in the fight against food fraud, and in many industries, it is considered the gold standard technique. Methods include Gas Chromatography (GC-MS), Liquid Chromatography (LC-MS), Isotope Ratio (IR-MS) and Inductively Coupled Plasma (ICP-MS). Once a targeted method is developed, mass spectrometry can provide a highly specific and sensitive technique that can quantify known analytes to sub-µg concentrations [68]. Although an expensive technique that requires significant expertise and laboratory surroundings, it is highly regarded as a confirmatory technique.

In the study by Black et al., Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) was used as part of a two-tier approach to detect the presence of adulterants in oregano with LC-HRMS used as a confirmatory technique [19]. The analysis was untargeted, and with the use of Principal Component Analysis (PCA) and Orthogonal Partial Least Squares – Discriminant Analysis (OPLS-DA) chemometrics, biomarkers specific to the classes (oregano and various adulterants) were identified. The identification of such biomarkers allowed further developments in the detection of adulteration with targeted mass spectrometry [69]. Wielogorska et al. [69] used targeted FTIR (Fourier Transform Infrared) and LC-MS/MS to quantitatively detect adulteration in oregano. These studies [19,69] were an improvement on the work of Bononi and Tateo [70] as they identified biomarkers for a number of adulterants, as well the development of a quantitative method. In the work by Bononi and Tateo [70], a targeted method was developed for

the detection of a characteristic marker of olive leaves, the phenolic compound oleuropein, in both oregano and sage with the use of Liquid Chromatography-Electrospray Ionization Mass Spectrometry (LC-ESI-MS/MS). This compound oleuropein was later found to be also present in myrtle leaves by Wielogorska et al. [69]. Similarly, the use of untargeted Ultra High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UHPLC-HRMS) merged with chemometrics, OPLS-DA proved to be a successful powerful tool in determining products from the PDO of saffron [33]. Falsely declared saffron from a PDO can be used in substitution of the authentic product.

GC-MS is another method that has been used to detect possible adulterants such as with the study carried out in 2015 investigating detection methods for known fruit adulterants in fennel seed [71]. Essential oils of fennel seed and two adulterants were profiled, and distinct differences between fennel seed and two of its adulterants were observed. Bononi, Fiordalise and Tateo were able to use GC-MS to detect olive leaves in oregano and sage by using GC-MS with a detection limit of 1 % [72]. The benefits of this method included the ease of use and reproducibility of the results. However, with regard to the detection of adulteration in herbs and spices, an issue that may occur with the use of GC-MS is that, only the volatile oils are investigated. Therefore, the addition of volatile oils to a product may cheat the GC-MS adulteration detection method.

ICP-MS along with PCA and Canonical Discriminant Analysis (CDA) was a method developed to detect falsely declared Szegdi paprika (PDO) [32]. The Sr isotopic composition and the multi-elemental analysis are indicative of paprika from the region.

Upgrades in mass spectrometry involve the use of real time analysis of samples by directly introducing the samples to the mass spectrometer. Ambient mass spectrometry is a relatively new analytical technique that gives comparable results to conventional techniques without complex sample preparation [73]. Examples of its use include the detection of the adulterant Japanese star anise in Chinese star anise using Direct Analysis Real Time-High Resolution Mass Spectrometry (DART-HRMS) by detecting the presence of anisatin [74]. Advances on this method involves the use of direct plant spray combined with orbitrap-HRMS [75]. This method can detect between the neurotoxic Japanese star anise and the Chinese star anise in seconds, and without sample pre-treatment. DART ionisation has slightly higher selectivity, no solvents added and the absence of high voltages when compared to direct plant spray. The benefits of direct plant spray over DART ionisation include the low cost, lower standard deviations and simplicity. Direct plant spray and DART ionisation techniques are more successful qualitative methods than quantitative methods.

Currently the disadvantages of mass spectrometry in comparison to spectroscopy are the cost and the requirement of a laboratory setting and highly trained analysts. However, advances to overcome this are ongoing with aims to miniaturize the instrumentation, and for the data to be presented so that it is easily interpreted. However, these developments require further optimization and are not readily available [68]. Similarly to spectroscopy, the validation procedure for non-targeted methods in mass spectrometry has not been standardised. This can reduce consistency between laboratories.

3.3. Spectroscopy

Vibrational spectroscopies, along with chemometrics, have become well known as rapid, non-destructive, fingerprinting techniques and are valuable screening tools in the detection of adulteration / authentication in the food industry. A range of spectroscopic analytical techniques

used in the food industry include FTIR, Fourier Transform Near infrared (FT-NIR), Raman, Hyperspectral Imaging (HSI) [76] and Nuclear Magnetic Resonance (NMR) [77].

In the detection of adulteration of herbs and spices for economic gain, a number of spectroscopic methods continue to be developed. Work has been carried out to develop competent models to detect cornstarch in garlic powder by FTIR [76] and onion powder by FTIR and NIR [78]. Raman has also been used to detect cornstarch in onion powder and garlic or ginger powder [79,80]. Starch may be added to white powders such as garlic and onion powder to add bulk to the product. In these studies, a quantitative model was built using the algorithm Partial Least Squares Regression (PLSR) in chemometrics. The Raman, FTIR and NIR spectral data based models described here are capable of detecting adulteration in onion powder, garlic and ginger with starch up to 35 %.

In a study by Black et al. on the detection of adulteration in oregano, FTIR was used alongside the confirmatory technique LC-HRMS [19]. Following the identification of biomarkers for both oregano and its adulterants, and the development of spectroscopic classification models using the unsupervised PCA and supervised OPLS-DA chemometric algorithms, a rapid screening method and confirmatory method was developed. The benefit of this method was that a number of different adulterants could be added to the database that was used to build the model. The developed screening technique therefore was robust and could identify numerous adulterants at each screening in the survey that was subsequently carried out. The results of the survey indicated that adulteration was ongoing, but also, it displayed the use of a rapid screening technique to help the fight against food fraud. Further development on these analytical techniques was carried out with the development of targeted quantitative methods using FTIR with PLSR and LC-MS/MS for the detection of adulteration in oregano [69].

Raman and FTIR methods analyse the sample in the mid infrared region of the electromagnetic spectrum. The spectral data consist of sharp bands representing inelastic scattering, or information on the fundamental vibrations of the sample respectively. This is in comparison to the vibrational overtones and combination peaks of the NIR, which does not provide as much information [68]. However, in the detection of starch in onion powder, NIR with PLSR chemometric algorithm was determined the most suitable method [78]. NIR has the ability to penetrate deeper into the sample and therefore is more suitable for bulk samples that have little or no sample preparation. Raman has advantages over NIR and FTIR as it is not affected by water, and inorganic materials can be analysed more easily. Analysis through packaging or glass is also a possibility [79]. Recent improvements to Raman also include the use of Surface Enhanced Raman Scattering (SERS) and Spatially Offset Raman Spectroscopy (SORS) which has shown its ability to detect counterfeit products through packaging [68].

The use of Proton Nuclear Magnetic Resonance (^{1}H-NMR) combined with chemometrics (PCA, OPLS-DA, O2PLS-DA) was investigated and was proven successful at determining the quality and authenticity of saffron [81], allowing the detection of common adulterants such as Sudan dyes [82], other dyers mixed with stabilizing agents or bio-adulterants such as gardenia, safflower or curcuma [77] whose specific markers have been identified. Additionally to these targeted studies (use of markers), some untargeted approaches coupled to chemometrics were also developed to assess saffron authenticity and detect the presence of unexpected adulterants [83]. That sort of approach can be transferrable to other spices, given the availability of consequent authentic database. Quantitative metabolomics analysis were also performed to distinguish cinnamon varieties and showed encouraging results [84]. Others spices such as safflower [85] were also studied by NMR. ^{1}H-NMR was shown to give reproducible results rapidly, however, this technique requires solvent extraction and is then limited to extracted metabolites. Additionally only organic compounds are visible with this technique. Further work carried out using DRIFTS on FTIR

minimized the process of sample preparation and proved to be successful along with PLS-DA classification and quantitative PLSR models at detecting six known saffron adulterants [86].

Although these spectroscopy methods are often successful on their own, further developments are being made to improve the methods by:

1) Combining data: Wang et al. [87] carried out a study that improved FTIR and NIR results for the detection of the adulterant *Iuicium lanceolatum A.C. Smith* (ILACS) in Chinese star anise. This method involved combining the NIR and FTIR spectral data and the use of PCA and Linear Discriminant Analysis (LDA) chemometric techniques. Although the FTIR performed better than NIR in this study when analysed separately, the classification results from the combined approach proved to be even more successful.

2) Increasing sensitivity: Vermaak et al. [88] used hyperspectral imaging with PCA and PLS-DA to distinguish between the neurotoxic Japanese star anise and Chinese star anise. This emerging method incorporates spectroscopy and imaging to produce both spatial and spectral data from a sample [89] (Gowen, O'Donnell, Cullen, Downey and Frias, 2007). This method is also non-destructive and rapid with the added advantage that with the acquisition of several predictions on the sample, the statistics are better [88]. The quantification of adulterants, buckwheat or millet, in ground black pepper was carried out using FTIR and NIR with hyperspectral imaging with PLSR chemometrics. NIR with hyperspectral imaging was seen to produce the best calibrations which, in this case was largely to do with the larger sample area used with NIR, and the spatial information from the imaging system used with it [90]. Galaxy Scientific's Classical Least Squares (CLS)-based Advanced-ID algorithm has been developed to detect screening samples to a level as low as 0.01 % [91]. When it was used to detect paprika adulterants, it detected Sudan 1 dye at 0.1 %, tomato skin at 0.5 % and brick dust at 5 %.

3) Analysis through packaging: Terahertz spectroscopy was used to overcome the barrier of common packaging materials such as plastics and papers [37]. This method is a promising non-intrusive technique that was used for the detection of yellow chalk powder in turmeric.

It is apparent that further improvements and developments are ongoing with the use of spectroscopy. Developments seen in benchtop spectroscopic instruments are also being transferred to handheld devices. An added benefit as discussed by Ellis et al. [68] would be to use the advantages of the NIR and FTIR combined, and developed into a handheld device. Overall, the ability to transfer this technology to portable and handheld devices allows the user to determine authenticity in the field, and can focus on vulnerable points of the supply chain. This not only allows improvements in traceability and detection of fraud, but at a basic level, it can also act as a deterrent. If food fraud criminals are aware of this possibility, they may be less likely to take the risks of committing a crime in the first place.

Limitations of spectroscopy must not be overlooked. Spectroscopy is used as a rapid screening technique and therefore, further investigations may need to be carried out by confirmatory techniques that require more expertise, time and cost more, such as mass spectrometry. This is also true when building models using chemometrics, the purity of samples needs to be assured in order to build accurate models. Another limitation of spectroscopy, as a non-targeted method, is the lack of a standardised validation procedure for all laboratories.

Following a review of more than sixty scientific publications, it was found that spectroscopic techniques are the major analytical techniques used to determine adulteration of herbs and spices in high concentrations [92]. Overall, these techniques provide a good first point of control in the

fight against food fraud. Although the use of other confirmatory techniques such as mass spectrometry may be required in some circumstances, the bulk of screening herbs and spices for EMA are possible with spectroscopy.

Although not a spectroscopic technique, an analytical screening technique called the 'electronic nose', capable of detecting aroma fingerprints, was used alongside PCA and Artificial Neural Networks (ANN) to detect adulteration in saffron. This technique was found to be promising, as detection was possible at higher than 10 % adulteration, enough to detect EMA [34].

3.4. Combination of detection methods

In some circumstances, there is a need to use more than one technique to verify results. Along with the combination of methods already described by Black et al. [19], the combination of microscopy and GC-MS was also carried out for the detection of adulteration of fennel seeds [71]. Screening tests are often carried out with rapid techniques, but they have their limitations. In 2014, the USA recalled over 675 products due to the presence of undeclared nut protein in cumin. In a study carried out by Garber et al. [22], it reported failings in the antibody-assay based technologies involved in screening products for allergens. Although these methods are robust, and can detect as little as 1µg of allergen, they are not always specific to the allergen they are developed to detect. Therefore, with this analytical weakness, DNA and mass spectrometry-based tests are often used for further investigations. With the use of DNA and mass spectrometry analysis, additional allergens were detected; however, further work on the development of biomarkers for accurate analysis of a range of possible allergens may improve detection. This case indicates the limitations of screening methods with single analyte testing in some cases, and the need for multiple testing methods to understand the adulteration further.

3.5. Chemometrics

Chemometrics is used to improve the chemical data obtained from analytical instruments and to correlate the properties of samples with the use of mathematics and statistical methods [76]. Chemometrics has been used in the calibration analysis of spectroscopic and spectrometric data. It has been used with both targeted and untargeted methods to detect the presence of fraud in food or to determine authenticity [92]. The use of pre-processing is carried out in chemometrics to amplify desirable information from raw data and reduce the effects of undesirable information in the spectra. There are three key stages in the use of chemometrics, data pre-processing, development of a robust model, and the validation of a model and the analysis of results. Two commonly used pre-processing techniques include scatter correction methods, and spectral derivatives. Scatter corrective techniques can include Multiplicative Scatter Correction (MSC), Standard Normal Variate (SNV) and, normalisation to reduce the effects of physical variability caused by scattering [93]. The two commonly used spectral derivatives are Norris-Williams (N-W) and Savitzky-Golay (S-G). The spectral derivatives aim to smooth the spectra without reducing the signal to noise ratio in the spectra too much.

The analysis of adulteration using spectroscopy and in some cases mass spectrometry requires further investigation with chemometrics. The most common algorithms used for the determination of authenticity or the detection of fraud are the classification/discrimination algorithms such as the unsupervised PCA, and the supervised LDA, PLS-DA or OPLS-DA. For the quantification of adulterant in a sample, PLSR analysis is used frequently.

3.6. Detection methods for the addition of illegal dyes

An extensive review of detection methods for illegal dyes has been carried out by Oplatowska-Stachowiak and Elliott [41]. Liquid Chromatography is the most common method of detection of illegal dyes. Other chromatography techniques were used with various detection methods including voltammetric, spectrophotometric and capillary electrophoresis. The use of Enzyme-Linked Immunosorbent Assay (ELISA) is also a common method of detection in this field.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Ingredient	Adulterant	Reference	Detection Methods	Chemo-metrics
Saffron	*Carthamus tinctorius, Chrysanthemum x morifolium, Zea mays, Nelumba nucifera*	[65]	DNA barcoding	
Black pepper	Chilli	[25]	DNA barcoding	
Saffron	Safflower	[59]	SCAR and ITS Multiplex PCR	
Saffron	Saffron	[94]	Barcoding Melting Curve	
Chilli	Dried red beet pulp and powdered *Ziziphus nummularia* fruits	[16]	PCR-SCAR markers	
Oregano	*Satureja montana* L. and *Origanum majorana* L.	[20]	SCAR-PCR	
Oregano	Olive leaves	[61]	SCAR-PCR	
Oregano	*Cistus incanus* L., *Rubus caesius* L. and *Rhus coriaria* L	[21]	SCAR-PCR	
Saffron	*Arnica montana* L., *Bixa orellana* L., *Calendula officinalis* L., *Carthamus tinctorius* L., *Crocus vernus* L., *Curcuma longa* L., and *Hemerocallis* sp.	[57]	SCAR-PCR	
Turmeric	*Curcuma zedoaria/Curcuma malabarica*	[36]	SCAR-PCR	
Cumin	Almond, peanut, tree nuts, peach and cherry	[22]	DNA analysis, Antibody based technology, Microscopy, Mass spectrometry	
Saffron	Saffron of unknown origin labelled as being cultivated in the PDO region in Spain can be used for substitution.	[33]	LC HRMS	PCA, OPLS-DA
Fennel seed	*Anethum graveolens* fruit (AGF) and *Cuminum cyminum* fruit (CCF)	[71]	Light microscopy, fluorescence microscopy, GC-MS	
Chinese star anise	Japanese anise	[75]	Plant spray DART-HRMS	
Chinese star anise	Japanese anise	[74]	DART-HRMS	

Ingredient	Adulterant	Reference	Detection Methods	Chemo-metrics
Oregano	Olive leaves, myrtle leaves, hazelnut leaves, sumac	[69]	LC-MS/MS, FTIR	PLSR
Oregano	Olive leaves	[70]	LC-ESI-MS/MS	
Sage	Olive leaves	[70]	LC-ESI-MS/MS	
Oregano	Olive leaves	[72]	GC/MS	
Paprika	Falsely declared Szegedi paprika substituted for *Szegedi Füszerpaprika* PDO	[32]	ICP-MS	PCA, CDA
Oregano	Olive leaves, myrtle leaves, cistus, hazelnut leaves, sumac	[19]	FTIR , LC-HRMS	PCA, OPLS-DA
Garlic	Cornstarch	[76,78]	Raman, FTIR	PLSR
Ginger	Cornstarch	[78]	Raman	PLSR
Onion Powder	Cornstarch	[78,79]	Raman, FT-NIR, FTIR	PLSR
Saffron	*Crocus sativus* stamens, turmeric, safflower, gardenia	[77]	^{1}H-NMR	PCA, OPLS-DA, O2PLS-DA
Saffron	*Crocus sativus* stamens, calendula, safflower, turmeric, buddleja, and gardenia	[86]	DRIFTS-FTIR	PLS-DA, PLSR
Chinese star anise	ILACS	[87]	NIR/MIR	LDA, PCA
Chinese star anise	Japanese star anise	[88]	SWIR-HIS	PCA, PLS-DA
Black pepper	Buckwheat or millet	[90]	NIR hyperspectral imaging, FTIR	PLSR
Paprika	Tomato skins, brick dust	[91]	FT-NIR & Advanced-ID algorithm	
Turmeric	Yellow chalk powder	[37]	Terahertz spectroscopy	
Saffron	Safflower dyed corn stigma	[34]	Electronic Nose	PCA, ANN

5. Conclusion

It is evident that EMA is a constant threat in the growing herb and spice industry. Cases of fraud have an economic impact on the industry as well as reducing consumer confidence. Potential public health risks following adulteration, such as the case of nut protein in cumin and paprika, are a major concern in the industry. Advances in DNA analysis include the use of SCAR-PCR and DNA barcoding provide faster and cheaper methods of analysis. Further advancement may include the use of NGS as it moves into the area of food fraud. Mass spectrometry, commonly used for the detection of food fraud is also improving by becoming faster and cheaper with the introduction of ambient techniques. Spectroscopic methods along with chemometric techniques are increasingly being used in the fight against food fraud and offer a rapid, robust screening technique that is cost effective and requires little expertise. There is an increasing need for screening techniques that can detect EMA over a range of products in the growing herb and spice industry.

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Saffron

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General overview of the product

Saffron, the most valued spice in the world, is the dried stigmas of the flowers of *Crocus sativus* L., and the only one able to impart colour, flavour and aroma to foods. Very often saffron is confused with other plants, sometimes due to lack of knowledge, but also, which is worse, adulterated for economic gain. For this reason, saffron deserves to be dealt with in a separate chapter in this book.

The cultivation of saffron has been known for more than 3700 years as illustrated in the frescoes of the Minoan goddess Thera where a crocus field appears with women picking and offering flowers of *Crocus sativus* or *Crocus cartwrightianus* [1]. Since those early days, the way the spice is obtained has not evolved as it is still manually processed, without the mechanisation seen for other agricultural products. Once the flower is collected and the stigmas removed, it will be handled differently according to country, production area or even culture; such differences contributing to the real value of the spice [2]. The most prized saffron origin on the international market is from La Mancha (Spain), since it traditionally imparts most colour, flavour and aroma to food [3]. Until the 1990s, Spain was also the largest producer, but following a massive abandonment of agriculture in this country, production fell drastically, and Spanish companies had to turn to Iran as a new supplier of the raw material. However, most of the world distribution continues to be from Spain, due to the know-how that the trading companies have maintained through family tradition.

World production of saffron remains unclear, but it is known that Iran is the largest producer in the world, exceeding 90 %. According to the Iranian Ministry of Agriculture in 2013, 280 tons were produced in this country [4]. Although internal consumption of saffron in Iran is high, it is the largest exporter of this spice with Spain as the largest importer. In Spain, the spice is stored under the suitable conditions and its quality determined. With little information available and its high market price, this spice has been an easy victim of adulteration since ancient times, offering the fraudster considerable economic returns.

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1. Product Identity

1.1. Definition of the product and manufacturing process

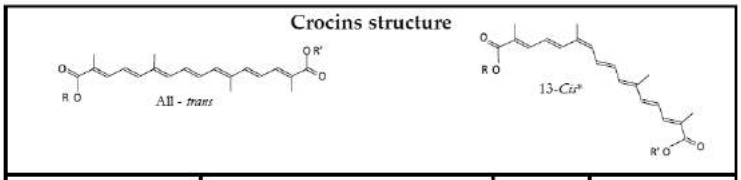
The flower of *Crocus sativus* grows from a corm in late October and early November in the northern hemisphere, and in late April and early May in the southern hemisphere. It is picked manually in the field, and depending on the tradition of the production area, either at dawn with the flower closed, or at noon when the flower is open. The stigma and part of the style are separated from the rest of the flower, to a greater or lesser extent according to tradition. Subsequently, the stigmas are dried either using a direct flameless heat source or by leaving them for several days in the sun or in the shade, depending on each producing area. In some places there is a flower market, where the farmer who collects the flowers is not the one who produces the spice. All these factors mean that there is great diversity among the products obtained in different areas and, therefore, different quality products [3].

In La Mancha saffron, the three filaments are joined together with a small part of the yellowish-white style that contributes nothing. Italian saffron is very similar and although in Greece stigmas are also held together, Greek saffron is usually accompanied by flower pollen which contributes other flavour and aroma characteristics. In Iran, traditionally, the stigma is accompanied by a long part of the style and is dried in the sun or in the shade, as in Morocco. In India, the stigmas, after being separated, are rubbed together to obtain a homogenous darker colour and therefore quality.

Saffron is marketed for its colour, flavour and aroma with major metabolites that determine the quality of saffron. These are currently controlled by ISO 3632 [5] and used in all commercial transactions with saffron. The substances responsible for the colouring properties of saffron are the glycosidic esters of the carotenoid dicarboxylic crocetin (2E, 4E, 6E, 8E, 10E, 12E, 14E)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid, $C_{20}H_{24}O_4$). The glycosides bound to crocetin are gentiobiose, glucose, neopolitanose and triglycose [6–9], which in saffron are found in their *trans* (majority) and *cis* (minority) forms. All these compounds are referred to in the literature as crocins, although in fact crocin is only *trans*-crocetin di (β-*D*-gentiobiosyl) ester. Figure 1 describes the names that have been accepted in recent years by the scientific community. The colouring strength of the spice depends on the concentration of these compounds, which ranges between 16-28 % in the dried stigma of *Crocus sativus* L., reaching concentrations up to 30 % in some years.

The substance responsible for the characteristic bitter taste of saffron is picrocrocin (4- (*β-D*-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, $C_{16}H_{26}O_7$). Up to now, this compound has not been detected in any other raw material, whether from plant or animal origin, and it is therefore considered to be a molecular marker of true saffron [11]. Its concentration is usually between 7-16 % [12], although in some samples it can reach 20 %.

With respect to saffron aroma, safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde, $C_{10}H_{14}O$) is the main compound [13] and the aglycone of picrocrocin. It has been detected in very few plant products and can be also generated when certain carotenoids undergo a thermal process. Safranal concentration is much lower than crocins and picrocrocin, usually between 0.1-0.6 % [14].



Nº Glucose Moieties (R + R′)	Moiety Distribution (R/R′)	Isomer	Named as
5	triglucoside/gentiobioside	*trans*	*trans*-5-tG
		cis	*cis*-5-tG
	neapolitanoside/gentiobioside	*trans*	*trans*-5-nG
		cis	*cis*-5-nG
4	neapolitanoside/glucoside	*trans*	*trans*-4-ng
		cis	*cis*-4-ng
	gentiobioside/gentiobioside	*trans*	*trans*-4-GG
		cis	*cis*-4-GG
3	gentiobioside/glucoside	*trans*	*trans*-3-Gg
		cis	*cis*-3-Gg
2	gentiobiose/hydrogen	*trans*	*trans*-2-G
		cis	*cis*-2-G
	glucose/glucose	*trans*	*trans*-2-gg
		cis	*cis*-2-gg
1	glucose	*trans*	*trans*-1-g
		cis	*cis*-1-g

Figure 1: Simplified names of the glycosidic esters, crocins, of the carotenoid crocetin introduced by Carmona et al. [3]. Meaning of the abbreviations: t is Triglucose; G is Gentiobiose; n is Neapolitanose; g is Glucose [10]

1.2. Current standards of identity or related legislation

1.2.1. ISO Standard

Major metabolites determine the quality of saffron, which is actually controlled by ISO 3632 [5], and used in all commercial transactions with saffron. The methodology still used to control saffron quality is UV-vis spectrophometry, although there are several scientific studies [14] that have shown that safranal and picrocrocin are overestimated by this methods. Despite this, no changes have been undertaken by ISO. Even in the draft amendment of Codex Alimentarius [5], the spectrophotometric methodology used in the ISO 3632 standard is proposed for the determination of saffron quality, but this time high performance liquid chromatography has been included in order to detect the adulteration of saffron spice with water-soluble dyes. The question that arises now is: Why not determine the quality of saffron, in terms of quantification of their metabolites, by means of liquid chromatography? There are enough scientific papers which show that such a technique can contribute to a correct quality determination [6,15,12,16]. A specific approach was carried out by García-Rodríguez et al. [17] who developed an analytical protocol, in which extraction of the compounds is based on ISO 3632 [5], but where identification and quantification of the saffron metabolites is done using liquid chromatography equipment with aligned diode detector (HPLC-DAD) and quantification performed using the commercial patterns (esters of crocetin *trans*-4-GG and *trans*-3-Gg, safranal) and the picrocrocin isolated by the same group using the methodology described by Sánchez et al. [11].

1.2.2. EU Protected Designations of Origin

In recent years, there has been increased interest in guaranteeing and defending the quality of saffron produced in certain historical regions. As a result, there are six Protected Designations of Origin (PDOs) in Europe: "Krokos Kozanis" in Greece [18], "Azafrán de La Mancha" in Spain [19], "Zafferano dell'Aquila" in Italy [20], "Zafferano di San Gimignano" in Italy [21], "Zafferano di Sardegna" in Italy [22] and "Munder Safran" in Switzerland. The drawback is that, to demonstrate their quality, most of these PDOs use the spectrophotometric method of ISO 3632, which is based on an erroneous analytical technique as already mentioned.

2. Authenticity issues

The main problem for the saffron consumer is the lack of knowledge about the shape of the product, which is the reason why some plant products such as *Carthamus tinctorius* are on offer on the market that are not true saffron. Also by not knowing the product in certain regions, it is easy to confuse consumers with fibres coloured with artificial dyes.

2.1. Identification of current authenticity issues

Throughout history, the adulteration of saffron has been prosecuted, and for many centuries and in diverse cultures, adulteration of the spice carried the death penalty [23]. Nowadays, the most frequent adulteration is to dye fibres, plants or animals, with food colorants, simulating the colour of saffron stigma [24]. True saffron is also frequently confused with the petals of the flowers of *Carthamus tinctorius,* sometimes referred to as "bastard saffron", which is also considered a spice but which does not impart to food the colour, flavour and aroma of true saffron [25]. However, the most difficult way of adulterating this spice is when the saffron is sold as whole, where the filaments that form the stigma are seen, and which are joined or cut according to the production area.

Adulterants and substitutes mainly consist of parts of the plant of *Crocus sativus* or from other plants such as marigold (*Calendula officinalis*) or arnica (*Arnica Montana*), which might have been dyed. Many diverse plant materials use the name “saffron” all over the world, for example safflower (*Carthamus tinctorius*) is called bastard saffron or saffron thistle; marigold is also known as Indian saffron, American saffron or Mexican saffron. These names contribute to the misidentification of saffron in filaments. Turmeric *(Curcuma longa* L.) may be misidentified as powered saffron. The mixture with extracted, recoloured exhausted saffron or old saffron also constitutes a fraudulent practice. In addition, increasing moisture and adding substances like honey, starch, meat fibres, coloured artificial fibres or even inorganic compounds to increase weight constitute known adulterations [25]. Nowadays, the addition of artificial colorants is the most common type of fraud. The aim of this practice is to mislead the consumer by improving or changing the appearance of old and low quality saffron, or of other extraneous materials added, to increase weight or use as substitutes. Gardenia has been found among the latest adulterants and substitutes of saffron due to its content in crocetin esters [25,26].

The content of crocins and picrocrocin in saffron is very high; there is no other spice that has such a high content of these metabolites, which is the reason why this spice is so appreciated by the consumer. As the crocin content is higher than 16 %, it would not be economically profitable to adulterate saffron with another product by adding crocins to reach these concentrations; the fraud

would be more expensive than saffron itself. In addition, given that the picrocrocin content has to be higher than 7 % to be considered saffron, and that this compound is a molecular marker since it is only found in saffron, the fraud would be even more expensive. In other words, simply by changing the method of determining the quality of the spice, all types of fraud could be avoided.

2.2. Potential threat to public health

Some adulterants may be dangerous for human health, as for example the so-called autumn saffron (*Colchicum autumnale*) which is extremely toxic. In principle, almost all adulterations of saffron can cause health problems. If saffron is adulterated with coloured fibres from other plants, allergy problems can occur since the consumer is not able to correctly identify the product being consumed which may be an allergen. If saffron is adulterated using artificial food colours to dye fibres that confuse the consumer, problems can be generated since their innocuous use is not admitted for this purpose. If it is with artificial colours, the problems can be greater, because these are not food grade products and can be toxic in varying degrees. The dust of metallic red oxides that are confused with ground saffron and that are very toxic and carcinogenic have also been detected. However saffron was and is still also valued as a medicinal plant with important biomedical applications. In the last 15 years, its bioactivity has been demonstrated and published in high impact scientific journals [10].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Non-saffron samples are not currently detected when evaluated by spectrophotometric measurements according to ISO 3632:2011 [5]. An approach to detect artificial colorants in saffron using derivative UV-Vis spectrometry was carried out in 2005, but the quantification limits reported were far from the actual market situation [27]. All this confusion would be greatly minimized, if the quality of the saffron were to be determined according to the content of crocins, picrocrocin and safranal by liquid chromatography.

As previously described, the method used in commercial transactions is the one described in ISO 3632-Part 2 [5], which is based on spectrophotometric measurements of aqueous saffron extracts. This methodology leads to erroneous results on two of the three main saffron metabolites due to the low selectivity in the determination of safranal and picrocrocin. This method is not selective because the absorbance at 330 nm and 257 nm, which are used to determine safranal and picrocrocin respectively, are wavelengths where other compounds also absorb. The misunderstanding due to the use of such methodology is producing significant errors. For example, in saffron-extract tablets used as dietary supplements, safranal is being quantified with a content higher than 5 % which means that the tablet would be toxic and taking several of them would lead to irreversible health problems for the consumer [14].

The problem about using the wrong standard (ISO 3632 Part 2 [5]) is that it has been extended to other official country standards, PDOs, Codex Alimentarius, etc. with the consequent global confusion of the chemical characteristics of the spice.

3.2. Other commonly used methods

The methods used that are proposed are not actually included in any of the official standards, as mentioned before, but could be used on a routine basis. Proper saffron quality determination should be carried out by the detailed quantification of the crocetin esters, picrocrocin and safranal metabolites by means of chromatography devices. LC-based methods with DAD detector may be used to determine water-soluble compounds such as crocins and picrocrocin. To determine the aromatic composition, GC-MS methods with different extraction and injection systems are used [15]. If only the most abundant volatile need to be quantified (safranal), HPLC-DAD methods can also be used [17]. An example of a saffron fingerprint, shown in Figure 2, which may help to define the authentication of the product [28] includes the chromatograms obtained at wavelengths of 440, 330 and 250 nm. At 440 nm, the fingerprint of the four mayor crocins, *trans*-4-GG, *trans*-3-Gg, *cis*-4-GG and cis-3-Gg, can be observed; the other peaks that appear at 440 nm are the rest of crocins named in the Figure 1, which may or may not be present. At 330 nm, the small peak of the safranal can be seen at the end of the chromatogram. At 250 nm, the peak of picrocrocin is observed.

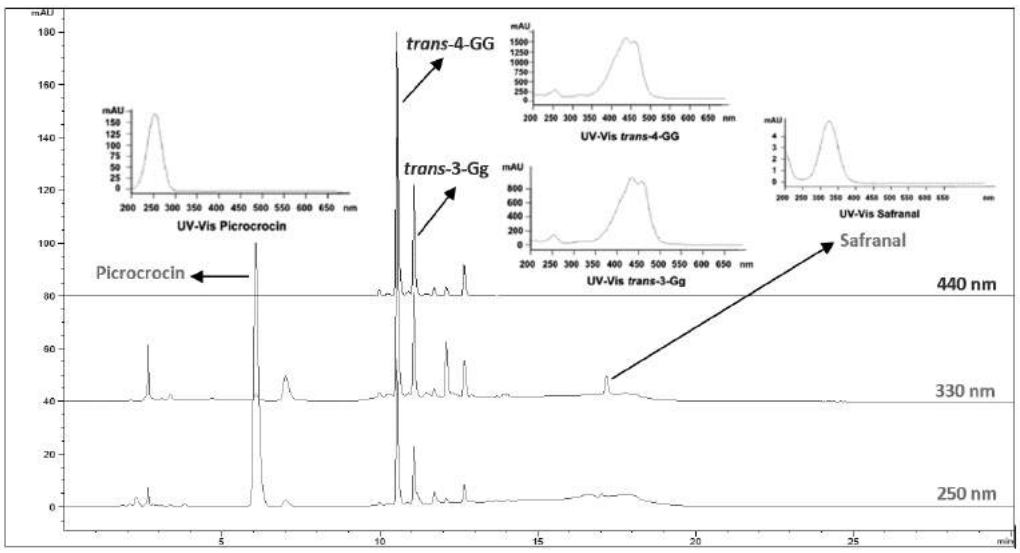


Figure 2: Saffron fingerprint obtained by HPLC, chromatograms at 440, 330 and 250 nm, and their UV-vis spectrum. The peaks corresponding to the major metabolites (crocins, safranal and picrocrocin) are indicated

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Spectrophotometry UV-Vis	Colouring strength	Authentication not possible / detection of artificial colorants in saffron (in g/kg)
Second derivative UV-Vis spectrophotometry	Artificial colorants water soluble	Detection of artificial colorants in saffron (in ppm)
HPLC-DAD	Crocins, picrocrocin and safranal	Saffron authentication (fingerprint) / quality
	Artificial colorants	Detection of artificial colorants in saffron
	Carminic acid	Not be present in Kosher and Halal foods
TLC	Artificial colorants	Detection of artificial colorants in saffron / disused technique
LC/DAD/MS/MS	Crocins, picrocrocin and flavonoids	Identification metabolites of saffron
UHPLC-MS/MS	Crocins	Differentiation of process obtaining saffron
DHS-GC-MS	Safranal and other aroma compounds	Fingerprint of saffron aroma / quality aroma
e-Nose	Volatiles of saffron as a whole	Determination geographical origin
Ultrasound extraction-GC-MS	Volatile compounds	Geographical differentiation of saffron
PTR-TOFMS	Volatile compounds	Identification volatile / quality aroma
Raman spectroscopy	Sum crocins and colouring strength	Quality of saffron
NIR spectroscopy	Saffron quality control	Determination of chemical composition and geographical origin
MIR spectroscopy	FT-IR spectra saffron filaments	Determination geographical origin
Tristimulus colorimetry	Colour	Quality of saffron
SBSE-GC-MS	Multi-residue	Contaminants and pollutants determination
Derivatisation-HPLC-DAD	Free amino acids and ammonium	Determination geographical origin
Stable isotopes H, C and N	Analysis of stable isotopes hydrogen, carbon and nitrogen	Determination geographical origin

5. Conclusion

To control the quality of saffron and avoid adulteration, it is necessary to introduce analytical methodologies in the compulsory standards, as well as in ISO 3632, which determine in detail the metabolites: crocins, picrocrocin and safranal, responsible for colour, taste and aroma, respectively. At this time and in the near future the methodology based on HPLC-DAD is the most appropriate, fastest and cheapest.

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Beverages and Related Products

Wine and must

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General overview of the product

Approximately 8 000 years ago and at the same time, in an area situated between the Black and Caspian seas (corresponding to modern Georgia and Armenia) and in Mesopotamia (modern Iraq and Iran) were once domesticated, or, at least grown as part of an ancestral cultivation, wild European grape vines (*Vitis vinifera*) [1]. Evidences of probably the earliest known winery, dating back 6000 years, hosting relicts of a press and several fermentation and storage vessels, were found in a cave in Areni (Armenia) [2].

https://doi.org/10.32741/fihb.12.wine

For its intoxicating and exciting properties, rapidly wine became far more than an ordinary beverage, and was often used as a ritual libation for priests and royalty in religious ceremonies, or as a votive offering to gods. Later, the expansion of the Greek civilization, and also that of the Roman Empire, led to the diffusion of the cult of Dionysus (or Bacchus for the Romans), the god of wine, and the vine growing culture, in all the coastal regions around the Mediterranean Sea. Under Celtic and Roman influence, viticulture was then introduced to the continental European temperate regions, notably to France and Germany. After the fall of the Roman civilization, when Europe was afflicted by mass migration and invasions, inside scattered monasteries was seeded and nursed the first embryo of modern winemaking knowledge.

Nowadays, the European Union is the world's largest wine producer and consumer, with roughly 70 % of global production and 60 % of global consumption. All 27 EU member states produce wine to some extent, and each has its own language, traditions and wine classifications. World wine production was around 246.7 mhl in 2017 (OIV report), with Italy, France and Spain as the leading world producers.

According to the European Commission's Directorate General for Agriculture and Rural Development (DG AGRI), European wine production can vary a lot from year to year (with yields ranging from +20 % to -20 %), highly influenced by weather conditions and/or the sanitary conditions of the vines. This has an important impact on price levels and hence on the number and types of adulteration. The price of wine also depends on its production area and label.

Wine exports are increasing year by year and accounted in 2017 for over 25 % of the volumes produced, whereas imports remain constant. Five main destinations (USA, Switzerland, Japan, Canada, China-Hong Kong) account for up 70 % in value of all wines exported outside the EU.

Outside Europe, the main wine producer is the USA, followed by Australia and China. Wine production in China is increasing year by year, from being absent in 2005 and taking its place as the world's 6th largest wine producer in 2016.

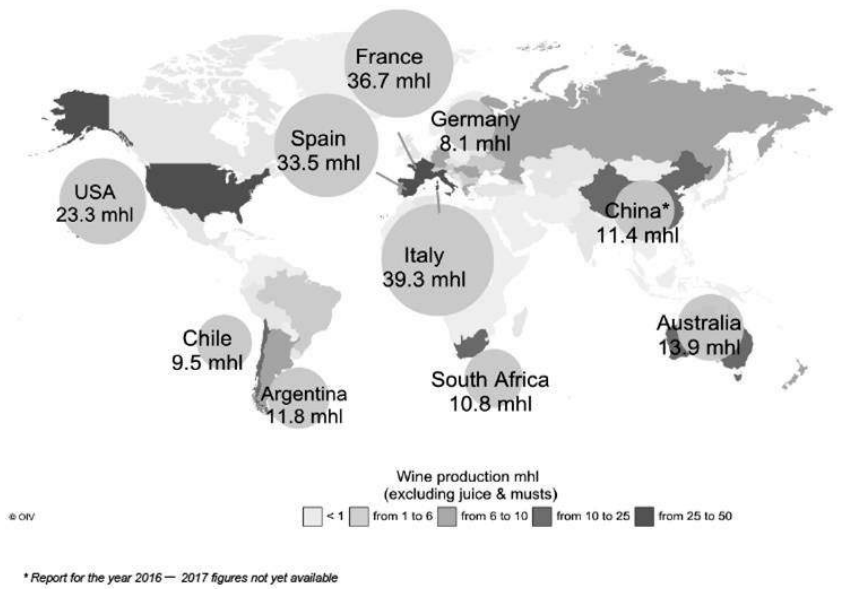

Figure 1: 2017 wine production, OIV Report

1. Product Identity

1.1. Definition of the product and manufacturing process

The most relevant constituents of must and wine are water, carbohydrates, acids, alcohols, phenolics, nitrogenous compounds (proteins, amino acids and ammonium salts), inorganic substances (metals and anions) and flavours. The chemical composition of grapes is affected by many factors, particularly grape variety or cultivar, environmental factors such as climate and soil (the concept of 'terroir'), viticultural management and seasonal variations (the concept of 'vintage'), and also on the variability of winemaking practices.

1.1.1. The winemaking process

Harvesting

Grapes are naturally rich in fermentable sugars, organic acidity, aroma precursors, protective tannins and coloured anthocyanins and flavonoids, making possible an easy transformation to a naturally stable beverage, wine. Moreover, grapes are rich in minerals and nitrogen compounds that are essential to promote the biochemical fermentation to wine. The choice of when to pick the grapes will determine acidity, sugar content and the potential richness in flavour of the musts. This decision was traditionally performed on the basis of a tasting directly in the vineyard, although today it is usually the result of a more conscious chemical evaluation of fruit composition.

Crushing and Pressing

After the grapes are sorted, if they are to be used in the production of white wines, they are generally destemmed and crushed, whereas for red wines, the stems are often not removed. Must is the freshly pressed grape juice that contains also the skins and seeds. For white wines, the juice is quickly separated from the skins and seeds, unless a greater extraction of aroma precursors from the skins is sought using a cold maceration technique. Red wines, on the other hand, are left in contact with their skins to extract as much as possible colour, tannins and aroma compounds. Nowadays, all these processes are automatically performed using mechanical equipment.

Fermentation

After crushing and pressing, the must can start to ferment at room temperature either within 8-12 hours when indigenous or wild yeasts are naturally present, or in a shorter time when selected yeast strains or a traditional 'pied de cuve' are added as inoculum. The latter practice provides an effective control of fermentation and prediction of the organoleptic features of wines, also reducing the risk of blocking and off-flavour deviations. Fermentation generally ends within 10-20 days, when fermentative sugars are totally converted into alcohol and a dry wine is obtained, whereas, for sweet wines, they are cooled to stop fermentation and filtered through a textile filter to remove yeasts. To create a sweet wine, wine makers will sometimes stop the process before all of the sugar is converted. Fermentation can take from 10 days to one month or more. Often, also a secondary bacterial fermentation of malic acid to lactic acid is promoted, especially for red wines or some specific white wines.

Clarification

This corresponds to the physical practices which are necessary at the end of fermentation to remove the solid fraction from the wine, such as dead yeast cells, precipitates of insoluble salts, and organic aggregates of polyphenols and proteins. After a period of static sedimentation, the

wine is periodically transferred into new containers, such as stainless-steel tanks or oak barrels. Wine can be also clarified using fining agents and filtration equipment.

Aging and Bottling

The ageing of wine, using variable periods of maturation in oak barrels or of aging in glass bottles, represents a crucial winemaking step, potentially able to improve the fineness of wine, making its aroma and taste more complex and pleasing to consumers. A shorter aging in steel tanks before bottling is instead commonly used for fresh white wines.

1.1.2. Legal definition

The legal definition of must and wine is provided by the OIV (The International Organisation of Vine and Wine), which is the body of reference in the area of vine and wine.

'**Grape must** is the liquid obtained from fresh grapes, whether spontaneously or by physical processes such as: crushing, removing stems from grape berries or crushed grapes, draining, pressing.' When alcoholic fermentation has been prevented by sulphiting or addition of carbon dioxide or by sorbic acid, the must is defined as **preserved grape must** and can contain up to 1 % vol ethanol. **Concentrated grape must** is obtained by its partial dehydration and has a density higher than 1.24 g/mL, whereas **caramelized grape must** is obtained by its partial dehydration on direct heat and has a density higher than 1.3 g/mL.

'**Wine** is the beverage resulting from the complete or partial alcoholic fermentation of fresh grapes, whether crushed or not, and from the grape must. Its acquired alcoholic strength should not be less than 8.5 p. 100 vol. Nevertheless, considering climatic conditions, soil or grape variety, special qualitative factors or individual traditions specific to certain vineyards, the total minimum alcoholic strength can be reduced to 7 p. 100 vol. by special legislation of the region in question'.
Wine is then defined as dry, demi-sec, semi-sweet and sweet, depending on the content of sugar, and still and semi-sparkling, depending on the carbon dioxide concentration.

As regards **wine labelling**, the EU classified wine quality into two categories: 'QWPSR' (Quality Wine Produced in a Specific Region) and 'Table Wine'. These were replaced in 2011 with PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication), as explained below.

PDO (Protected Designation of Origin) wine are "produced, processed and prepared in a given geographical area, using recognised know-how". Their quality and properties are significantly or exclusively determined by their environment, in both natural and human factors. Each EU country has its own quality categories which correspond to PDO. The most significant are: France: AOC (Appellation d'Origine Contrôlée); Italy: DOC (Denominazione di Origine Controllata) and DOCG (Denominazione di Origine Controllata e Garantita); Spain: DO (Denominación de Origen) and DOCa (Denominación de Origen Calificada).

PGI (Protected Geographical Indication) wine is linked to the geographical area in which it is produced, processed or prepared, and has specific qualities attributable to that geographical area. The category is named VDP (Vin de Pays) in France, IGT (Indicazione Geografica Tipica) in Italy and VT (Vino de la Tierra) in Spain.

Table Wine and Table Wine with a Geographical Indication were collectively replaced by PGI in 2011. The aim of this was to remove the word 'Table', along with its connotations of low quality, from the EU wine nomenclature. Thus the phrases Vin de Table (France), Vino da Tavola (Italy), Vino de Mesa (Spain), Vinho de Mesa (Portugal) and Tafelwein (Germany and Austria) are now legally obsolete.

1.2. Current standards of identity or related legislation

The International Organisation of Vine and Wine, originally named 'International Wine Office' was created in 1924 as an agreement among eight nations, but today accounts for 46 international member states.

OIV activity is focused on publishing methods of analysis and quality assurance in oenological laboratories, for the determination of the analytical composition of wines, musts and spirit beverages of vitivinicultural origin and wine vinegars. The first collection of analytical methods, the Compendium of International Methods of Wine Analysis, was published in 1962, while the present Compendium of International Methods of Wine and Must Analysis is annually revised and amended since 2000.

Many member countries, in order to facilitate international trade, have adopted the Compendium introducing its definitions and methods into their own regulations. In this way, the European Union (Regulation No 479/2008) recognised all the methods of the Compendium making them binding in all Member States for establishing the composition of the products covered by that Regulation. Regulation (EC) No 606/2009, laid down that the list and description of these analysis methods must be published also at Community level (C Series of the Official Journal of the European Union).

2. Authenticity issues

2.1. Identification of current authenticity issues

Food and beverage authenticity issues fall into one of the following categories:

i. Non-compliance with the established legislative standards,

ii. Adulteration of high value products, through substitution by cheaper but similar ingredients or extension adulterant

iii. Misdescription and/or mislabelling of geographical, botanical or species origin.

In the case of wine/must, category (i) corresponds to the non-compliance with the legislative reference standards and limits of European regulations and OIV, Codex and specification rules of each PDO or IGP in terms of the chemical-physical composition of the product. Some examples are given in Table 1. The authenticity of the samples is determined by using quantitative analyses which quantify the amount of the compounds present: if the actual values are outside the limits quoted in the table, the samples are non-authentic.

The category (ii) relates to the unpermitted addition of exogenous sugars and water in order to increase the alcoholic degree and the yield of the product, and the unpermitted addition of exogenous compounds, such as flavours, glycerol, dyes, tartaric acid and CO_2 in order to improve the poor quality of the product.

In these cases, the authenticity of the product is evaluated using analytical approaches able to trace the source of the compound (from grape, from exogenous products or synthetic). Maximum acceptable limits do not exist, but a reference database on the basis of the analysis of authentic samples has to be built.

Table 1: Maximum acceptable limits of various substances contained in wine (mainly from Compendium of International Methods of Analysis-OIV, 2015/1 Issue)

Substance	Maximum acceptable limits	Notes
Citric acid	1 g/L	
Volatile acidity	20 milliequivalents/L	The volatile acidity of various specially fortified old wines (wines subject to special legislation and controlled by the government) may exceed this limit.
Arsenic	0.2 mg/L	
Borom	80 mg/L (expressed as boric acid)	
Bromine	1 mg/L	Limit exceeded by way of exception in wines from certain vineyards with a brackish subsoil.
Cadmium	0.01 mg/L	
Copper	1 mg/L 2 mg/L	For liqueur wines produced from unfermented or slightly fermented grape must (Oeno 434-2011)
Diethylene glycol	≤ 10 mg/L, to the Quant. Limit	
Malvidol diglucoside	15 mg/L	
Silver	< 0.1 mg/L	
Total sulphur dioxide (at the time of sale to the consumer)	150 mg/L	For red wines containing a maximum of 4 g/L of reducing substances.
	200 mg/L	For white and rosé wines containing a maximum of 4 g/L of reducing substances.
	300 mg/L	For red, rosé and white wines containing more than 4 g/L of reducing substances.
	400 mg/L	In exceptional cases some sweet white wines.(Oeno 9/98)
Ethanediol/Ethylene glycol	≤ 10 mg/L	
Fluoride	1 mg/L	Except for wines coming from vineyards treated in conformity with national law, with cryolite in which case, the level of fluoride must not exceed 3 mg/L (Oeno 8/91)
Methanol	400 mg/L	For red wines
	250 mg/L	For white and rosé wines(Oeno 19/2004)
Ochratoxin A	2 µg/L	For wines obtained as from the 2005 harvest (CST 1/2002).
Lead	0.15 mg/L	For wine made, starting from the 2007 harvest year (Oeno 13/06).
Propan-1,2-diol Propylene glycol	150 mg/L	Still wines
	300 mg/L	Sparkling wines (Oeno 20/2003)
Excess sodium	80 mg/L	(Oeno 12/2007)
Sulfates	1 g/L (expressed as potassium sulfate)	

Misdescription and mislabelling (iii) concern false declaration of origin and grape variety, harvest year and wine category. The aim of this adulteration is to give premium price and value to products with low quality.

In addition, for these types of adulteration, reference databases have to be built on the basis of the analysis of authentic samples in order to define the ranges of values that are characteristic of a particular production area, vintage or variety.

2.2. Potential threat to public health

In the very long history of wine fraud several adulterations have posed severe health risks and harm to consumers. One of the oldest examples is the addition of lead acetate (sugar of lead) as a sweetener, which was already reported in Ancient Rome and again in the 17th century. This practice particularly occurred when "good" wine was rare and led to severe health damage by lead intoxication. The determination of lead acetate addition by the precipitation of black lead sulphide was one of the early official test methods established in Germany (1788: "Württembergische Weinprobe") in the fight against food fraud.

More recent examples of health risks related to wine fraud were the addition of diethylene glycol and methanol in the mid-1980s. In 1985 it was uncovered that diethylene glycol (an anti-freeze agent) was added to Austrian wine in a large scale in order to imitate a better wine quality by its sweet taste and increasing the extract. Acute diethylene glycol intoxications lead to nephrotoxic effects. In 1986, several cases of death and severe intoxications were reported after the consumption of Italian wine which contained high concentrations of methanol. Methanol, cheaper and free of tax compared to ethanol, was added intentionally in order to reach the former required minimum alcoholic degree for table wine with low-grade starting material.

As for allergens, according to the European Regulations, there are maximum limits for sulphur dioxide content depending on the type of wine, and wines containing sulphite must be labelled with "contains sulphites". Moreover, if egg or dairy products are used, these must be declared on the label.

For wine there is risk of contamination with Ochratoxin A and lead. Ochratoxin A is formed when grapes are contaminated by certain mould species and its maximum allowed level is 2.0 µg/kg. For lead there is a threshold limit of 0.20 mg/kg.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

According to the Resolution OIV Oeno 9/2000, analytical methods are classified in 4 categories on the basis of criteria of robustness and metrological traceability (I, Criterion Benchmark Method; II, Benchmark Method; III, Approved Alternative Methods; IV, Auxiliary Method) and they should be recommended for different uses: from tests in cases of disputes or calibration purposes, to monitoring, inspection and regulatory purposes.

The OIV Compendium consists of 5 sections and 6 annexes, where physical and chemical analyses are grouped in the second and third sections, respectively.

Physical tests are used to define very different characteristics of wines and musts. Some of these methods are basic and principally devoted to checking the general compliance with legal or trade specifications: Density, Total Dry Matter, Ash and its alkalinity, Chromatic Characteristics, Folin-Ciocalteau Index, and Turbidity. Others are very specific, such as the determination of the $^{18}O/^{16}O$ isotope ratio of water from wine and must after equilibration with CO_2, using isotope ratio mass spectrometry (IRMS).

The chemical tests of section 3 are divided into 2 subsections: Organic compounds (Sugars, Alcohols, Acids, Gas, Other organic compounds) and Non-organic compounds (Anions, Cations, Other non-organic compounds).

3.1.1. Sugars

The determination of fermenting sugars in must and wine represents a fundamental issue for oenology. Different approaches are provided: the most practical for use in the winery, but not very accurate, is the determination of reducing sugars as an estimation of fermentable ones. It is indeed of the lowest category. The determination of glucose and fructose by an enzymatic method, and the determination of sugars, including glycerol and sucrose, by HPLC, are both regarded as being of superior accuracy and selectivity, and are considered as belonging to category II. Of a lower classification are the two approaches that use differential pH sensors for the joint determination of glucose and fructose or, separately, of glucose, fructose and sucrose.

Polyols derived from sugars and residual sugars in dry wines (fructose, glucose, mannitol, sorbitol, dulcitol, and mesoinositol) are determined using gas chromatography after formation of their trimethylsilylated derivatives.

The source of sugar (whether from grape or from cane or beet) is determined using Site Specific Nuclear Isotope Fractionation Nuclear Magnetic Resonance (SNIF-NMR) which determines the deuterium distribution and the D/H ratios in the methylic and methylenic sites of ethanol derived from the fermentation of grape musts, concentrated grape musts, grape sugar (rectified concentrated grape musts) and wines. The $^{13}C/^{12}C$ isotope ratios of glucose, fructose, glycerol, ethanol in products of vitivinicultural origin (dry wine, sweet wine, grape juice, and rectified concentrated must) are determined by HPLC/IRMS. This method belongs to category II for glucose, fructose and glycerol, and III for ethanol.

3.1.2. Alcohols

Accurate measurement of alcoholic strength (by volume) was, for a long time, both a technical challenge and a practical need for establishing the commercial value of wine. Two methods (categories I and IV) are available. The first measures the alcoholic strength of wine determining the density of its distillate using, alternatively, a pycnometer, an electronic densimeter, or a hydrostatic balance. The second method, definitely less accurate, uses a hydrometer or refractometer to determine the alcoholic strength of the wine distillate.

Two possible methods for methanol quantitation are also considered. The first determines methanol in the wine distillate using GC/FID, while the second measures it on the base of the violet colour intensity at 575 nm after its oxidation to formaldehyde by potassium permanganate and reaction with chromotropic acid in a sulphuric medium.

In this section are also reported 2 isotopic methods. The first determines the $^{13}C/^{12}C$ isotope ratio of wine ethanol or that obtained through the fermentation of musts, concentrated musts or grape sugar by IRMS, enabling the detection and quantification of sugars of C_4 origin (sugar cane or corn isoglucose) which are added to products derived from grapes. The second method is for the determination of the $^{13}C/^{12}C$ isotope ratio of glycerol in wines by GC/C or HPLC coupled to IRMS, and which is used to detect the addition of glycerol from maize (C_4 plant) or from synthesis (fossil sources) to wines or to spirit drinks.

Moreover, the absolute content of glycerol in wine can be investigated using two different approaches: one method based on the colorimetric measure at 480 nm of the reaction products of formaldehyde, obtained by the oxidation of glycerol, with phloroglucinol; or using an enzymatic approach.

3.1.3. Acids

Total and volatile acidity (and their difference, fixed acidity) methods both belong to category I, and are based on titrimetric measurements, directly or after distillation of the wine.

For the determination of the single organic acids, several chromatographic approaches are proposed: by thin-layer chromatography (sorbic acid); by HPLC (tartaric, malic, shikimic, lactic, acetic, citric, succinic and fumaric acids; sorbic, benzoic and salicylic acids; shikimic acid; L-ascorbic acid and D-iso-ascorbic acid); by GC (sorbic acid); by Capillary Electrophoresis (sorbic acid; tartaric, malic and lactic acids) and by ionic chromatography (malic, citric and tartaric acids).

Enzymatic methods are also provided for the selective measuring of enantiomeric forms (D-lactic and L-lactic acids, D-malic and L-malic acids, L-ascorbic acid) and citric acid.

A method for the identification of L- tartaric acid origin (plant or fossil) using ^{14}C activity is also proposed.

3.1.4. Carbon dioxide

Carbon dioxide content in still, semi-sparking and sparkling wines can be measured by titration and is carried out using an acid solution in the presence of carbonic anhydrase, while a direct overpressure measurement in bottles of semi- sparkling and sparkling wines can be performed, after thermal stabilisation and agitation of the bottle, using a specific pressure gauge (aphrometer).

An IRMS method can also be used to discriminate the origin of CO_2 in the headspace of bottled sparkling wines on the basis of stable carbon isotope ratio ($^{13}C/^{12}C$).

3.1.5. Other organic compounds

The main compounds of this class are detected using chromatographic methods: by thin-layer chromatography (artificial sweeteners such as saccharine, dulcin, cyclamate, and P4000), by HPLC (hydroxymethylfurfural by reversed-phase chromatography at 280 nm; ochratoxin A using an immunoaffinity column and fluorescence detection; 9 anthocyanins on reverse phase column and VIS detection at 518 nm; lysozyme on reverse phase column with combined spectrophotometric and spectrofluorimetric detection; 17 biogenic amines on reverse phase column after ophthalaldehyde derivatisation and fluorimetric detection, or 8 of the most frequently present in wine, on reverse phase column after derivatisation with diethyl 2-(ethoxymethylene)malonate (DEEMM) and UV detection at 280 nm; α-dicarbonyl compounds, such as glyoxal, methylglyoxal, diacetyl and pentane-2,3-dione, on a reverse phase column after 1,2-diaminobenzene derivatisation and UV detection at 313 nm), by GC (ethyl acetate on wine distillate using flame ionisation detection; ethyl acetate after purification on a solid phase extraction column and mass analysis; 3-methoxypropane-1,2-diol and 6 cyclic diglycerols, as impurities of 'synthetic' glycerol (plant and animal triglycerides), after extraction and mass analysis; polychlorophenols and polychloroanisols after pre-concentration on head space/solid phase microextraction or solid/liquid extraction, and mass analysis or electron-capture detection; α-dicarbonyl compounds after 1,2-diaminobenzene derivatisation and mass analysis; 27 volatile compounds in wines using flame ionisation detection; 1,2-propanediol and 2,3-butanediol after 'salting out' extraction and mass analysis).

Capillary electrophoresis is proposed for: glutathione using fluorimetric detection; lysozyme using high performance capillary electrophoresis and UV detection at 214 nm). Immunoblotting test permits to check the presence of plant proteins in must and wine, while residues of allergenic

proteins from fining agent can be detected in wine applying the direct and indirect ELISA methods. Immunological methods of immunoprinting are also available for testing the presence of unstable proteins in white wines.

3.1.6. Non-organic compounds

Specific methods are indicated for single anions: by colorimetric test at 590 nm after ashing and treatment with chloramine T and phenolsulfonephthalein (total bromide); by titrimetry using Ag/AgCl electrode potentiometry (chlorides); by selective ion electrode (fluorides); by colorimetry measuring the yellow phospho-vanadomolybdate complex (total phosphorous); and by gravimetry (sulphates).

Different analytical methods are also proposed for cations: by atomic absorption spectrophotometry (AAS) or by flame photometry (FP) (potassium, sodium); by AAS (calcium, iron, cooper, magnesium, zinc, silver); by graphite furnace atomic absorption (GFAA) (cadmium); and by a method that fulfils required performance criteria (e.g. GFAA or ICP-MS; lead). A multi-element method using inductively coupled plasma / atomic emission spectrometry is also provided for potassium, calcium, magnesium, sodium, iron, copper, zinc, manganese, strontium, aluminium, and barium.

A final section is also provided for 'other non-organic compounds' analysis: arsenic can be analysed by atomic absorption spectrometry after ethyl alcohol evaporation, As (V) and As (III) reduction to hydride, or by flameless atomic absorption spectrophotometry after acid mineralization and reduction to arsenic hydride; total nitrogen by direct Dumas method or, after acid mineralization and basic distillation, by titration of ammonia; boron by spectroscopic analysis at 420 nm after alcohol evaporation distillation, decolouration on polyvinylpolypyrrolidone, and complexation with azomethine H; mercury by florescence after wine mineralisation and its reduction with stannous chloride; natamycin by HPLC in combination with DAD or MS detection; phthalates in wines after extraction by gas chromatography/mass spectrometry.

For sulphur dioxide in wine, 2 different approaches are proposed: by titration with sodium hydroxide, after 10 °C and roughly 100 °C distillation (free and total sulphur dioxide, respectively) and oxidation; by titration, direct and after alkaline hydrolysis, of wine with iodine (free and total sulphur dioxide, respectively).

Multielement quantitative determination of aluminium, boron, bromine, cadmium, cobalt, copper, strontium, iron, lithium, magnesium, manganese, nickel, lead, rubidium, sodium, vanadium, and zinc in wines (after mineralisation of the sweetest ones) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is also defined.

Finally, for pesticide residues in wine, the OIV have adopted the extraction method QuEChERS (Quick Easy Cheap Effective Rugged and Safe) necessary to prepare the sample before GC/MS and/or LC/MS-MS analysis.

These methods allow in the majority of cases to detect adulteration linked to the (i) category: non-compliance with the established legislative standards, based on the comparison of data with reference limits (cf. Table 1). Some of these methods are also used commonly to verify other types of adulteration belonging to the other 2 categories (cf. section 3.2.1).

3.2. Application and interpretation of official methods

Some of the officially-recognised methods listed above have been the subject of studies investigating the factors involved in the variability of their data or concerning their application for detecting mislabelling of must and wine, in terms of declared grape variety or geographic origin or unpermitted addition of exogenous components.

3.2.1. Stable isotope ratio analysis

The stable isotope ratios of H, C and O have been analysed using IRMS and SNIF-NMR in wine and must since 1987, using official standards that are listed as OIV methods. They are expressed in D/H ppm when analysed using SNIF-NMR [$(D/H)_I$ for the methylic site and $(D/H)_{II}$ for the methylenic site] and in $\delta^{13}C$ ‰ and $\delta^{18}O$ ‰ when analysed using IRMS. This analysis enables the detection of sugar and water addition as well as mislabelling, on the basis of a comparison of data with an official reference databank, set up according to the current European Regulation 555/2008. According to this, every year a number of samples that are representative of the wine production of each Member State are officially collected by the relevant national competent authority. The sampling design has to take into account both the geographical distribution and the harvest period due to the geographical and climatic variability of the isotopic values. For each sample, about 10 kg of fresh grapes are harvested, vinified under controlled conditions and the resulting reference wines analysed in accredited laboratories. The data plus a number of metadata related both to the harvest and the vinification are registered in one official databank that is managed by the European Directorate General, Joint Research Centre (DG JRC). The isotope databank comprises reference data for each year. This allows definition of limits for authentic wines and musts in terms of isotopic data, for each country, each sub-area (e.g. region) and each protected designation of origin (PDO-IGP) as well as general limits [3].

Recently the effect of some oenological practices, such as dealcoholisation, grape withering and the stopping of fermentation on these isotopic ratios has been investigated. The variations in wine water $\delta^{18}O$ and $\delta^{13}C$ ethanol encountered have to be considered when interpreting the isotopic values of actual samples.

The reduction of ethanol levels in wine (= dealcoholisation) is today an important topic for many different reasons, including climate change, health and social matters. Dealcoholisation of up to 2 % vol. is allowed by the legislation (EC Reg. 606/2009). Of the available dealcoholisation techniques, the membrane contactor is one of the most efficient and commonly used. The physical phenomena occurring is called osmotic distillation as the compounds extracted are actually migrating through the membrane pores in a gas physical state. In recent works [4,5] variations of wine water $\delta^{18}O$ of up to -1 ‰ and of ethanol $\delta^{13}C$ of up to +1 ‰ have been encountered for 2 % v/v dealcoholisation. The drop in $\delta^{18}O$ water is mainly caused by isotopic diffusion, which involves $H_2{}^{18}O$ migration from the wine to the extracting solution. The increase of $\delta^{13}C$ is due to the fact that ^{13}C has vapour pressure lower than the ^{12}C, and this causes a prevalent transfer of ethanol with ^{12}C.

Withering involves postharvest drying of grapes and can be performed in a dedicated ventilated or unventilated fruit drying room, (called a “fruttaio”) during autumn-winter, or withering grapes on the plant (‘plein-air’). In both cases, during this period the grapes lose water, and this causes a variation of wine water $\delta^{18}O$ [6]. In ‘fruttaio’, $\delta^{18}O$ decreases significantly from fresh to dry grapes, with differences from -2 to -6 ‰. The decrease in $\delta^{18}O$ is coherent with the decrease in temperature and is due to a chemical exchange between grape water and atmospheric water vapour according to equilibrium isotope fractionation. For Passito produced ‘en plein air’, $\delta^{18}O$

increased with withering in southern Italy, since, due to the relatively higher temperature in these areas, kinetic evapo-transpiration takes place.

Stopping of alcoholic fermentation up to 4.5–10 % of ethanol, is used for the production of some traditional Italian sweet wines (such as Moscato d'Asti) in order to leave a pleasant amount of residual sugar in the wine. It was found [7] that the $\delta^{13}C$ and, in particular, the $(D/H)_{II}$ values of ethanol of wine were positively related to the stage of fermentation, while $(D/H)_{I}$ and $\delta^{18}O$ of ethanol were not. The partially fermented musts were characterized by lower isotopic values, which, in the case of $(D/H)_{II}$, are outside the range of variability of natural wines.

Moreover, more innovative isotopic methods, based on the analysis of the stable isotope ratio of other elements or of other components have been developed.

The $\delta^{18}O$ of wine ethanol was measured directly on dry-ethanol using TC/EA-IRMS in pyrolysis conditions, after having trapped residual water using a molecular sieve [8]. It was found to be significantly correlated with the $\delta^{18}O$ of wine water and can therefore be considered as an internal reference to improve the detection of wine watering, as is the case for fruit juice [9]. As the addition of water to wine changes only the $\delta^{18}O$ of water and not that of ethanol, the watering of wine changes this relationship, which can then fall outside the threshold value, even if the water $\delta^{18}O$ is not outside the limit defined by the wine databank. Thus, measuring the $\delta^{18}O$ of ethanol improves the detection of the watering of wine.

Internal reference was found also for $\delta^{13}C$ to potentially improve the detection of sugar or alcohol addition to wine [10]. The compound specific analysis of the main higher alcohols in wine showed indeed a strong relationship between their $\delta^{13}C$ and that of ethanol, that might help to identify exogenous ethanol sources. However, additional experiments verifying the possible refinement were not performed.

Recently also a method to measure $\delta^{15}N$ in must, wine and in the extracted proline was developed [11]. For proline, the most abundant amino acid in grape and wine and not used by yeast as nitrogen source, $\delta^{15}N$ was measured after N-acetylisopropyl derivatisation using gas chromatography − combustion − isotope ratio mass spectrometry (GC-C-IRMS). $\delta^{15}N$ values of leaves, grapes, wine and particularly must and wine proline were found to be related to those of $\delta^{15}N$ in the growing soil. The addition of inorganic or organic adjuvants was able to influence the $\delta^{15}N$ of bulk wine, but not the $\delta^{15}N$ of wine proline, which is therefore the best marker for tracing the geographical origin of wine.

A GC-c-IRMS method for analysing vanillin in distillates after dichloromethane extraction was developed [12]. Storage in oak barrels release different degradation products such as vanillin, which plays an important role in the flavour and aroma of the distillates. The addition of vanillin, as well as other aroma compounds, of different origins is prohibited by European law. $\delta^{13}C$ values are able to distinguish natural vanillin extracts (-21.0 ‰ to -19.3 ‰), vanillin from lignin and also from tannin (-29.5 ‰ to -26.7 ‰) and synthetic vanillin (-32.6 ‰ to -29.3 ‰).

3.2.2. Trace element profile

Several studies have shown that the trace element profile can be used to classify wines according to their geographic provenance [13,14]. Factors such as soil geochemistry influence the elemental composition of crops. On the other hand, anthropogenic factors such as viticultural practices and processing methods have a strong effect as well.

In 1994, Latorre et al. [15] differentiated the PDO Rias Baixas Spanish wine from Galicia from its imitations. Pattern recognition analysis, performed on ICP-MS data, revealed that Li and Rb were the most discriminating variables. Similar studies were carried out by Baxter et al. [16] on wines from different regions of Spain and England. Taylor et al. [17] studied soils and wines from the Canada's two major wine-producing regions. They found that, among trace elements, strontium was able to differentiate both soils and wines from the two regions. The fingerprint of REE was kept unaltered in the passage soil–grapes–must, while fractionation occurred in wine [18] after the clarification with bentonites. In addition, analysis of Moscato musts from 102 samples showed that is possible to classify their geographic origin, building a basis for identification of possible addition of foreign musts.

3.2.3. Shikimic acid content

Shikimic acid occurs naturally in wine in different concentrations. It is derived from caffeic acid and is a precursor of different amino acids in the biochemistry of the grape plant. Its concentration in wine has been linked to its grape variety.

The analytical method originally proposed by Holbach et al. [19] became an officially-recognised Category II method adopted by the OIV in 2004 (OIV-MA-AS1-02, Oeno 33/2004) [20], fully validated in a collaborative trial.

Based on the publication of Holbach 2001 [19], the official German wine control authorities published reference data for the so-called burgundy group of varieties which are characterized by a low content of shikimic acid (< 30 mg/l) in 2003. Since then, in 2018 the data collection consists of almost 14 000 data – including a broad range of varieties, entries which still the early findings. Wines from many growing regions over the world are implemented, although some sample collectives for certain more local varieties derive mainly from Germany.

For some questions the shikimic acid concentration gives interesting information on the authenticity of the wine variety. For example, Riesling wines are characterised by a high content (with an arithmetic mean of 58 mg/L, n=3346) in contrast to the burgundy wines which show a low concentration of shikimic acid. Therefore, shikimic acid is an indicator for certain varieties and can be indicative for some others. Further authors have confirmed the suitability of shikimic acid for the verification of certain wine varieties [21], reported for low shikimic acid concentrations for the variety Semillon, and Merlot showed a lower shikimic acid concentration than Cabernet sauvignon. Furthermore, the authors showed that the combination of shikimic acid with protein/anthocyanin profiles led to a successful verification of different varieties grown in France (Chardonnay, Chenin, Petit Manseng, Sauvignon Semillon and Ugni Blanc). The low content of shikimic acid for Burgundy varieties was confirmed also for Chilean wines [22].

3.2.4. Anthocyanin composition

The analysis of anthocyanins and particularly their ratios has been successfully used for verifying the identity of grape varieties. Although similar types of anthocyanins are found in different grape varieties, the relative amounts of the individual compounds and their ratios differ. The analytical method was adopted by the OIV as a Category II method (reference method) with Resolution 22/2003: "HPLC-Determination of nine major Anthocyanins in red and rosé wines" (MA-E-AS315-11-ANCYAN) [23].

Thus Individual grape varieties can be verified from one or more anthocyanin compounds in some cases. The acylated anthocyanins have proven to be particularly characteristic for certain grape varieties, with considerable practical significance being attached to the ratio of acetylated to p-

coumaroylated anthocyanins (Rac/cou) and the sum of acylated anthocyanins (Sac) in the assessment of the variety ([18]. For example, it has been noted that Pinot Noir grapes contain no acylated anthocyanins and this feature of burgundy wines is successfully applied for their variety control. For example, the German speciality “Weißherbst” which is a rosé wine produced from 100 % Pinot Noir grapes, should show no significant proportion of acetylated anthocyanins. It should be noted, however, that measurement uncertainty of the wine in question, the typical authorized blending (e.g. 15 % in the EU) and, in the case of sweetened wines, the addition of further products (such as must), should all be considered appropriately before drawing conclusions on the variety in question. In addition, the ageing of wine gives rise to the degradation and polymerisation of the anthocyanins which leads to the absence of the analytes.

Brunello di Montalcino, one of the flagship products of Italian oenology, must be produced from Sangiovese grapes grown in Montalcino, a specific area in Tuscany. Sangiovese grapes are poor in acetylated anthocyanins, one property that in principle promotes the makes it possible to authenticate these premium wines by analysis of the anthocyanins, but as these wines typically aged up to 10 years, sophisticated mass spectrometric approaches give more reliable results as shown by Arapitsas et al. 2012 [24].

One example related to grape variety fraud -the so-called “Pinotgate” incident- was uncovered 2010 in California where Pinot Noir wine imported from France sold in the United States was identified to contain large amounts of Merlot and Syrah [25]. According to information available, the wine was first suspected because of its sensory properties.

3.3. New prospective

There are moreover analytical methods in the literature that are showing promising applications for wine characterization mainly in terms of its geographical and varietal origin.

3.3.1. NMR profiling

^{1}H NMR spectroscopy in combination with multivariate data analysis can be successfully used also to achieve information on various aspects of wine quality such as the authenticity, grape variety, geographical origin, and the year of vintage [26].

This technology, called Wine-Profiling™, has been developed and validated in a joint effort by Bruker BioSpin GmbH and a consortium of analytical laboratories with expertise in wine analysis. The comparison of the spectroscopic fingerprint obtained for each individual sample with that of a large database of authentic wine samples provides answers to questions on the composition, geographical origin, grape variety and vintage. This procedure had been already developed with success for fruit juice analysis (SGF-Profiling™), and it was further optimized for wine and alcoholic beverages in general. In particular, to overcome the need to eliminate the major signals (water and ethanol), a methodology was developed which can suppress both signals from water and ethanol during the NMR experiment without losing signals outside those regions. Similarly to SGF-Profiling™, Wine-Profiling™ provides both targeted and untargeted analysis. The former is performed through the quantification of 56 parameters per sample and their comparison with official reference values, while the latter is carried out through verification models able to detect any deviation from authentic reference data. Models are still under construction to enlarge and maintain the database but at the date of this publication the methodology is well established to control the origin for the major producing countries (France, Italy, Spain, Germany, Chile, Austria), even at the regional level for some major regions (France: Bordeaux, Burgundy, Languedoc, Rhone

Valley, Loire Valley ; Italy : Piemonte, Toscana, Sicilia, Puglia ; Spain : La Rioja, Ribera des Duero) and also to control the major varieties (Red : Pinot Noir, Tempranillo, Garnacha Tinta, Syrah, Merlot Noir, Cabernet Sauvignon, Sangiovese, Nebbiolo, Montepulciano, Primitivo, Dornfelder, Portugieser Blau, Zweigeltrebe Blau ; White : Chardonnay Blanc, Sauvignon Blanc, Riesling, Pinot Blanc/Gris, Silvaner, Verdejo, Mueller Thurgau, Veltliner, Moscatel, Welschriesling. This analytical technique gives information on unforeseen deviations and is a multivariate untargeted analysis useful for a screening control of the market. Interlaboratory comparison is monitored with a dedicated Proficiency Testing Scheme, Pro-PTS, organised by Eurofins Analytics France which controls not only the quantitative parameters but also the interpretation of the sample.

The Wine screener provides additional answers in the control of the authenticity of wines, and in combination with other methods, such as stable isotope analysis as described above, it can offer a performant solution using data fusion. The benefit of fusing NMR data with alternative techniques has been provided by [27]. The authors evaluated the combination of discrete isotopic data with the untargeted NMR spectrum to have better control of wines. Both techniques are known to provide useful information to the characterization of wine: ^{1}H NMR spectroscopy can be used to build robust classification models for grape variety, year of vintage and geographical origin, while stable isotope ratio analysis is a good source of chemical information for the authenticity assessment of food products. By combining these two methodologies, an improvement of classification rates of wine was achieved: 100 % for the determination of geographical origin (60–70 % correct prediction was obtained with stable isotope data alone and 82–89 % with ^{1}H NMR spectroscopy) and 99 % for the vintage of wine (from 88 to 97 % with ^{1}H NMR).

3.3.2. MS metabolomics

Metabolomics represents one of the most recent analytical approaches used in wine authentication. Since wine is a very complex matrix and all its metabolites are physically and chemically diverse, it is not possible to identify all of them in a single platform measurement. Therefore, it is necessary to use different, complementary analytical techniques. Besides NMR mentioned above, mass spectrometry (MS) is frequently used, either ambient or coupled to separation techniques such as gas chromatography (GC) and liquid chromatography (LC) [28–30].

Among mass spectrometry techniques used in wine metabolomics, LC-MS is the most common. It is suitable for determination of non-volatile, thermolabile compounds (e.g. phenolic compounds). One of its main advantages is, that before analysis of the wine, no pre-treatment or extraction of the sample is necessary. However sometimes, simple steps like filtration, dilution or pre-concentration of the sample might be desirable [28].

LC provides metabolite separation based on the different distribution between the mobile (liquid) and stationary phases. For this purpose, the LC system can be equipped with different types of columns, although usually reverse phase columns are used. The ionisation sources frequently used in conjunction with LC-MS are electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI), or atmospheric pressure photoionisation (APPI), however, ESI is the most common. Considering that most metabolites ionise in one ionisation mode (positive or negative), not in both, it is necessary to analyse the samples in both of them, in order to cover a wider metabolome. After ionisation, ions pass through the mass analyser. Since the metabolomics approach is focused on characterisation of the entire composition of small metabolites (metabolome), mass analysers capable of whole metabolome analysis within single analytical run (with good dynamic range, fast scan speed, sensitivity and high mass resolution and mass accuracy) such as Time of flight (TOF) and orbital ion traps are usually used. The resolution achieved is closely associated with the ability of the instrument to measure the accurate mass,

which is crucial for the identification of unknown compounds. The mass error is usually below 5 ppm in the case of TOFs and below 2 ppm in the case of orbital ion traps. Extensive technological improvements have been achieved with the new generation of hybrid instruments, Q-TOFs and Q-Orbitraps, allowing performance of the specific ion fragmentation, bringing an additional dimension by enabling the identification of unknown compounds (MS/MS spectra, i.e. spectra of fragment ions by HRMS) [31]. It should be mentioned that by metabolomics analysis large amount of data is obtained. In order to extract valuable information from the data, pre-treatment steps (data mining, retention time and m/z alignment etc.) and also effective statistical software tools are required for effective data handling (not only for LC-MS, but also for GC-MS and ambient MS) [28,30,32,33] .

LC-MS in combination with metabolomics could be used for different wine authentication purposes, such as classification/discrimination of wine samples according to their variety [34–36], origin/producer [34,37], vintage [34], and quality [34].

Since in most of the cases, this goal is achieved using statistical evaluation of data, marker identification does not represent a necessary step in non-targeted metabolomics studies aimed at sample classification/discrimination [36]. However sometimes, it might be useful to know the identity of a compound related to the sample differentiation. In the following paragraph, examples of several markers are listed.

In the study of Rubert et al. [35], astilbin was identified as a marker of Pinot Noir and different flavonol glucosides as markers of Merlot and Tempranillo (among varieties Pinot Noir, Tempranillo, Merlot, Shiraz, Riesling, Sauvignon Blanc, Silvaner and Chardonnay Blanc). In the study carried out by Roullier-Gall et al. [37], polyphenols, fatty acids, carbohydrates, and amino acids were identified as markers of different wine samples according to the geographical origin/producer (among Chablis, Meursault 1, Meursault 2, and Corton Charlemagne wines).

Another technique frequently used in metabolomics is GC-MS. Unlike LC-MS, this technique is limited to detection of thermostable, sufficiently volatile compounds. Therefore, the main drawback of GC-MS-based metabolomics is the need for sample handling prior to the analysis. The most important of these are procedures enhancing the volatility and the thermal stability of the metabolites (e.g. derivatisation) and procedures (extraction processes) isolating metabolites and enhancing their concentration (e.g. liquid-liquid extraction - LLE, solid-phase extraction - SPE, solid-phase microextraction - SPME) [28,38].

As with LC-MS, a chromatographic separation based on different distribution between two phases is used. However this time, the mobile phase is gaseous. The ionisation source dominantly used in conjunction with GC-MS is electron ionisation (EI) with an ionisation energy of 70 eV. In combination with standardised protocols of data acquisition, the use of EI results in reproducible, rich fragmentation mass spectra. These can then be recorded in large user libraries (e.g. NIST 14 Mass Spectral Library) or compared (matched) with mass spectra (and other additional information) already present in the libraries, in order to confirm the compound identification. This is undoubtedly one of the main advantages of the GC-EI-MS-based techniques [30,38].

Since the requirements for the mass analysers capabilities in GC-MS are similar to LC-MS, mass analysers such as TOF or hybrid Q-TOF are suitable for wine metabolomics. However, the most frequently used mass analyser (due to its relatively low price, high sensitivity and good dynamic range) in GC-MS-based metabolomics is the quadrupole [30,38].

GC-MS in combination with metabolomics is often used to authenticate wine variety [39,40], producer[39] and vintage [39]. In the following paragraph, examples of several markers are listed.

In the study of Kruzlicova et al. [39], various terpenes and alcohols (e.g. α-terpineol, linalool, 1-hexanol and (E)-3-hexen-1-ol) were identified to be the most important markers for wine classification according to the wine variety (among varieties Welsch Riesling, Gruener Veltliner and Mueller Thurgau) Most important markers for wine classification according to the producer/origin (producers located in West and South West Slovakia) were esters, alcohols and carboxylic acids (e.g. diethyl succinate, 2-ethylphenylacetate, (E)-3-hexen-1-ol, 1-hexanol and hexanoic acid) and according to the vintage (years 1996, 1997 and 1998) were alcohols, esters and carboxylic acids (e.g. (E)-3-hexen-1-ol, hexanoic acid, 1-hexanol and ethyl-3-hydroxy butanoate).

Ambient mass spectrometry represents a group of MS-based techniques, which are not coupled with chromatographic separation. In other words, analytes are directly injected / transferred into the mass spectrometer, without prior separation. In comparison with the GC- and LC-MS based approaches, ambient MS is far less informative. It is not capable of isomer separation, quantification of individual metabolites is less accurate and it does not provide additional data such as retention time/factor/index etc. Also, the absence of chromatographic separation prior to MS analyses may increase matrix effects and cause ionisation suppression. However, ambient MS is much faster than GC- and LC-MS-based approaches and for large sample sets analysis it is often the only possible/reasonable option [30].

The ionisation sources frequently used in ambient MS are desorption ESI (DESI), extractive ESI (EESI), direct analysis in real time (DART) or matrix-assisted laser desorption / ionization (MALDI). For the purposes of ambient MS analysis, advanced high-resolution tandem MS instruments such as Fourier-transform ion cyclotron resonance MS (FT-ICR-MS), TOF-MS or Orbitrap MS are usually used [30,41].

Ambient MS was used for example for the authentication of wine variety or to detect adulteration of wine by illegal wine mixing or by colouring [42]. In the following paragraph, examples of several markers are listed.

In the study of Hartmanova et al. [42], different anthocyanins (e.g. malvidin-3,5-diglucoside, malvidin-3-acetylglucoside and peonidin-3-acetylglucoside) were used for authentication of wine according to the variety.

3.3.3. DNA Molecular analysis

The metabolic composition of grapes and wines depends on external factors whereas each grapevine cultivar displays a unique genotype which is independent of growing conditions, such as soil composition, environmental conditions, vintage and cultural practices. For this reason, DNA is the ideal target molecule for efficient variety identification also of wines as an alternative to, or in combination with, chemical profiling.

Identification of grapevine varieties from direct plant material - leaves, fruits, canes and roots - through DNA-based markers is a well-established practice. Simple Sequence Repeats (SSRs or microsatellites) have proved to be the best genetic markers for grapevine DNA typing because of their high degree of polymorphism, species-specificity, co-dominant Mendelian inheritance, reproducibility and simple data. Due to the extensive use of this fingerprinting technology worldwide, large international *Vitis* databases of SSR profiles are now available as references for grapevine varietal identification (Vitis International Variety Catalogue, VIVC - www.vivc.de).

Wine is a complex matrix where the DNA found comes not only from the grapes used for its elaboration, but also from the spontaneous microbiota, or which have been inoculated for alcoholic and malolactic fermentations, as well as from the additives of biological origin and the

concentrated musts that may have been used. The potential of a genetic traceability approach in a such heterogeneous matrix, indeed, is almost unlimited, since the molecular analysis would enable the identification, not only of the grape varieties from which it has been produced, but also the yeasts and/or bacteria strains used for fermentation and to establish if genetically modified organisms (GMO) have been used [43–45]. Thus, the development of genetic analysis would make possible the traceability of a wine at all levels and in all stages of the winemaking process.

However, winemaking implies several processing steps which limit the quantity and quality of DNA available in wine. On one hand the DNase from the microorganisms degrade DNA during fermentation generating denatured and fragmented residues. On the other hand, decantation, clarification, filtration and other fining treatments may contribute to the decrease of the final DNA concentration available. In addition, the co-existence of polysaccharides and proteins interfere with DNA isolation, and other substances — such as polyphenols — act as inhibitors of the polymerase chain reaction (PCR) methodology used for genetic fingerprinting analysis.

Genotyping for grapevine varietal identification can be roughly described in four main steps: DNA isolation from plant material, DNA markers amplification by PCR, analysis of the PCR products by capillary gel electrophoresis and results interpretation [46]. This methodology was first applied for grape juice varietal identification by [47], and then for varietal wine authentication by Siret el al. [48,49], who analysed experimental wines from the start to the end of fermentation. These authors performed successful varietal identification by SSR genotyping in musts, but reported difficulties for the authentication of the cultivars in finished wines due to the scarce DNA isolated. Successive studies have been performed in order to improve DNA isolation from wine, but in all cases, although varietal identification of musts was possible, reproducibility problems for the systematic authentication of finished experimental and/or commercial wines were always reported again due to the extraction of low DNA quantity and quality from a wine matrix [50–60]. Analysis of other marker types, such as chloroplast SSR markers or Single Nucleotide Polymorphism (SNP) markers, has been proposed [51,59]. Multivarietal must mixtures and blended experimental wines have been analysed as well to detect the discriminatory power of the DNA marker technology for determining the varieties used in the mixtures [49–51,55,61]. Although it could be determined if more than one variety was used, the identification of unknown additional cultivars used became impossible, especially when the blends consisted of more than two varieties. Preliminary results obtained using a TaqMan SNP genotyping approach highlighted the potential of Real Time PCR for wine varietal authentication and quantification [59]. The TaqMan assay is much more sensitive than SSR genotyping, not only because it requires a smaller amount of DNA for the analysis, but also because it is based on the analysis of cultivar-specific SNPs. Moreover, this method is more sensitive and precise for relative quantification of each variety in a mixture because is based on specific allele probes.

Despite all the studies performed up to date, the main limiting factor for the development of a standard method for wine varietal authentication remains the quality of grape nucleic acids extracted from wine. The PCR amplification of shorter fragments of DNA that allows access to minute traces of nucleic material seems more promising at least for authentication purposes.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Enzymatic, HPLC, differential pH sensors	Sugars (glucose, fructose, sucrose)	Non-compliance with the established legislative standards, based on the comparison of data with reference limits
Pycnometry, electronic densimeter, hydrostatic balance	Alcoholic strength	
GC-FID	Methanol	
Colorimetry, enzymatic	Glycerol	
Titrimetric measurements	Total and volatile acidity	
TLC, HPLC, GC, CE, IC	Organic acids	
Aphrometer	CO_2	
HPLC	Biogenic amines	
Colorimetry, titrimetry, gravimetry	Anions	
AAS, FP, GFAA, ICP-MS	Cations	
Direct & indirect ELISA	Proteins	Residues of allergenic proteins from fining agent
TLC	Saccharine, dulcin, cyclamate	Use of artificial sweeteners
Stable isotope analysis SNIF-NMR & IRMS	(D/H)I ppm, $\delta^{13}C$, $\delta^{18}O$	Detection of sugar and water addition; application to withering, dealcoholisation, stopping of alcoholic fermentation
IRMS	$\delta^{13}C$ of CO_2	Origin of CO_2 in sparkling wines
ICP-MS	Trace element profile	Geographical provenance
HPLC/UV	Shikimic acid	Grape varieties
HPLC	Anthocyanins	Grape varieties
^1H NMR screening	Overall profile of ^1H NMR spectrum + selection of compositional parameters	Grape variety, vintage, geographical origin
Metabolomics using LC or GC-MS with ESI, APCI, APPI ionisation and TOF, Q-TOF, Q-Orbitrap mass analysers	Various metabolites in wine	Classification of wine samples according to variety, origin/producer, vintage, quality
DNA-based techniques (SSR, microsatellites)	DNA extracted from wine	Grape varieties ; yeast/bacterial strains

5. Conclusion

The authenticity of oenological products appears to be well guaranteed by a complex and robust analytical control system. However, the high value of these commodities generates continuous attack to their genuineness. Botanical and geographical, as well as varietal origins, probably represent the main issues for the sector. Further innovative methods using isotope, mineral and metabolomic profiles integrated with DNA molecular analysis can represent the future of this challenge. Availability of specific and extensive compositional databases and of validated and recognised analytical protocols are required. However only a higher awareness of these new approaches from the competent governmental control bodies and courts will make it possible to reach a superior control of frauds and mislabelling.

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Spirit drinks

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General overview of the products

Spirit drinks are a significant category of food product when considered economically, legally and culturally. Economically, spirit drinks represent an important outlet for agricultural production, and generate considerable revenues for the public purse via excise duty and other taxes. Their cultural and economic importance is reflected both in the large number of key rulings in the development of European Union (EU) food law that relate specifically to spirit drinks, and the unique protection their geographical indications hold within trade law.

The economic significance of spirit drinks can be seen in a number of metrics. In the EU, spirit drinks are the largest agri-food export with almost two-thirds of the sector's production being exported, contributing to a positive balance of trade of around EUR 9 billion [1-3]. The spirit drinks sector also contributes around EUR 23 billion annually to the EU in excise duties and VAT and around 1 million jobs can be linked to the production and sales of its products [2].

Foods associated with specific geographical areas are of great economic importance and this has led to the introduction of systems by which geographical indications (GIs) can be protected. The cultural significance of many spirit drinks is indicated by the large number of associated GIs registered in this sector ([4, Recital 2]). At an international level, the agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs) [5] is arguably the most important international treaty offering protection for GIs. This notably offers advanced protection to the GIs for spirits (and wines) compared to other agricultural products, underlying the national importance of these products.

To sustain its importance in global trade, the spirit drinks industry needs to maintain consumer confidence in its products. One of the key areas in supporting this position is by offering assurance that spirit drinks sold in global markets are authentic. Spirit drinks are excisable food commodities and often command premium price tags, which adds considerably to their allure to the counterfeiter. Excise duty is added to alcoholic products in most countries [6], and spirit drinks are often taxed at higher levels than other alcoholic beverages [7,8]. Counterfeit products will be produced without consideration of excise requirements and consequently offer large profits to the

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counterfeiter. In the UK, it has been estimated that the Treasury loses GBP 1.3 billion annually through alcohol fraud [9].

An effective legal framework for tackling the production and marketing of fraudulent spirit drinks requires two elements. The first element is a clear and enforceable set of definitions of the spirit drinks categories, including their production processes and specific analytical and organoleptic characters. The second element is a range of appropriate analytical methods that will help confirm that a suspect spirit drink product meets its labelling claims, according to the legally established definitions. These two elements, as well as an overview to the common spirit drink frauds such a system is designed to tackle, are explored in the rest of the chapter.

1. Product Identity

1.1. Definition of the product and manufacturing process

Spirit drinks can be simply defined as alcohol beverages created from the distillation of fermented agricultural raw materials. Exact terminology and definitions will vary on jurisdiction, but these key elements, along with the requirement that the distillate be intended for human consumption (potable), will be common to most markets.

1.2. Current Standards of Identity and Related Legislation

1.2.1. European Union – Spirit Drink Categories

In the European Union, the current definition of a spirit drink is contained in the Spirit Drinks Regulation 110/2008 [4] on the definition, description, presentation, labelling and the protection of geographical indications of spirit drinks[1]. This regulation (Article 2) defines a spirit drink as an alcoholic beverage that is:

(a) intended for human consumption;

(b) possessing particular organoleptic qualities; and

(c) having a minimum alcoholic strength of 15 % vol[2].

and contains a distillate of a naturally fermented agricultural product.

None of the alcohol contained in a spirit drink shall be of synthetic or non-agricultural origin (Article 3(4)). The nature of the raw material that may be considered agricultural in origin is contained in the Treaty on the Functioning of the European Union (TFEU) [10] in Annex I.

There are three types of distillate defined within the Spirit Drinks Regulation [4]. The first is one that meets the definition of a spirit drink, as outlined above. The second is ethyl alcohol of agricultural origin (EAAO), a highly rectified distillate meeting specific technical requirements [4, Annex I(1)], including a minimum alcoholic strength of 96.0 % v/v[3]. This creates a distillate that is

[1] Regulation 110/2008 is, at the time of writing, currently undergoing revision. However, it is assumed that most, if not all of the points noted in this chapter will be retained in any forthcoming legislation.

[2] With the exception of egg liqueur or advocaat or avocat or advokat where the minimum strength is 14 %.

[3] The EU regulation does not specifically state that EAAO has to be a *distillate* of agricultural ethanol, but (given the minimum strength) this is effectively the case.

designed to be low in compounds other than ethanol and water, and consequently neutral in its flavour profile. The last category of distillate is a “distillate of agricultural origin”. This covers any agricultural distillate that does not meet the criteria for a spirit drink or ethyl alcohol of agricultural origin. Spirit drinks can be created directly from a distillation of naturally fermented products, or can be produced from appropriate treatments of EAAO, distillates of agricultural origin or other spirit drinks [4, Article 2].

Within the Spirit Drinks Regulation [4, Annex II] there are 46 defined categories of spirit drinks. The first 14 of these have certain restrictions placed on their production [4, Article 5]. These include the sole use of the raw material contained within the category definition for the production of alcohol, a prohibition on the use of EAAO and flavourings, and restrictions in relation to colouring and sweetening. Examples of such spirits include rum, whisky and brandy. Unless otherwise stated in their category definitions, the remaining 32 categories may use any agricultural raw material as the origin for the alcohol, EAAO, as well as any permitted flavourings, colourings and sweeteners. All alcoholic beverages which meet the definition of one of the 46 spirit drink categories must “bear in their description, presentation and labelling the sales denomination assigned therein”; for those spirit drinks that do not fall into one of the 46 categories, they “shall bear in their description, presentation and labelling the sales denomination ‘spirit drink’.” [4, Article 9(1-2)].

It is notable that the European legislation for the definition of spirit categories are typically process definitions. Whilst all spirit drinks categories specify minimum alcohol strength, few additional analytical parameters are set in the legislation against which compliance can be judged - examples include:

- limits for anethole concentration in pastis, pastis de Marseille, sambuca and Mistrà;
- limits for sugars in various liqueurs and the spirit drink Berenburg/Beerenburg; and
- a minimum egg yolk content in Egg liqueur/advocaat/avocat/advokat or liqueur with egg.

1.2.2. European Union – Geographical Indications

The Spirit Drinks Regulation provides for the ability to apply stricter definitions for locally produced spirit drinks [4, Article 6(1)]. This typically applies to the production of geographical indications, for example United Kingdom legislation [11] defines a tighter production specification for whisky produced in Scotland. The sales denomination as described above may be replaced or supplemented with a geographical indication [4, Chapter III; 12]. Given the economic importance of GIs, their use is often subject to fraud, and any additional production specifications should be considered when determining whether a suspect product is consistent with a GI sales denomination. The Spirit Drinks Regulation requires that GIs are produced in accordance with the specifications contained within an associated technical file and that this is verified by an appropriate body [4, Article 22].

The Spirit Drinks Regulation [4] and the associated regulation detailing Union Reference Methods for spirit drinks [13] provide the basis for authenticating spirit drinks in the EU. GI verification, however, has also been exploited in the protection of the sector. The Scotch Whisky Verification Scheme [14], for example, comprises three interlocking elements offering additional protection to this whisky GI. Firstly, the scheme provides an audit of all production facilities, including importers of bulk Scotch Whisky (on a 2-year cycle) via documentary and physical checks according to defined requirements [14, Annex B]. Secondly, a tight chain of authenticity is provided by the requirement that a production facility, in whatever location, cannot pass product to another unless it has been verified. Finally, a register that lists all the verified production facilities and importers is

available on the Her Majesty's Revenue and Customs website [15], as well as a register of all brands produced at verified sites. Such a scheme offers both protection against fraud, and a market opportunity for guaranteeing authenticity, and has been looked at by other EU spirit producers, e.g. Swedish vodka and Dutch gin [16].

1.2.3. Spirit Drink Legislation outside the European Union

The harmonisation of spirit drink definitions across the European Union (first introduced in 1989) and the introduction of geographical indications can be seen as necessary support measures for rural communities and an important sector of agricultural produce [17]. This has led to a detailed set of regulations, with specific process definitions for categories and geographical indications.

Other jurisdictions typically have significantly less extensive ranges of spirit drink categories and fewer restrictions on methods of production. Unsurprisingly, a range of conflicts are established by different cultural expectations of the characteristics possessed by certain spirit categories. This can be seen by considering one of the category definitions – whisk(e)y – in some key pieces of national legislation that apply to spirit drinks.

According to the European Union definition [4, Annex II (2)]:

(a) Whisky or whiskey is a *spirit drink produced exclusively by:*

- *(i) distillation of a mash made from malted cereals with or without whole grains of other cereals, which has been:*
  - *saccharified by the diastase of the malt contained therein, with or without other natural enzymes,*
  - *fermented by the action of yeast;*
- *(ii) one or more distillations at less than 94.8 % vol., so that the distillate has an aroma and taste derived from the raw materials used,*
- *(iii) maturation of the final distillate for at least three years in wooden casks not exceeding 700 litres capacity.*

The final distillate, to which only water and plain caramel (for colouring) may be added, retains its colour, aroma and taste derived from the production process referred to in points (i), (ii) and (iii).

(b) The minimum alcoholic strength by volume of whisky or whiskey shall be 40 %.

(c) No addition of alcohol as defined in Annex I(5)[4]*, diluted or not, shall take place.*

(d) Whisky or whiskey shall not be sweetened or flavoured, nor contain any additives other than plain caramel used for colouring.

1.2.3.1. Canada

The Canadian Food and Drug Regulations [18] define 8 different spirit categories (whisky, rum, gin, brandy, liqueurs and spirituous cordials, vodka, tequila and mezcal), although provision is made for the protection of a number of geographical indications, such as Scotch Whisky, Bourbon Whiskey, Cognac, Armagnac and Grappa. The Canadian definition of whisk(e)y is an example of a specification that approximates closely to the European Union definition.

[4] This is *ethyl alcohol of agricultural origin* (EAAO), a highly rectified distillate meeting specific technical definitions and requirements (Regulation 110/2008, Annex I(1)), including possessing a minimum alcoholic strength of 96.0% v/v.

For example, in Canada, whisk(e)y is defined as:

"*a potable alcoholic distillate, or a mixture of potable alcoholic distillates, obtained from a mash of cereal grain or cereal grain products saccharified by the diastase of malt or by other enzymes and fermented by the action of yeast or a mixture of yeast and other micro-organisms and may contain caramel and flavouring*" [18, B.02.010]

and

"*no person shall sell for consumption in Canada any whisky that has not been aged for a period of at least three years in small wood*" [18, B.02.023(1))]

where small wood is defined as "wood casks or barrels of not greater than 700 L capacity" [18, B.02.002]

However, though many of the provisions are similar there are notable differences: the Canadian whisk(e)y definition does not define a maximum distillation strength, thus allowing the inclusion of highly rectified alcohol, akin to EAAO; there is no minimum alcohol strength (although Canadian Whisky itself and other geographical indications, such as Scotch, all set a minimum of 40 %); malt is not an essential component of the saccharification process; and limited flavouring of whisky can take place. Such differences will alter the range of flavour and analytical profiles covered by the definition.

1.2.3.2. The United States

The spirit drinks definitions in the United States are within Part 5.22 in Title 27 of the Code of Federal Regulations, which governs food and drugs [19]. As with the European Union and Canada, categories of spirit are defined by their manner of production. These include: neutral spirits (the US equivalent of EAAO, including vodka), whisk(e)y, gin, brandy, blended applejack (apple brandy), rum, tequila, cordials and liqueurs, flavored spirits (brandy, gin, rum, vodka, and whisky). There are a number of geographical indications specifically referenced within these categories (e.g. Scotch Whisky, Canadian Whisky, Pisco and Cachaça) as well as some standards of identity that are culturally significant to the U.S. market (e.g. blended applejack and corn whisky).

As with the Canadian definition, the U.S. standard of identity for whisk(e)y is very similar to the European definition. It states that (5.22 (2)(b)):

"'Whisky' is an alcoholic distillate from a fermented mash of grain produced at less than 190° proof in such manner that the distillate possesses the taste, aroma, and characteristics generally attributed to whisky, stored in oak containers (except that corn whisky need not be so stored), and bottled at not less than 80° proof, and also includes mixtures of such distillates for which no specific standards of identity are prescribed."

In this case, there is a minimum alcohol strength equivalent to 40 % alcohol by volume and a maximum distillation strength of 95 % alcohol by volume (just 0.2 % above the EU limit). It is also slightly more specific in the wooden containers used for maturation, although the worldwide use of wood other than oak for maturation is negligible. However, there is increased latitude in other areas of the definition compared to the European Union. No minimum maturation time is specified for whisk(e)y in general, and whilst American straight whiskies require at least 2 years storage in oak containers, this is less than the 3 years associated with European whiskies. Corn whisky does not need to be matured at all. An additional section (5.23) also provides for the qualified addition of harmless colouring, flavouring, or blending materials such as caramel, sugar and wine, a much wider range of materials than the sole permitted additive of plain spirit caramel in the EU.

1.2.3.3. Australia

Other jurisdictions have much looser definitions than those seen above. An example is Australia, where there are certain provisions under the *Australia New Zealand Food Standards Code* which govern spirits where these are manufactured or imported into Australia. Principally, Standard 2.7.5 [20] defines brandy, liqueurs and spirits in general. All definitions are light on details of production, in particular the definition for spirits (2.7.5-2), given that only 2 categories are specifically referred to in the same legislation. This states that a spirit means an alcoholic beverage consisting of:

(a) a potable alcoholic distillate, including whisky, brandy, rum, gin, vodka and tequila, produced by distillation of fermented liquor derived from food sources, so as to have the taste, aroma and other characteristics generally attributable to that particular spirit; or

(b) such a distillate with any of the following added during production:

(i) water;

(ii) sugars;

(iii) honey;

(iv) spices.

In addition, all spirits have a minimum alcohol strength of 37 % alcohol by volume (2.7.5-3). As can be seen, such a definition, for whisk(e)y say, provides little in the way of specifics about the production methods of the spirit, so long as a subjective organoleptic assessment indicates the standard of identity has been met, and there is a lower minimum strength than in Europe. In addition, a number of additives such as sugar, honey and spices are permitted under the definition, contrary to European legislation for whisk(e)y.

Some additional process information can be found in the Excise Act 1901 [21, Section 77FI] and the Customs Act 1901 [22, Section 105A]. However, these are similarly light on detail compared to their European definitions. Both pieces of legislation provide minimum maturation requirements for brandy, rum and whisky. However, these are limited to the requirements that these spirit types are stored for a minimum of 2 years in wood. They also define the materials for the production of brandy (grape wine), rum (a fermented liquor derived from the products of sugar cane) and whisky (a fermented liquor of a mash of cereal grain).

However, the Australia New Zealand Food Standards Code does contain explicit protection for spirit drink geographical indications, including a specific requirement that products produced in accordance with a geographical indication, but shipped and bottled elsewhere, must meet the minimum alcohol strengths of the laws relevant to the geographical indication.

1.2.3.4. India

India has (based on the regulations to be enforced from April 2019 onwards) a number of spirit category definitions [23], including brandy, gin, rum, vodka, liqueurs/cordials/aperitifs and whisk(e)y. Like other jurisdictions, a number of culturally significant definitions are included: country liquors, fenny and pot distilled spirits. A key example of the conflict between different cultural perceptions of a spirit category can be seen in the whisky definition [23, Section 2.8]). Whilst placing an emphasis on cereal being the raw material for whisky production, it is clear that whisky can also be made from neutral spirit, which can be made from fruits, vegetables, molasses or any other source of carbohydrates of agricultural origin, as well as grains and has a minimum alcohol strength of 96 % alcohol by volume [23, Section 1.2.9]. This is a clear contradiction to most other definitions of whisk(e)y, which require a cereal substrate, a maximum distillation strength (to retain an appropriate level of organoleptic character from the raw material) or both.

India is also a country that has imposed a number of analytical limits on the spirit beverages it defines [23 Table 1]. These are individually tailored to each category of spirit. Some of these limits are obviously intended to act as a general restriction on compounds of public health concern, such as the levels for heavy metals, although it is unclear why spirits produced according to good distilling practice should ever be at risk of exceeding such limits, and thus why such category-based limits are required.

The inclusion of other varying limits based on spirit category (such as total esters and higher alcohols) are more typical of quality-based specifications, but unlike the European regulations that relate limits to some characteristic of a particular product category (e.g. the high sugar content of liqueurs or the specific flavouring requirements of aniseed spirits such as Pastis) these are applied to each category in turn. Thus, the limits in effect represent an attempt to provide an analytical definition to a product category. Such limits, whilst seemingly providing some guidance as to appropriate analytical range for authentic products, should be treated with caution. Whilst usually covering a large proportion of a category, they do not always include all the various styles and variations contained within a spirit drink category definition. They can therefore act as misleading guides to authentic database ranges and may also restrict trade in genuine products.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Brand and Generic Counterfeits

There are many forms of spirit drink fraud. Some frauds may be simple product substitutions, where authentic spirit drink bottles are refilled with cheaper, poor quality replacements. Other counterfeiting operations can be very sophisticated, involving products deliberately created to avoid detection by the analytical investigator. However, when considering spirit drink fraud there are two broad categories of counterfeit produced, brand counterfeits and generic counterfeits[5].

A **brand counterfeit** fraudulently trades on the reputation associated with a particular brand of spirit drink. It could involve the direct copying of the brand packaging and filling with non-authentic liquid. Alternatively, brand counterfeiting could comprise the collection of authentic used packaging, refilling it with counterfeit product, and application of new closures.

A **generic counterfeit** product fraudulently trades on the premium quality associated with a defined category of spirit drink, e.g. Scotch Whisky, Cognac or Vodka. Whilst not claiming to be a brand with a recognised reputation in the marketplace, it will use a familiar category definition of spirit drink in its labelling to command added value to which it is not entitled. Geographical indications are often the targets of generic counterfeiting, due to the associated reputations of these spirit drinks. Such frauds may be explicitly signalled in the labelling by use of the regional name (e.g. Armagnac, Scotch Whisky) or implicitly indicated using brand names or imagery associated with that area.

[5] Counterfeit alcohol is just one type of illegal alcohol. For information on the correct terminology to use when discussing legal and illegal alcohol refer to the resources produced by The International Alliance for Responsible Drinking [24], in particular the section on taxonomy of the alcohol market [25].

The two cans shown in Figure 1 claimed to be Scotch Whisky and are examples of generic counterfeits, trading on the goodwill associated with that geographical indication. These products were canned in Austria and sold in the Middle East. They were manufactured from industrial alcohol and flavouring. In total, it has been estimated that 15 million of these cans were sold over a period of a few years, which provides an indication of the scale of some of these spirit drink counterfeiting operations.

For both generic and brand counterfeiting, the liquid inside the bottle is often the extension (dilution) or replacement of the authentic product with: (i) water; (ii) cheaper locally produced spirit, (iii) neutral alcohol (a highly rectified spirit lacking in flavour, used as a base to produce many genuine spirits) or (iv) an alternative alcohol. These products may also contain added sweetening or flavourings to mask the inferior flavour of the counterfeit spirit or to mimic aromas of the genuine spirit.

Figure 1: Examples of Counterfeit Scotch Whisky

2.1.2. Subsitution with cheaper brands and water

Aylott [26], in his review of modes of spirit drink counterfeiting, highlights the practice of product substitution, the swapping of a higher value branded product for a value product of the same category. Previous references have been made in his work to both gin and whisky product substitution [27,28]. Lachenmeier [29] notes that brand fraud has been observed in restaurants and bars, especially in establishments that sell very cheap alcohol. In such cases, the bar operator may refill bottles of branded spirits with cheaper brands of the same type of spirit (in Germany often from so-called discount stores) [30]. Additionally, dilution of the branded spirits or cheaper spirits with water may occur. Spirit products may gain significant additional value by virtue of their rarity or age. Counterfeit spirits may be produced to take advantage of such elevated prices paid for in rare spirit auctions. Both the packaging and the liquid may be analysed to identify whether their ages are consistent with any labelling claims.

2.1.3. Subsitution with other forms of alcohol

The illegal production of spirit drinks is often carried out by simple substitution or dilution of authentic beverages with alcohol, adjusted to the appropriate strength with water, or a mixture containing such alcohol with colouring and/or flavourings ([31,32]. Different types of alcohol may be used in this process: distillates of agricultural origin, including highly rectified products such as

neutral spirit or ethyl alcohol of agricultural origin; synthetic alcohol; some alternative alcohol such as methanol; or industrial alcohol.

The alcohol used for extension or substitution may be from non-permitted agricultural substrates, i.e. the botanical origin of the alcohol is incorrect. For example, rum can only be created from sugar cane by-products or sugarcane juice, according to most spirit drinks legislation. The identification of alcohol from different agricultural origins will signify a fraudulent product [33, pages 18-19]. Whilst constituents of the distillates of the incorrect agricultural origin may make their presence detectable in a fraudulent product [34] much work has been undertaken on the use of the stable isotopic ratios of ethanol and water to detect this fraud [35-38]. Isotope ratios are of particular importance when the product is fraudulently substituted or diluted with highly rectified neutral spirit and for reasons of natural variability the levels of components in the authentic product are insufficient to detect this practice [39]).

The use of alternative alcohols added to potable ethanol from agricultural substrates is particularly attractive to spirit drink counterfeiters, since there is no excise duty to be paid. Synthetic alcohol has been used to produce counterfeit spirits. For example, tequila made from synthetic alcohol (probably derived from petroleum) has been identified [33, page 20], as has the falsification of vodka using synthetic ethanol [40]. Denatured alcohol is used in a number of industrial applications. This product, exempt from excise duty after the addition of specific chemicals (denaturants) designed to render the alcohol non-potable, has also been used as the base alcohol for counterfeit products [33, page 10]). Whilst the denaturants are often added to specifically mark a product as denatured alcohol, counterfeiters will often attempt to remove these compounds, thus recovering the alcohol in an unmarked form and making its presence in counterfeit spirits hard to identify [41].

2.1.4. Additives

Counterfeit products may also contain added sweetening or flavourings to mask the inferior flavour of the counterfeit spirit or to mimic aromas of the genuine spirit. Depending on the legislation relevant to the spirit category, these additives may not be permitted in the genuine products. An example of this is where sugars are illegally added to whisky [31].

2.2. Potential threat to public health

In some cases, counterfeit product can be dangerous and pose a risk to consumer health, particularly when non-potable alcohols such as methanol or denatured alcohol are employed.

Methanol (methyl alcohol) is potentially toxic. Its consumption can cause blindness, other severe health complications and death. A maximum tolerable concentration for methanol in alcoholic beverages has been estimated as 2 % by volume in a 40 % spirit drink [42]. Methanol occurs naturally in most alcoholic beverages at levels without any danger to public health. However, there are many recorded incidents of its harmful presence in counterfeit spirit drinks [43]. Its presence is most likely to have been introduced to the illicit beverage by a counterfeiter who hopes to profit from methyl alcohol's lower cost compared to ethyl alcohol [29,44].

In 2011, in the UK, 10 people became ill after consuming vodka that smelled of nail varnish remover. Twelve thousand litres were seized, and the product was found to have contained high levels of methanol that had been diluted into the final product [45]. In 2012, methanol poisoning incidents were reported in the Czech Republic from the consumption of deliberately adulterated

spirits. To contain the problem the Czech authorities temporarily banned the consumption of spirits above 20 % alcohol by volume. At least 36 deaths were related to this incident [29].

Another health risk that can arise is from metals used in illicit stills and other production materials that are unfit to come into contact with food products. Genuine producers will take steps to prevent any unwanted contamination from metals and plastics or other food contact materials that could leach into the final products. Counterfeiters are either unaware of these risks or are not concerned enough for the health of their customers. Where industrial alcohol has been substituted into the food chain similar concerns occur, with the added health impact of the chemicals used to denature the alcohol. Elevated levels of metals in illicit alcohol include metals such as lead, arsenic and mercury. These have been linked to makeshift illicit distilling apparatus using a variety of reused metal components that may leach harmful toxins into the distillate, including the aforementioned metals [43,46-48].

Chloroform has been detected at high levels in illegally produced alcoholic products [49], creating an increased risk to the public. This could be due to a process used by some counterfeiters to remove the common denaturant denatonium benzoate, which has a unpalatable bitter taste, from denatured alcohol, via the addition of hypochlorite [50,51]; chloroform is known to be a product of hypochlorite and ethanol [52]. Other denaturants may also have health impacts, to a greater or lesser degree. The impact of methanol has already been noted; other compounds, whilst not exhibiting acute toxicity would still be regarded as unwanted contaminants of toxicological significance [43].

3. Analytical methods used to test for authenticity

There are many well-established methods available for authentication of spirit drinks. However, not all methods will be applicable to every authentication challenge. As noted previously, there are two principal types of counterfeit activity within the spirit drinks sector, generic counterfeits and brand counterfeits. A variety of analytical techniques can be employed to confirm whether a suspect sample is consistent with its production requirements, for the detection of generic counterfeits. This includes a set of official EU reference methods contained within their own regulation [13] and developed specifically to test parameters listed within the EU regulation that defines spirit drinks categories [4]. Some spirit categories are often too variable in terms of composition to permit generic authentication, although if some form of analytical limit is defined in legislation, this can be of assistance. The EU definition of liqueurs, for example, allows for a wide variety of product formulations; despite the limits on minimum sugar content, this will be insufficient to define such a varied category.

The analytical methods frequently used to authenticate spirit categories can be applied to a more tightly defined set of parameters associated with a particular brand to identify brand counterfeits.

Spirit drinks authenticity analyses can identify with certainty if a product is not genuine (e.g. because the results are inconsistent with a particular brand or the production parameters contained within a spirit category definition). However, they will never be able to confirm with certainty that a suspect product is genuine, only that, based on the tests undertaken, the suspect product is *consistent* with a genuine product. Similarly, spirit drinks authenticity analyses will be unable to confirm, with certainty, the actual nature of a non-genuine product.

Category authentication, brand authentication and screening technologies all have their specific uses and applications. The aim of this section is to assist in spirit drink authentication by providing supplemental information, references and guidance for analytical methods commonly employed. The officially recognised methods referred to herein relate to three main sources of reference methods: Commission Regulation (EC) No 2870/2000 (Union reference methods of analysis) [13], the OIV Compendium of International Methods of Spirit Beverages of Viticultural Origin [53] and the AOAC International Official Methods of Analysis [54], which are typically the methods of analysis referred to by the U.S. Alcohol and Tobacco Tax and Trade Bureau (TTB). The methods in the OIV are often aligned with those in the Union reference methods. Some national markets will have their own official methods, which will often be variations of the techniques referred to in the above standards. Where significantly differently methods are employed, it would be advisable to demonstrate equivalency to the common methods referred to here.

3.1. Officially recognised methods

3.1.1. Alcoholic Strength

The measurement of alcoholic strength is important for quality control and product integrity, but also has implications in terms of regulation and excise duty. As noted, all the spirits defined within the EU spirit drinks definitions have a minimum alcohol strength requirement with which genuine products need to comply. Dilution with water below the minimum alcohol strength limit, or significant differences between the label and measured alcohol strength (e.g. outside acceptable tolerances as given in [55, Annex XII]) are key indicators of counterfeit products.

Accepted reference methods are based on the measurement of liquid density (densitometry), following a prior distillation step. Key reference methods are documented in the Union reference methods (Annex I) and the aligned OIV methods (OIV-MA-BS-01 to OIV-MA-BS-05). Following the distillation step, samples can be analysed by one of three types of densitometry methods (pycnometry, electronic densitometer, and hydrostatic balance). Pycnometry and densitometer methods are detailed in the TTB recommended AOAC methods for distilled spirits (942.06, 945.07, 982.10 and 983.12).

The distilled samples under analysis are assumed to be mixtures of pure ethanol and water; hence the density of the liquid can be directly related to the alcohol strength. Conversion to alcoholic strength is carried out using official alcohol tables (manually or automatically). In the EU, all measurements are based on the density of alcohol and water at 20°C. The principle tables for conversion in the EU are the International Alcoholometric Tables prepared by the International Organisation of Legal Metrology (OIML) [56]. The AOAC density measurements are however all taken at 15.56°C (60°F) which produces a slightly different alcohol strength by volume from the EU measurements (0.07 % difference at 40 % alcohol by volume).

The identified reference methods are by default measured after a distillation step. This is referred to as a real, true or actual alcohol strength measurement. Direct analysis of the spirit has been found to be satisfactory for samples such as vodka, gin and whisky. However, it cannot be used for samples that contain high levels of non-volatile material such as sugars, creams or wood extractives, as these compounds affect the density measurement and thus the reported alcohol strength. Such products need be distilled prior to density measurement to obtain the actual strength. If a high degree of accuracy is required or there is some uncertainty as to whether the product contains non-volatile material that might obscure the density measurement, an actual or real strength should be carried out.

Spectroscopic methods involving near-infrared (NIR) are commonly used within the industry to provide alcohol strengths, since real strengths can be obtained for many distilled spirit matrices without the need for a distillation step. However, only the OIV currently has an official method detailing the application of NIR spectrometers (OIV-MA-BS-08). This method of determining the real alcoholic strength is based on the physical principle of the spectral analysis of materials with absorption bands in the near infrared range. The key point about the use of such apparatus is that, as noted in the method, the NIR equipment needs to be appropriately calibrated and verified against an appropriate reference set of samples, measured using an approved reference method for real strength as referred to above.

3.1.2. Major Volatile Congeners

The major volatile congeners are principally produced during the fermentation stage of spirit production and carried over via the distillation process to the resulting spirit. They represent, as the name suggests, a subset of compounds at relatively high levels in distilled spirit (typically at ppm levels). The level of these congeners and their proportions to each other can lead to conclusions regarding the production process, hence their use in authentication and quality control. A smaller subset of these compounds are the higher alcohols, which are often used for the same purposes.

The officially recognised methods of analysis use Gas Chromatography (GC) with Flame Ionisation Detection (FID) for detection of most of the major volatile congeners. The Union reference version (Annex III.2) measures selected aldehydes (acetaldehyde and ethanal), higher alcohols (propan-1-ol, butan-1-ol, butan-2-ol, 2-methylpropan-1-ol, 2-methylbutan-1-ol and 3-methylbutanol-1-ol), ethyl acetate and methanol. The OIV aligned method is OIV-MA-BS-14. The AOAC methods 968.09 and 972.10 measure specific higher alcohols and ethyl acetate; a separate method measures methanol (972.11). The Union reference methods also includes volatile acidity (measured as acetic acid) in its measurement of volatile substances (Annex III.3), the levels of which, whilst affected by fermentation and distillation, are also strongly influenced by any maturation that certain spirit categories may be required undergo (see also OIV-MA-BS-12 and AOAC 945.08).

A common mode of adulteration for some spirit categories is the prohibited addition of neutral spirit (or EAAO) which is often used in product ‘stretching’. The high distillation strength of neutral spirit leads to a concomitant reduction in levels of many of the major volatile congeners. Hence, the illegal dilution of certain spirits with this product may often lead to an observable reduction in key compounds, particularly the higher alcohols.

Major volatile congener concentrations can also provide information on the raw material from which the spirit is made. The ratios of 2-methylbutan-1-ol and 3-methylbutan-1-ol (also known as the amyl alcohols) can differ between different fermentation substrates such as cereals, sugar cane and grape [31]). Trace methanol concentrations are useful as grape fermentations produce more methanol than cereals and cereals produce more methanol than molasses [26].

Once established ranges have been set, major volatile congener profiles are particularly effective for brand authenticity, as often tight ranges can be obtained. Generic authenticity such as spirit category identification and classifications within a spirit category (such as Single Malt Scotch Whisky) are also possible, although wider tolerances will need to be set.

3.1.3. Maturation Related Congeners

A number of spirit category definitions (such as whisky and brandy in the EU), involve maturation in wooden casks as part of the requirement of their production. During maturation, a number of compounds are extracted from the wood into the spirit. The maturation related congeners are quantified by liquid chromatography with detection via ultraviolet spectrophotometry, and spectrofluorimetry. The Union reference method (Annex X) and OIV-MA-BS-16 are aligned, having been validated for whisky, rum, cognac, bourbon and wine spirit.

Maturation can theoretically take place in different types of wood, but oak is by far the predominant material of choice. Extracted wood congeners are present at consistent ratios to each other and at concentrations that increase with maturation time, representing age. Data on the consistency of profile for spirits matured in oak is demonstrated in the published literature [31,57-59]. Ratios and ranges of compounds can be used to determine if a product is authentic by comparison with those observed in genuine products. This will rely heavily on databases generated through analysis of authentic samples and is applicable for both category and brand authentication. The maturation congener profile can also be used to detect where wood extracts or flavourings have been added, often to cover up the absence of a maturation period [60].

3.1.4. Sugars

Sugars may be found in a variety of different spirit types. The individual composition and levels observed will be related to the spirit category and how that spirit is produced. In the EU (Regulation No 110/2008), some spirit categories such as liqueurs require the addition of a minimum concentration of sugars for sweetening; others allow the addition of sweetening sufficient to round off the final taste of the product. Still other categories (e.g. whisky) prevent *any* sweetening by the addition of permitted carbohydrate sources. Trace levels of certain sugars can be naturally present in some spirits as a result of the post-distillation manufacturing procedures of maturation and addition of caramel colouring.

It is common for counterfeiters to add sugars to poorly produced, fraudulent products to try and improve the taste or mimic the natural sweetness of a genuine product. To confirm if sugars are present naturally as opposed to adulteration, the sugar profile of the suspect product should be compared with the known ranges and ratios encountered within the spirit category or brand. For example, analysis of genuine Scotch Whisky products has shown that, where sucrose is present, the level is considerably less than the concentration of glucose and fructose [31].

Liquid chromatography (LC) with Refractive Index (RI) detection is a common technique for sugars analysis. This technique is principally used for quality control of distilled spirits containing high (g/L) levels of sugar content such as liqueurs and pastis. The Union reference method (Annex VIII) and the OIV method (OIV-MA-BS-11) for measurement of total sugars (glucose, fructose, sucrose, maltose and lactose) are aligned. LC-RI is not suitable for identifying sugars adulteration in spirit categories that contain low levels of sugars such as vodka, gin and whisky. A much more effective method is Ion Chromatography (IC), typically used in conjunction with a pulsed electrochemical detector (PAD) [31]. This technique can allow trace levels of individual sugars present naturally in certain spirits to be distinguished from higher levels that can only be achieved by adulteration.

3.1.5. Stable Isotope Ratio Analysis

The applicability of stable isotope ratio measurements to the detection of spirit adulteration will be dependent on the individual spirit and its method of manufacture. Uses include the detection of alcohol from a botanical origin that is not specified in the product definition, and the addition of synthetic alcohol or flavours. The success of these techniques is dependent on the isotope ratios in the natural product being sufficiently different from those in the adulterant.

Two stable isotope ratio analysis methods are currently officially recognised for the application to distilled spirits by the OIV. These are the analysis of the $^{13}C/^{12}C$ ratio of ethanol using stable isotope ratio mass spectrometry, SIRMS, (OIV-MA-BS-22) and the determination of the deuterium distribution of ethanol using nuclear magnetic resonance, SNIF-NMR (OIV-MA-BS-23). Such techniques have also been officially recognised by the EU, OIV and AOAC for determining the origin of sugars in other matrices [37]. Both Carbon-13 SIRMS and deuterium SNIF-NMR have been shown to differentiate between spirit samples containing ethanol from different sources of fermentable sugars. For example, Carbon-13 SIRMS can differentiate between sugars coming from C_4 metabolism plants (e.g. cane, maize, etc.) and C_3 metabolism plants (e.g. wheat, barley, grapes), or Crassulacean acid metabolism plants (e.g. agave) and synthetic ethanol derived from petroleum; deuterium SNIF-NMR can demonstrate similar differentiations [33,61].

In addition to the $^{13}C/^{12}C$ and $^{2}H/^{1}H$ ratios of ethanol, the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios of water have been used for the determination of geographical origin, where this can be definitively linked to the area of production. The OIV have a recognised method for the analysis of $^{18}O/^{16}O$ ratios of water in wines and must, rather than for distilled spirits, in its Compendium of International Methods of Wine and Must Analysis Vol. 1 [62]. This technique and others, such as the $^{18}O/^{16}O$ ratio of ethanol [63] or the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios of bulk spirit [38] may be applied to spirit drink authentication. Whilst not an officially recognised method, Carbon-13 SNIF-NMR has also recently been introduced to allow the practical separation of C4 metabolism plants from some Crassulacean acid metabolism plants, for example ethanol from cane or corn and ethanol from agave [37].

3.1.6. Metals

There are several recognised methods for the analysis of metals in distilled spirits. This reflects both quality control measures and potential concern from external regulators about the levels of such metals in the food chain. (Based on the low risk presented by the sector's products, however, the EU assigns no analytical limits to distilled spirits.) The OIV has four recognised methods for metals analysis by atomic absorption spectroscopy (AAS): calcium, copper, iron and lead (OIV-MA-BS-29 to OIV-MA-BS-32). The AOAC also has methods for distilled spirits using similar methods such as atomic absorption techniques for copper (967.08) and iron (970.12).

Modern laboratories however can employ a variety of techniques to measure metal ions. Typically, they will employ methods that allows the detection of a number of metals within the same analysis, for example inductively coupled plasma optical emission spectrometry (ICP-OES) [64], inductively coupled plasma mass spectrometry (ICP-MS) and IC. Brand owners often have to measure the concentrations of a number of metals to complete certificates of analysis for markets outside the EU. Hence, a database of genuine products can be built up by brand owners, where expected ranges for a number of metals can be set and compared against unknown or suspect samples. This technique is naturally more challenging for generic authentication, particularly where the product is not limited to being bottled in a particular location.

3.1.7. Carbon Dating

The OIV methods include the determination of the ^{14}C content in ethanol by liquid scintillation counting (OIV-MA-BS-24) to help determine between alcohol derived from fossil raw materials (synthetic alcohol) and alcohol made from recently grown plant materials. An alternative technique uses accelerator mass spectrometry (AMS). Both methods may also be used for shorter term dating. This approach can be used to help confirm whether a spirit is consistent with a stated extended maturation age, or whether a bottle claiming to be of historical significance (e.g. pre-1900), and thus potentially meriting a premium price at auction, contains liquid that is consistent with that claim [65].

3.1.8. Additional Officially Recognised Methods

There are a number of additional methods that are listed in the Union reference methods, OIV or AOAC that can be used for spirit drinks authentication. Indeed, any parameter can be employed if there is a natural range for a brand or category and it is likely that this parameter in a fraudulent product may fall outside that range. Those recognised methods listed above reflect commonly used methods for authentication purposes, but the following also deserve attention:

- The determination of pH is a quick measurement that can provide information on the maturation of a sample (OIV-MA-BS-13).
- There are several specific categories of spirits with defined analytical limits in EU legislation to ensure conformity with the definition (see Union reference methods). For example, aniseed flavoured drinks require certain levels of trans-anethole (Union reference methods Annex V; OIV-MA-BS-15).
- The OIV methods contain a method for the measurement of isopropanol (propan-2-ol) (OIV-MA-BS-20). This is not a natural fermentation product for grape based products; it may be added to alcohol during its denaturation. Its presence would thus indicate a fraudulent product.

3.2. Other commonly used methods

3.2.1. UV-Vis Spectroscopy

UV-Vis spectroscopy is a well-established technique for brand authentication both in the laboratory and in the field [28,66]. This method relies on the construction of a large database of individual brands for comparison with suspect samples, and thus tends to be best undertaken by brand owners. In general, UV-Vis spectroscopy works best for the more chemically complex, usually darker spirits such as whisky, brandy and rum. However, the technique can also be applied to clear spirits like vodka. In addition to brand profiling, an abnormal UV-Vis spectrum may indicate the presence of non-permitted compounds [26,67].

3.2.2. Flavourings and Extended Congener Profiles

The EU Spirit Drinks Regulation states that, for a number of spirit categories, the addition of flavourings is prohibited [4, Article 5(1)(c)]. This makes flavouring compounds or flavouring carriers ideal analytical markers for the identification of fraud. Flavouring carriers for spirit drinks are solvents which are used to dilute a flavouring and to facilitate its incorporation and dispersion into the product. To assess whether a flavour compound is naturally present in a spirit product or has

been added, knowledge of the individual spirit category and its production practices is required. Knowledge of common flavourings, flavouring carriers and additives used in the food and drinks industry will also assist in the detection of counterfeit products.

If a suspected flavouring additive is detected, it is often beneficial to identify if the chemical compound is synthetic (man-made). Synthetic compounds are not found in nature hence they will not be naturally occurring as part of standard production practises [68]. Flavouring additives often need only to be present at trace levels to be able to influence the aroma and flavour of a product, therefore sensitive techniques are required. GC-MS and LC-MS are commonly used for the detection of volatile and non-volatile flavouring additive compounds. The analysis of anethole has already been noted as a necessary flavouring constituent of aniseed flavoured products; however its presence in other spirits, such as whisky, would indicate a non-genuine product. Other examples of added flavouring seen in counterfeit products include the synthetic flavouring ethyl vanillin [69] and the flavouring carrier propane-1,2-diol [40].

For certain spirit categories and brands, it will be necessary to extend the range of compound information over and above that provided by the standard major volatile congeners and maturation related congeners methods that are often employed for spirit drink authentication. Such extension will be category specific; for example, the characterisation of gin brands, which uses EAAO (neutral spirit) as its base, is typically free of most major volatile congeners listed, bar methanol. Different entities, such as a range of terpenic compounds, will be more suitable for brand authentication [70]. GC-MS and LC-MS will often be used to increase both the range and sensitivity of compound information obtained from the volatile and non-volatile fractions of a spirit, thus improving differentiation, but also increasing complexity of analysis. MS based techniques (GC, LC or direct injection) can also be used for fingerprinting/non-targeted analysis [71,72]. This will require the creation of large databases gathered from genuine products as well as the use of multivariate statistical analysis. Such techniques have the advantage of identifying when a profile deviates from the expected and may identify the contaminants, or lack of expected congeners, resulting from adulteration/counterfeiting.

3.2.3. Denaturants

Ethanol is produced on a large scale for a variety of industrial uses. To aid in the differentiation of potable alcohol from industrial alcohol and its products, ethanol is “denatured” to make the liquid non-potable and excise duty exempt. The denaturants can act as useful markers for identifying instances where industrial alcohol may have been used in the production of illicit spirits. The chemicals used, and the proportions of denaturants, have traditionally varied by country. In 2008, the EU Commission started a review that has led to a reduction and harmonisation of denaturants in use within Europe. A new “Euro” denaturant formulation is now established, designed to help prevent fraud. This consists of isopropanol, methyl ethyl ketone and denatonium benzoate [73].

Denaturants vary in the ease with which they can be differentiated from constituents of spirit drinks; they will also vary considerably depending on location. Outside the EU, different formulations will be used. In the US, these can be found in Title 27 of the Code of Federal Regulations Part 21 [19]. Methanol has been a commonly used denaturant, which can be potentially fatal contaminant in a fraudulent spirit. In addition to the denaturants themselves, secondary markers resulting from attempts made by counterfeiters to remove denaturants from industrial alcohol may indicate denatured alcohol in a fraudulent spirit [51]. Methods of analysis used to detect denaturants will be targeted to the specific alcohol denaturants. The OIV already have a method for isopropanol; the Customs Laboratory European Network is also due to implement methods to measure the three constituents of the “Euro” denaturant.

3.2.4. Sensory Analysis

Sensory analysis by assessment of aroma (nosing) can be used to assist in spirit drink authentication by identifying suspect samples with atypical aromas. This requires trained and experienced sensory panelists who are familiar with the spirit category or brand's major sensory attributes. Although sensory analysis cannot determine if a product is genuine, it is a useful tool for identifying instances where non-permitted flavourings may have been used in production. It should be noted that sensory analysis is generally considered a subjective technique and confirmatory chemical analysis is always recommended.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Densitometry	Alcohol Strength (not suitable for spirits with significant levels of dissolved solids e.g. sugars)	Dilution
Distillation and Densitometry	Alcohol Strength	Dilution
GC-FID	Major Volatile Congeners (e.g. higher alcohols, methanol)	Category and brand discrimination
GC-FID	Denaturants (Methanol, isopropanol, methyl ethyl ketone etc.)	Detection of non-potable alcohol
(U)HPLC-UV	Maturation Congeners	Category discrimination, lack of maturation, addition of flavouring
(U)HPLC-RI	Sugars	Brand discrimination
IC-PAD	Sugars	Addition of sweetening
GC-MS	Flavourings, Denaturants, Fingerprinting	Brand discrimination, addition of flavourings, detection of non-potable alcohol
LC-MS	Flavourings, Denaturants, Fingerprinting	Brand discrimination, addition of flavourings, detection of non-potable alcohol
UV-Vis Spectroscopy	Spectroscopic profile	Brand discrimination
^{13}C SIRMS, ^{18}O SIRMS, ^{2}H SNIF-NMR, ^{13}C SNIF-NMR,	Ethanol	Botanical origin of ethanol, detection of synthetic alcohol
^{18}O SIRMS	Water	Category and Brand Discrimination, Adulteration (addition of synthetic alcohol)
^{14}C dating by Liquid Scintillation Counting or Accelerator Mass Spectrometry	Ethanol	Date of production
pH	pH	Lack of maturation
AAS, ICP-OES, ICP-MS, IC	Trace Metals	Brand Discrimination

5. Conclusion

There are considerable financial incentives to create fraudulent spirit drink products. The prices commanded by premium spirit drinks and excise duty combine to offer a lucrative opportunity, especially where excise exempt alcohol can be used in its creation. Excessive taxation is often quoted as being a key contributor to the production and consumption of illicit alcohol. For example, in Indonesia many local people cannot afford to purchase genuine spirit drinks as they are heavily taxed, leaving them to risk drinking unregulated products. In 2018 more than 100 people in Indonesia were killed by one poisoning outbreak [74]. Another potential issue is the ease with which denatured alcohol can enter the potable supply chain. Efforts are being made to address this, such as the changes in European legislation designed to reduce the wide range of denaturants in use, and to focus on formulations which prove hard to remove.

The detection of counterfeit spirit drinks can be challenging. Spirit drinks are characterized by two major constituents: ethanol and water. The other compounds present, which provide differentiation in terms of flavour and identity, are generally present at low levels. Many compounds, such as proteins and DNA that are associated with the raw materials (cereals, grapes etc.), are removed during the distillation step. As a result, techniques used to identify counterfeit spirit drinks are typically based on profiles of flavour and other constituents present at trace levels (ppm to ppb), such as the measurement of major volatile or maturation related congeners. Certain properties of the whole spirit, such as pH, UV spectrum and alcohol strength can however prove useful in identifying frauds.

Looking to the future, there are several trends apparent in spirit drink authentication. The first is the drive for portability in analytical measurements, allowing rapid evaluations to take place at key points in the supply chain, for example at point of sale. Portable pH and conductivity meters can already be employed [75]. The use of portable UV-Vis for brand authentication is common, but it can also be used for detection of specific compounds such as sugars [76]. Raman and NIR spectroscopy are also being explored for their potential [77-79]; the opportunity of analysis through spirit drinks bottles using such techniques is an attractive option for fraud detection.

Another trend is the increasing availability of more conventional laboratory techniques in machines with a smaller footprint. These offer the potential for the both the quantitative profiling of key marker compounds (of either genuine or counterfeit products) where chromatography is involved [80] or a rapid assessment of authenticity based on a chemometric model of a particular brand or category [72]. Finally, advances in laboratory authentication of spirit drinks will most likely result in more detailed analysis (increased number of compounds and/or increased sensitivity) becoming more routine and more rapid. The application of NMR as a routine technique for both targeted and untargeted analysis of spirit drinks is one possibility [81].

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Fruit juices

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General overview of the products

Fruit juices (100 % fruit) and nectars (25-99 % fruit) are an important sector of the food industry with a global consumption volume of fruit juice and nectars in 2017 of 36 247 million litres. The 28 countries of the European Union (EU-28) accounted for 9 187 million litres, with North America not far behind with 8 629 million litres followed by the Asia-Pacific region with 8 159 million litres [1].

In the EU, five countries account for over 70 % of the total fruit juice and nectar consumption, with Germany coming out on top (2 342 million litres), followed by France (1 406 million litres), the UK (1 079 million litres), Poland (820 million litres) and Spain (808 million litres). Total consumption of juices and nectars has been slowly declining over the last decade, particularly in the area of nectars, and ambient/from concentrate juices. One of the key drivers of fruit juice consumption has always been its nutritional image, as a natural product high in vitamins, anti-oxidants and other nutrients. However the juice sector has recently come under fire due to its relatively high content of sugar, one of the reasons responsible for the decline in juice, and particularly nectar, consumption.

Although orange and apple still top the popularity list, the number and diversity of types of fruit juice available in the market has changed considerably over the last few decades, with the regular appearance of novel exotic fruit types each claiming new health benefits. To counteract the downward trend in juice consumption, the industry is turning to new innovative and sophisticated flavours and mixes. Efforts have focused on the NFC (not from concentrate) and smoothies sectors with fruit and vegetable mixes that are lower in sugar, and the addition of functional ingredients such as proteins. Further insight into the juice market including an overview of the trends, opportunities, and threats facing the fruit juice industry is given in reference [2].

All these factors, in the changing landscape of the fruit juice sector, make ensuring the authenticity of what is available to the consumer an ongoing challenge for both the industry and the regulators.

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1. Product Identity

1.1. Definition of the product and manufacturing process

The type of fruit juice found on the market is generally conditioned by the processing method used to get the product from its growing region to the supermarket shelf and by the specific regulations in force in the country where it is sold. Fruit juice is traded either as natural strength juice or purée or as concentrated fruit juice or purée from which the water has been extracted. The latter greatly reduces storage space requirements and cuts transport costs considerably. The concentrate is stored at very low temperatures or in aseptic drums and transported in bulk from the production area to the main markets where it is reconstituted to single strength juice by adding water. The juice is then pasteurised, bottled and sold as "100 % fruit juice made from concentrate" or "100 % fruit juice made with concentrated fruit juice".

Natural strength fruit juice is obtained directly from the fruit, pasteurised and bottled ready to be sold or kept in sterile tanks at low temperature for packing at a later date. It is sold as "100 % fruit juice" and sometimes as "direct juice".

Chilled (refrigerated) short shelf life juices

- Chilled freshly squeezed juice: single strength juice not made from juice concentrate with a shelf life of between 3 days and 3 weeks depending on fruit type, storage temperature 0-5 °C
- Pasteurised 100 % (direct) juice: single strength juice not made from concentrate with shelf life of about 24 days
- Pasteurised juice made with concentrated fruit juice: reconstituted from juice concentrate (generally frozen), shelf life of about 24 days.

Pasteurised, ambient juices

- Pasteurised direct juice/freshly squeezed: long shelf life (6 to 12 months depending on fruit type)
- Pasteurised juice made with concentrated fruit juice: reconstituted from frozen concentrate with a long shelf life (6 to 12 months depending on fruit type).

Fruit nectars are also popular in Europe. These are blends of fruit juices (between 25 – 90 % juice content depending on the fruit type), water and sugar.

Fruit purées and pulps are ideal raw materials for soft fruits such as strawberry and raspberry that are prone to physical damage during transport. Such products are used in fruit juice blends, drinks and nectars as well as in jam and marmalade manufacture.

1.2. Current standards of identity or related legislation

1.2.1. Codex Alimentarius

The Codex Alimentarius (CODEX STAN 247-2005 [3]) gives the following definition for a fruit juice:

"Fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means

including post-harvest surface treatments applied in accordance with the applicable provisions of the Codex Alimentarius Commission.
[….]

The juice is prepared by suitable processes, which maintain the essential physical, chemical, organoleptical and nutritional characteristics of the juices of the fruit from which it comes…"

Definitions given by the Codex Alimentarius can be taken as a general basis for export purposes. However these guidelines may differ from those of specific countries.

1.2.2. European Union

The EU "Fruit Juice Directive" 2001/112/EC [4] provides a slightly different definition to that given by Codex Alimentarius.

"The fermentable but unfermented product obtained from the edible part of fruit which is sound and ripe, fresh or preserved by chilling or freezing of one or more kinds mixed together having the characteristic colour, flavour and taste typical of the juice of the fruit from which it comes…"

Taking into account all other stipulations and paragraphs of both documents there is no relevant difference. However, according to the Codex Standard physical, chemical, organoleptic and nutritional characteristics of the fruit from which the juice comes should be maintained, whereas the EU Directive limits this aspect to colour, flavour and taste. This is somehow closer to industrial reality since a juice cannot have the same physical, chemical and nutritional characteristics as a fruit. A juice is liquid and a fruit is partly solid and has more fibre. In addition, the chemical structure cannot be the same because the use of authorised enzyme treatment changes the chemical composition and some substances are discarded with the solid fruit parts during the extraction process. However, fruit juice remains a healthy product with nutritional benefits through its constituents.

Fruit juices and related products like fruit juice concentrates are natural products. According to the applicable legislation only a very limited range of additives and further ingredients are allowed.

Similar properties are defined for fruit purees.

Any legal framework is national or regional specific. In Europe the above mentioned "Fruit Juice Directive" and other food related laws are applicable. Differences to other regional legislation exist and must be taken into consideration when interpreting analytical results. For example, it is not accepted in the EU to blend orange juice with juice from *Citrus reticulata* hybrids (mandarin and others). In most other parts of the world such blending is allowed up to a certain amount. Furthermore the use of conservation agents is regulated differently in some countries and differences exist in the expected minimum content of solid solids (density/Brix) that must be present in a juice or a juice reconstituted from juice concentrate.

For the EU an industrial Code of Practice has been developed by the European Juice Association or AIJN [5] which is regularly updated. This Code of Practice provides analytical reference data for specific fruit types and gives comments for interpretation. Some of the guide values listed are obligatory such as minimum Brix values, limits of heavy metals and spoilage parameters. On the other hand, analytical parameters which are used for authenticity assessment are generally indicative. Case specific interpretation through experts is always necessary.

A regional specific data base is accessible in the member portal of SGF International e.V. [6].

1.2.3. In the United States

The US Food and Drug Administration (FDA) regulations on foods are established in a Code of Federal Regulations (CFR) under Title 21. Under this, juices must conform to FDA standards of identity 21 CFR part 146 for fruit juices and to 21 CFR part 156 for vegetable juices [7]. In addition 21 CFR part 101.30 provides regulations for percentage juice declaration for beverages that contain fruit or vegetable juice [8].

The USA has always been primarily focused on food safety and in 2001, the FDA brought in a ruling requiring a mandatory HACCP (Hazard Analysis and Critical Control Points) plan for fruit juice (21 CFR part 120, the Juice HACCP regulation [9]). The regulation requires that processors apply HACCP principles if they make juice or juice concentrates for subsequent beverage use. In 2011, the FDA brought in its Food Safety Modernization Act (FSMA [10]), specific legislation that puts into place mandatory prevention-based controls across the food supply to protect public safety and prevent illness. And in 2013 it issued its final rule, as part of FSMA, for Mitigation Strategies to Protect Food Against Intentional Adulteration [11]. The FDA has recently issued guidance to address any discrepancies arising from the new FSMA regulations in relation to the juice HACCP regulation [12].

2. Authenticity issues

2.1. Identification of current authenticity issues

There are various potential frauds possible as regards fruit juices. The most important authenticity issues are listed below:

- **Water addition**: there is a natural variation in fruit juices for the ratio between soluble dry matter and water. However it is not allowed to add water, even if the fixed minimum density or Brix are lower than any naturally obtained product not from concentrate. Also, for reconstituted juice from concentrate, legislation stipulates that the quantity of water added to reconstitute the juice must be the same as that removed during concentration. As mentioned earlier, the AIJN, has laid down guidelines for minimum density and its corresponding Brix value, a measure of the soluble solids content. One of the simplest forms of adulteration is dilution of a concentrate to below the permitted minimum Brix.
- **Sugar addition**: As a commodity, sugar is much cheaper than fruit juice, and therefore its addition to the latter to increase Brix values can be an economic advantage. Sugar can be added as beet, cane or corn sugars, or as modified sugar syrups such HFCS (high fructose corn syrup) or BMIS (beet medium invert syrup).
- **Complete or partial replacement of juice by juices made from concentrate**: in some countries consumer preference has shifted in recent years to freshly squeezed or not from concentrate (NFC) juices, conferring a higher premium on these products. It is therefore not permitted to pass reconstituted juices off as NFC or to add a proportion of water or reconstituted juice to the direct product.
- **Added products from undeclared cheaper fruits**: the prices of certain fruit types can fluctuate widely from one season to another, affected by poor harvests, gluts, and trade regulations. The addition of a cheaper fruit alternative to stretch one in short supply and/or high demand is another fairly common form of adulteration. Examples include the addition of orange to passion fruit, apple to red fruit, grape to pomegranate

- **Addition of undeclared ascorbic acid/vitamin C**: some fruit types are naturally high in vitamin C and use this as a selling point.
- **Addition of undeclared organic acids (e.g. citric acid, malic acid)**: in some cases the acidity of a juice can be corrected by the addition of organic acids within the limits tolerated by the legislation, and with suitable mention on the label. Specific practices such as addition of malic acid to apple juice is not permitted.
- **Addition of flavour compounds (natural or synthetic)**: authenticity issues arise when the product claiming to be "natural" contains flavours not from the named fruit or that have been chemically synthesised. Food fraud through addition of unauthorised flavour compounds is covered in the “flavourings” chapter.
- **Colourings (e.g. anthocyanin extracts, cochenille red, beetroot)**: the colour of fruit juices is an important part of the product's appeal. Colourings may be added to meet colour intensity specifications, to restore natural colour lost during processing conditions or storage, or to darken colour when the authentic product has been extended with a less pigmented fruit type.
- **Addition or over-proportional use of fruit extracts which were produced by non-authorised technology (water extractable solids)**: water extractable solids is the material obtained from washing the remaining pulp and cell membrane material after extraction of orange juice. Its overuse in juice is not permitted under the legal definition of the EU for citrus fruits.
- **Texture influencing agents (e.g. pectins)**: texturisers can enhance body and mouthfeel in juices that are less than 100 % fruit. This is authorised for specific fruit types only and provided that the compounds are mentioned on the product label.
- **Declaration of wrong origin**: the country of origin of a fruit product can become an authenticity issue if it is falsely declared on the juice label or in the product's trade specifications. This could be the concern of customs and excise authorities, if the fruit origin in question is subject to preferential import duties. Certain geographical origins may also carry a premium on the market
- **Declaration of wrong fruit variety**: this could be an issue where a specific variety is prized for its flavour or processing qualities

In particular if the fruit content in the falsified product is lower than in an authentic one the fraud could be covered up by adding other ingredients to adjust the analytical profile to the expected picture of an authentic product. Therefore minerals, organic acids, amino acids or a combination of different materials from other fruits can be added as part of the fraudulent practice.

2.2. Potential threat to public health

Most instances of fraud in fruit juices have no real food safety impact. However every food fraud is a potential health risk through the use of undeclared ingredients. Allergen issues or contamination with unexpected agrochemicals for wrongly declared origins or other contaminants present in an ingredient are possible. As analytical techniques become more and more sophisticated fraudsters are also pushed to better mask the analytical profile of the falsified product. This could lead to a vicious circle by increasing the risk of unexpected ingredients. An example is given in the following.

An orange juice concentrate is diluted with deionised well water and after respective flavour addition commercialised as juice not from concentrate. In this case an analysis of the isotope ratio $^{18}O/^{16}O$ of the water in the juice can detect the fraud. To avoid being discovered, the water used for dilution could be replaced by the dishonest juice producer by water obtained from the concentration process of grape juice. The $^{18}O/^{16}O$ isotope ratio – which is in many companies the routinely applied control parameter - would then be insufficient to detect the fraud. The risk increases that the fraud remains undiscovered. However [22]as most grape juices used for the production of concentrate are stabilised by sulfiting, the presence of sulphur dioxide (SO_2) in the final product is possible. SO_2 is listed as an allergen and represents a health risk for sensitive consumers.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. IFU Methods

The IFU (International Fruit and Vegetable Juice Association) has published a number of analytical methods to test for both juice authenticity and quality [13]. These methods include traditional wet chemistry methods such as refractometric index, density measurement, titratable acidity, formol index or photometric methods. In addition a list of enzymatic tests (e.g. organic acids, sugars), mineral determination (AAS, flame photometry) and different chromatography methods, e.g. for phenolic compounds, are suitable IFU methods to assess authenticity (cf. Table 1).

Methods are classified as IFU recommendation if no ring test results are available.

3.1.2. CEN Methods

The European Normalisation Committee, CEN, has also, through its technical committee CEN/TC 174, published a number of methods applied to the authenticity testing of fruit juice. A selection is listed in Table 2 below.

3.1.3. AOAC methods

The AOAC (Association of Official American Chemists) has also published a number of analytical methods for the authentication of fruit juices. These are listed in the Table 3.

Table 1: Available IFU methods and recommendations for fruit juice authenticity testing

IFU Method	
IFU 01	Determination of Relative Density (Pycnometer Method)
IFU 01A	Relative Density (Method using Density Meter)
IFU 02	Determination of Ethanol by Gas Chromatography
IFU 03	Determination of Titratable Acidity
IFU 05	Determination of Volatile Acids
IFU 07A	Determination of Total Sulphur Dioxide (SO2)
IFU 08	Determination of Soluble Solids (Indirect Method by Refractometry)
IFU 09	Determination of Ash
IFU 10	Determination of Ash Alkalinity
IFU 11	Determination of pH Value
IFU 12	Determination of Hydroxymethylfurfural (HMF)
IFU 12A	Microbiogical detection of taint alicyclobacillus
IFU 17A	Determination of ascorbic acid by HPLC
IFU 18	Fermentation Test (Screening Test for the Presence of Preservatives)
IFU 21	Determination of L-Malic Acid, Enzymatic
IFU 22	Determination of Citric Acid, (enzymatic)
IFU 24	Detection of Artificial Water-Soluble Artificial Colorants
IFU 25	Organoleptic Examination
IFU 26	Determination of Pectin
IFU 28	Determination of Total Nitrogen
IFU 30	Determination of Formol Number
IFU 33	Determination of Sodium, Potassium, Calcium and Magnesium
IFU 36	Determination of Sulphate
IFU 37	Determination of Chloride
IFU 45	Determination of Essential Oils (Bromate Method)
IFU 46	Determination of Pectin Esterase (PE) Activity in Citrus Juices and their Concentrates
IFU 49	Determination of Proline
IFU 50	Determination of Phosphate
IFU 52	Determination of Alcohol, Enzymatic
IFU 53	Determination of Lactic Acid, Enzymatic
IFU 54	Determination of D-Isocitric Acid, Enzymatic
IFU 55	Determination of Glucose and Fructose, Enzymatic
IFU 56	Determination of Sucrose, Enzymatic
IFU 57	Determination of Free Amino Acids
IFU 58	Determination of Hesperidin and Naringin, HPLC
IFU 59	Determination of Total Carotenoids and Individual Carotenoid Groups
IFU 60	Determination of Centrifugable Pulp
IFU 61	Determination of Total Dry Matter
IFU 62	D-Sorbitol (Enzymatic)
IFU 63	Preservatives (HPLC)
IFU 64	D-malic Acid (Enzymatic)

IFU Method	
IFU 65	Tartaric Acid in Grape Juice (HPLC)
IFU 66	Acetic Acid (Enzymatic Method)
IFU 67	Determination of Sugars and Sorbitol (HPLC)
IFU 68	Test for Pectin (Turbidimetric)
IFU 69	Determination of Hydroxymethylfurfural (HPLC)
IFU 70	Cell Content of Pulps and Juices
IFU 71	Anthocyanins by HPLC
IFU 72	Fumaric Acid (HPLC)
IFU 73	Detection of Starch in Fruit Juices
IFU 74	Determination of Nitrate by Ion Chromatography
IFU 76	Determination of D-Gluconic Acid in Grape Juice (Enzymatic)
IFU 77	Determination of Glycerol in Grape Juice (Enzymatic)
IFU 78	Determination of Galacturonic Acid using High Performance Anion Exchange Chromatography
IFU 79	Measurement of Polyols in Fruit and Vegetable Juices using Electrochemical detection
IFU 80	Measurement of the Colour of Clear and Hazy Juices (Spectrophotometric Method)
IFU 81	Determination of Ergosterol by HPLC (Provisional)
IFU 82	Determination of Nitrate (Provisional)
IFU 83	Colour measurement of blood orange juices
IFU 84	Stability test for clarified juices

IFU Recommendations	
IFU R01	Detection of Invert Syrup Addition by Oligosaccharide Analysis
IFU R02	Recommendation for the Determination of Patulin
IFU R03	The Use of Isotopic Procedures in the Analysis of Fruit Juices
IFU R04	Detection of Syrup Addition to Juices by Capillary Gas Chromatography
IFU R05	Recommendation for Vitamin C Analysis
IFU R06	Determination of Heavy Metals in Fruit Juices
IFU R07	Recommendations for Turbidity Measurements
IFU R08	Recommendations for Analysis of High Intensity Sweeteners
IFU R09	Recommendation for Colour Measurements in Cloudy Juices
IFU R10	Recommendations for Analysis of Ochratoxin in Fruit Juices
IFU R12	Methods for the Conformation of Country of Origin
IFU R13	The use of DNA Methods in the analysis of fruit juices, purees and concentrates
IFU R14	Recommendation. Methods to assess the organic or bio nature of juices
IFU R15	Recommendations, Basic quality systems for juice laboratories

Table 2: CEN methods for the authenticity testing of fruit juices

Reference	Application
EN 1131:1994	Determination of relative density
EN 1132:1994	Determination of the pH-value
EN 1133:1994	Determination of the formol number
EN 1134:1994	Determination of sodium, potassium, calcium and magnesium content by atomic absorption spectrometry (AAS)
EN 1135:1994	Determination of ash
EN 1136:1994	Determination of phosphorus content - Spectrometric method
EN 1137:1994	Enzymatic determination of citric acid (citrate) content - NADH spectrometric method
EN 1138:1994	Enzymatic determination of L-malic acid (L-malate) content - NADH spectrometric method
EN 1139:1994	Enzymatic determination of D-isocitric acid content - NADPH spectrometric method
EN 1140:1994	Enzymatic determination of D-glucose and D-fructose content - NADPH spectrometric method
EN 1141:1994	Spectrometric determination of proline content
EN 1142:1994	Determination of the sulphate content
EN 12133:1997	Determination of chloride content - Potentiometric titration method
EN 12134:1997	Determination of centrifugable pulp content
EN 12136:1997	Determination of total carotenoid content and individual carotenoid fractions
EN 12137:1997	Determination of tartaric acid in grape juices - Method by high performance liquid chromatography
EN 12138:1997	Enzymatic determination of D-malic acid content - NAD spectrometric method
EN 12143:1996	Estimation of soluble solids content - Refractometric method
EN 12144:1996	Determination of total alkalinity of ash - Titrimetric method
EN 12145:1996	Determination of total dry matter - Gravimetric method with loss of mass on drying
EN 12146:1996	Enzymatic determination of sucrose content - NADP spectrometric method
EN 12147:1996	Determination of titratable acidity
EN 12148:1996	Determination of hesperidin and naringin in citrus juices - Method using high performance liquid chromatography
EN 12630:1999	Determination of glucose, fructose, sorbitol and sucrose contents - Method using high performance liquid chromatography
EN 12631:1999	Enzymatic determination of D- and L-lactic acid (lactate) content - NAD spectrometric method
EN 12632:1999	Enzymatic determination of acetic acid (acetate) content - NAD Spectrometric method
EN 12742:1999	Determination of the free amino acids content - Liquid chromatographic method
ENV 12140:1999	Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruits juices - Method using isotope ratio mass spectrometry
ENV 12141:1996	Determination of the stable oxygen isotope ($^{18}O/^{16}O$) of water from fruit juices - Method using isotope ratio mass spectrometry
ENV 12142:1996	Determination of the stable hydrogen isotope ratio ($^{2}H/^{1}H$) of water from fruit juices - Method using isotope ratio mass spectrometry
ENV 13070:1998	Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) in the pulp of fruit juices - Method using isotope ratio mass spectrometry

Table 3: Available AOAC methods and recommendations for fruit juice authenticity testing

Reference	Application
2004.01-2004	Carbon stable isotope ratio of ethanol derived from Fruit Juices and Maple Syrups
2005.02-2005	Total Monometric Anthocyanin Pigment Content
969.30-1980(1998)	Organic acids (foreign) in fruit juices.
971.18-1980	Carbohydrates in fruit juices. Gas chromatography
981.09-1983(1997)	Carbon stable isotope ratio of apple juice
982.21-1983(1997)	Carbon stable isotope ratio of orange juice
986.13-1989(1996)	Quinic, Malic and Citric acids in cranberry juice cocktail and apple juice. Liquid chromatography
986.14-2008	Orange Pulpwash and/or Added H_2O in Processed Florida Orange Juice Spectral Characterization
991.30-1994	Polydimethylsiloxane in pineapple juice. Atomic Absorption
991.46-2008	Glycerol in wine and grape juice. Liquid chromatography
992.09-1997	Sugar-Beet-Derived Syrups Frozen Concentrated Orange Juice $\delta^{18}O$ Measurements in Water Stable Isotope Ratio Mass Spectrometric Method
993.05-1997	L-malic/total malic acid ratio in apple juice.
995.06-1998	D-malic acid in apple juice. Liquid chromatography
995.17-1998	Beet Sugar In Fruit Juices Site Specific Natural Isotope Fractionation– Nuclear Magnetic Resonance Method
999.05-1999(2002)	Naringin and neohesperidin in orange juice. Liquid chromatography

3.2. Strategy for the authenticity assessment of fruit juices

The authenticity assessment of fruit juices is complex and does not follow the same strategy for all types of fruits and products. As shown above, a considerable number of analytical methods and associated parameters make up the official methods for fruit juice analysis. It is not realistic to assume that all possible analytical checks to cover all aspects of food fraud can be applied for each individual sample. A typical authenticity assessment of a fruit juice sample without former information about possible fraud consists of two steps. First the analyst tries to get an overview of the overall analytical profile and decides in a second step which aspect should be checked by specific analyses. Ideally a vulnerability assessment on the selected product should be carried out first, in order to better orientate the analytical focus.

The analytical strategy for the authenticity assessment of fruit juice can be classified into different groups of analyses:

- Metabolomic fingerprinting, e.g. proton-NMR Juice Screening (SGF-Profiling^{TM})
- Parameters obtained via chromatographic techniques, enzymatic tests, atomic absorption spectrometry, Inductively Coupled Plasma (ICP)-spectrometry, classical wet chemistry methods; named here "**conventional methods**".
- Detection of marker substances for specific adulterants
- Stable Isotope analyses
- Chromatographic fingerprinting

For all analyses the quality and specificity of reference databases is important. A critical consideration is recommended in this regard. In many cases region-specific reference data help to better recognise food fraud or to avoid false positive interpretation. Regional differences are mainly due to weather conditions, cultivated varieties and process techniques. If one of these parameters changes, then an incorrect analytical evaluation could result. However experience has shown that such changes are relatively seldom, and when they do, the reference databases must be adjusted by addition of appropriate authentic reference samples when they do occur.

To obtain an overall profile of the fruit juice, the best choice today would be, where available, an untargeted proton NMR juice screening combined with selected conventional parameters. If the NMR screening is not possible, a larger set of conventional analyses is needed to cover a maximum of fraud possibilities. The comparison in table 4 below shows how conventional parameters can be replaced by the NMR screening for a minimum scope to check the authenticity for apple and orange juice. Some conventional parameters are not necessary because the NMR screening gives the same information as these parameters.

Wherever possible, positive fraud detection should be confirmed by at least a second analytical approach.

Table 4: Extract from the SGF-Conformity matrix for apple and orange juice/-concentrate. The columns indicate the analytical order with or without SGF-Profiling™

Analysis	Apple		Orange	
	Without SGF Profiling™	With SGF Profiling™	Without SGF Profiling™	With SGF Profiling™
SGF-Profiling ™		x		x
Relative density 20/20	x	x	x	x
Brix (table)	x	x	x	x
Soluble solids	x		x	
Glucose	x		x	
Fructose	x		x	
Sucrose	x		x	
Titrat. acidity expr. as tart. acid pH 7.0	x	x	x	x
Titrat. acidity expr. as citric acid pH 8.1	x	x	x	x
L-malic acid	x		x	
Citric acid	x	x	x	
Isocitric acid			x	x
L-ascorbic acid			x	x
Sodium	x	x	x	x
Potassium	x		x	
Calcium	x	x	x	x
Magnesium	x		x	
Nitrate	x	x	x	x
Phosphate	x		x	
Sorbitol	x	x		
Formol number	x		x	
Proline			x	
Water-soluble pectins			x	
Lactic acid	x		x	
HMF (5-hydroxmethylfurfural)	x		x	

3.2.1. SGF-Profiling™ ^{1}H-NMR-Juice Screening

As a powerful fingerprint method, proton-NMR juice screening SGF-Profiling™ [14–16], which stands for Spin Generated Fingerprint Profiling, is used for authenticity control enabling the screening of a large range of potential adulterations. It is a non-targeted metabolomics application and quantification is performed with a high throughput. The presence and quantity of natural fruit compounds for which the chemical structure is unknown are used in the same way as signals from those compounds that can be identified. The statistical evaluation and quantitative determination of several parameters is done with one single 400 MHz NMR experiment. Analysis time is about 15 minutes per sample and the process can be automated to facilitate a large sample throughput.

Sample preparation consists of centrifugation and diluting a juice or a concentrate with a buffer containing TSP (sodium salt of 3-(trimethylsilyl)-propionate acid-d_4) as an internal standard and sodium azide for preservation. The pH of the buffer solution is 3.1 or 3.4 depending on fruit type. Use of these specific buffer solutions is essential so that the required reproducibility is achieved due to the pH sensitivity of the chemical shifts of some of the polar compounds (e.g. organic acids).

A full proton-NMR spectrum is recorded at 300K. A NOESYPRID™ pulse sequence with continuous wave pre-saturation of the water resonance is used. Baseline and phase corrections can be included in an automatic data treatment. The whole instrument configuration can be designed as a push button system with a flow cell or individual tubes.

A standard routine includes J-resolved NMR spectroscopy (JRES) which allows better signal assignment to molecular structures which show interferences with other compounds in the one-dimensional spectrum along the chemical shift axis [14,17]. JRES spectra are obtained by suppressing J-coupling to separate chemical shifts and J-coupling; both are projected on two different axes which results in a two-dimensional spectrum.

For those products for which SGF-Profiling™ models exist it is recommended to base further analytical choice for juice authentication on the outcome of this screening. It uses a spectral data base of authentic reference material. Different independent verification and classification models are applied. A number of analytical aspects such as type of product, origin or mixture with other varieties are checked in parallel. For some fruits a check of fruit content in a sample is possible too. However, not all products have the same possibility to be analysed. Depending on fruit type, the analytical possibilities and available models may differ. With increasing amount of collected reference samples a development of new and improved models is permanently ongoing.

Table 5 summarises the currently available classification models, and Table 6 lists the quantitative parameters provided. Depending on the type of fruit more or less of the listed substances are determined by automatic quantification routines.

Despite its growing importance, this proton-NMR screening technique is not recognised as an official method yet. However, all measurements and the statistical treatments are accredited according to ISO 17025 in certain laboratories.

Table 5: Classification models by SGF-Profiling™

Analytical aspect	Classes to differentiate
Origin Apple Juice	Poland/Germany, Turkey, China, Hungary, Spain/Italy
Origin Orange Juice	Brazil, Spain, Greece, Belize/Costa Rica/Cuba, Mexico, USA, Argentina/Paraguay/Uruguay
Origin Lemon Juice	Spain/Italy, Argentina
Origin Pineapple juice	Asia, Middle America, Brazil
Origin Sour Cherry Juice	Poland, Turkey
Origin Mango puree	Mexico, India
Product Type Apple Juice	Direct juice, Juice made from Concentrate
Product Type Orange Juice	Direct juice, Juice made from Concentrate
Product Type Lemon Juice	Direct juice, Juice made from Concentrate
Product Type Pineapple Juice	Direct juice, Juice made from Concentrate
Citrus type	Orange, Blood Orange, C. reticulata

Table 6: Parameters which can be automatically quantified from proton-NMR spectrum of SGF Profiling™

4-aminobutanoic acid	chlorogenic acid	gluconic acid	proline
acetaldehyde	citramalic acid	glucose	pyruvic acid
acetoine	citric acid	HMF	quinic acid
Alanine	ethanol	isocitric acid	shikimic acid
Arbutin	formic acid	lactic acid	sorbic acid
arginine	fructose	malic acid	succinic acid
benzaldehyde	fumaric acid	methanol	sucrose
benzoic acid	galacturonic acid	phlorin	xylose

Calculated values

glucose/fructose	% sucrose	total sugar	malic acid/quinic acid

3.2.2. Authenticity strategy using conventional methods

Authenticity patterns for fruit juices are generally composed of a considerable number of analytical figures, many of which have guidance ranges in the AIJN Code of Practice (3). For a number of types of fraud, the overall profile obtained by different conventional analyses provides a first indication and sometimes even clear evidence of adulteration. In any case, if there is no particular authenticity indicator or suspicious parameter, an analytical pre-screening is recommended to provide some idea of suitable follow-up specialised analyses. This can consist of a compilation of typical analytical data, sometime also called “full analyses” combined with an expert interpretation. The value of the analyses depends strongly on available reference databases and the ability to interpret the analytical data. In fact, producing correct analytical results is often not the main challenge in fruit juice authenticity control, but the interpretation of obtained data.

Every laboratory and its analysts have to develop a way of judging the whole analytical pattern as such and not only value by value. Therefore no standardised procedure exists, however hereafter follows an attempt by the chapter's authors try to give a rough description of one way of proceeding.

1. Check the Brix value or density and Brix/acidity ratio.
   - High values of Brix or density in single strength products stand for high sugar content and high degree of ripeness. Ripe products would probably tend to have a low acid content and high Brix/acidity ratio. Particularities of certain varieties should be taken into account.
   - Single strength products have natural variations, official reference values are generally set at the lower limit. If products from one source are regularly close to this minimum level without variation, a systematic standardization through water addition is to be considered.
2. Check the sugar profile.
   - The glucose/fructose ratio is specific for different fruit types.
   - The glucose/fructose ratio generally decreases with microbiological spoilage. Check consistency with other metabolites indicative of spoilage (e.g. lactic acid, ethanol, volatiles acids)
   - Sucrose content is typical for different fruit types, some fruits do not have any. The step of invertase deactivation in the process flow plays an important role.
   - A product which has undergone heat stress, a long storage time or inappropriate transport conditions can show sucrose inversion to glucose and sucrose in equal quantities. Inversion is also favoured by high acid content.
3. Check the sugar free extract and its relation to the total amount of sugar.
   - The sugar free extract contains all soluble compounds which are not the main sugar compounds glucose, fructose and sucrose. This would decrease if external sugar is added.
   - The relation between total sugar and the sugar free extract would shift towards the sugar content for products which are supposed to derive from very ripe fruits.
4. Try to explain the sugar free extract with available data.
   - An analytical profile will never cover the total soluble extract. Depending on the type of product and the chosen analytical parameters the gap between measurable compounds of the sugar free extract and the total sugar free extract is more or less great. However, the sum of measured concentrations for organic acids, minerals, sorbitol (if present) could show experienced analysts whether the usually expected gap is in the natural range. If the gap is too small, an adjustment of the analytical profile to mask a low fruit content would be expected.
   - If the gap is too big unexpected compounds must be present (e.g. sorbitol, solubles from mash extraction, starch or other polysaccharides)
5. Check relation of compounds inside the sugar free extract.
   - Group of organic acids: the ratio of citric acid and D-isocitric acid is typical for every fruit type. High values indicate the addition of citric acid.
   - Group of minerals: higher sodium and nitrate values can indicate the presence of minerals from process water or process agents. Regional exceptions are possible.

- In particular, for products not from concentrate high values of sodium or nitrate – even below the AIJN-Code of Practice maximum guide values – could hint at added water or the use of reconstituted concentrates.
- Group of flavonoids, anthocyanins: different fruit types have different patterns.

6. Check individual marker substances. Examples:
   - Some substances are untypical for certain fruit types, e.g. sorbitol, sucrose,
   - Some substances can indicate the presence of another fruit type, e.g tartaric acid indicates grape, phloridzin indicates apple, arbutin indicates pear, naringin indicates grapefruit.
   - Lactic acid and ethanol indicate fermentation.
   - 5-hydroxy-methylfurfural (HMF) is a typical Maillard product and indicates heat stress and/or long-term storage in inappropriate conditions.

There is no obligation to follow this list in this order or limited to the afore-mentioned examples. However it represents a useful approach to interpretation for the less experienced analyst, while building up his/her own referential of importance for any parameter. Table 7 provides an idea of which authenticity aspect a parameter could contribute to.

An authenticity check based on the overall analytical profile is particularly efficient if the analyst has a good idea about the product: its ripeness, applied process, microbiological status, etc. Meta data information with influence to the analytical profile can be counter checked.

Table 7: Choice of conventional parameters and their use for interpretation (frequent analytical targets are indicated; fruit and product type specific deviations are possible)

Parameter	Fruit content	Sugar addition	Organic acid addition	Foreign fruit	Water addition	Technology (citrus juice)
Brix / Density					X	
Total titratable acidity			X			
Potassium	X					
Formol number	X					
L-Malic acid	X		X			
D-Malic acid			X			
Magnesium	X					
Calcium	X					X
Phosphorus	X					
D-isocitric acid	X		X			
Proline	X			X		
Citric acid			X	X		
Ratio citric / isocitric acid			X			
pH			X			
Maltose/Isomaltose		X				
Chloride					X	
Sulphate					X	
Glucose		X				
Fructose		X				
Ratio Glucose/Fructose		X		X		
Sucrose		X		X		
Total sugar (glucose, fructose, sucrose)		X				
Sucrose versus total sugar		X				
Sodium					X	
Nitrate					X	
Carotenoid profile (diff. fractions)				X		
Total Carotenoids, β-Carotene				X		
Sorbitol	X	X		X		
Water soluble pectins						X
Centrifugeable pulp						X
Phlorin						X
Ascorbic acid			X			

3.2.3. Detection of marker substances for specific adulterants

A specific marker substance for an adulterant can be used to highlight the fraud. Methods for the detection of these markers can be selective or specific, and the limit of detection of any adulteration depends on the sensitivity of the method being used. A list of marker substances for undeclared fruit types is given in Table 8.

In most cases marker substances are used to identify so called “foreign fruits”, or a cheaper fruit type added to the declared one. It is therefore necessary to know the concentration of a certain marker substance in both the declared fruit juice and in the adulterant.

Table 8: Marker substances of undeclared fruit types

Adulterant	Marker substance	Suitable for	Method
Pear	Arbutin	Most other juices	HPLC/UV phenolic compounds, proton-NMR
Apple	Phloridzin	Most other juices	HPLC/UV phenolic compounds
Pome and stone fruits	Sorbitol	Citrus, most berry fruits, tropical	HPLC/IC, enzyme test
Lime	Iso-pimpinellin, Bergapten 7-methoxy- coumarin	Lemon juice	HPLC/UV(DAD) and or MS
Lemon	Eriocitrin	Citrus juices	HPLC/UV Flavonoid glycoside (IFU 58)
Grapefruit	Naringin	Citrus juices	HPLC/UV Flavonoid glycoside (IFU 58)
Orange	Hesperidin	Passion fruit juice	HPLC/UV Flavonoid glycoside (IFU 58)
Grape	Tartaric acid	Other juices	HPLC/IC (IFU 65)

The example of sorbitol as a marker substance for the presence of undeclared fruit types is discussed here. It can be used to detect apple, pear, aronia and certain other fruits which might be added to blackcurrant juice. Apples, pears and aronia naturally contain sorbitol, whereas blackcurrant does not. Therefore positive detection of sorbitol in blackcurrant juice would clearly show that the juice is not authentic, but it would not differentiate which type of fruit is the adulterant. A more fruit specific indicator for added apple juice would be the phenolic compound phloridzin, which is a typical marker for apple and not present in pears or aronia.

If the marker substances are present in low concentrations, the possibility of unintentional product cross-contamination must be considered and manufacturing practices should be investigated. Furthermore, the natural occurrence of traces of sorbitol through naturally present micro flora should be looked at. A reference value to which a measured concentration remains acceptable is complex and needs more investigation. Official guidelines such as the AIJN Code of Practice have to apply relative high uncertainty margins in the benefit of the doubt, whereas individual company policies can be different.

Marker substances are also used in chromatographic fingerprint methods such as anthocyanin or flavonoid profiles. The occurrence of fruit specific substances allows the differentiation and identification of undeclared fruit types present in a sample. Important methods are shown in Table 9.

Furthermore there are also methods which use typical by-products from the production of sugar syrups as marker substance for added sugar. Such marker substances could be oligosaccharides. Maltose, maltotriose and higher starch degradation products can be detected with ion HPLC. Typical by-products of inversion or degradation of polysaccharides give characteristic peak patterns in GC chromatograms obtained after silylation [18].

Table 9: Fingerprint methods for fruit juice authentication

Fingerprint markers	Sugar addition	Acid addition	Foreign fruit	Applicable in juices/purees of…
Amino acid - HPLC or Amino Acid Analyser (IFU 57)			X	All fruits
Flavonoids - HPLC (IFU 58)			X	Citrus fruits
Anthocyanins - HPLC (IFU 71)			X	Red-coloured fruits
Polymethoxylated flavanones – HPLC			X	Citrus fruits
Oligosaccharides – HPLC / IC	X			Most fruits
Oligosaccharides – GC	X			Most fruits
Phenolic Fingerprint - HPLC			X	All fruits
Carotenoid profile - HPLC			X	Yellow/orange coloured fruits
Organic acids – HPLC		X	X	All fruits

3.2.4. Isotopic methods

The isotopic profiles of juice constituents are often “the ultimate weapon” for confirmation of a supposed adulteration. In some cases they are the only means of identifying a certain type of food fraud, especially when it is masked by “cocktails” of typical components (e.g. minerals, organic/amino acids, etc.)

A recent revision of the IFU recommendation explains the use of isotopic procedures in the analysis of fruit juices [19]. The possibilities offered by these parameters are summarised in Table 10.

Besides the determination of the isotopic ratios of juice water or sugars, refined approaches have been developed during the last three decades to enhance the sensitivity of isotopic methods, using:

- Multi-component approaches (looking at inner correlations between sugars, acids, etc.): [20–26],
- Multi-element approaches (combining several isotopes) [21],
- Site-specific approaches [27–29].

Stable isotopes can also help for origin confirmation [19]. In particular the use of strontium (^{87}Sr & ^{86}Sr) has been shown to be quite useful as it can be a very good marker for the “age”, in the geological sense, of rocks, which can also be used for origin assessment. However, it is much harder to use them in a predictive sense, that is to infer an origin of a product purely from analytical data. This is due in part to the overlapping of ratios seen for many geographical regions around the world.

Table 10: Available stable isotope analyses for the authentication of fruit juices

Parameter	Water addition	Sugar addition	Organic acid addition	Origin	Fertilisation regime
Oxygen or hydrogen isotope ratios of water (and ethanol from fermentation)	X				
Carbon and hydrogen isotope ratios of sugars or ethanol from fermentation		X			
Carbon and hydrogen isotope ratios of citric acid			X		
Global & positional carbon isotope ratios of malic, tartaric and ascorbic acids			X		
Carbon, hydrogen, oxygen, nitrogen, sulphur and strontium isotope ratios of bulk juice / juice components				X	
Nitrogen isotope ratio					X

3.3. Other commonly used methods

Not all aspects of fruit juice authenticity are controlled with required precision with methods described above. The identification of different fruit types and varieties is one of them. There DNA analyses are used successfully in a few cases. The analytical challenge is the low amount of DNA in the product and its degradation due to low pH and processing conditions. If a juice is clarified there is even no chance to obtain any exploitable DNA information. Nevertheless with advanced techniques the following differentiations have been validated successfully [30]:

- *Citrus sinensis* (orange) and *Citrus reticulata* (mandarin and other hybrids),
- Different varieties of mango.

The quantification of the adulterant is difficult because the available amount of DNA differs significantly from one sample to another. Results are semi-quantitative or qualitative only.

New DNA approaches will certainly increase the range of applications in the near future.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address. More details are given in the tables in the relevant sections.

Analytical Technique	Indicative data or Analyte or Parameter	Authenticity issue or information
Conventional methods	Main compositional parameters (Tables 1,2,3,7)	Compositional parameters are out of set specifications or AIJN limits
Metabolomic fingerprinting using ^{1}H NMR (SGF-Profiling^{TM})	Overall profile from 1H NMR spectrum + selection of compositional parameters (Tables 4,5,6)	Fruit type, geographical origin, addition of other fruits
Chromatography (HPLC/UV, HPLC/IC, HPLC/UV(DAD) or MS)	Marker substance (Table 8)	Undeclared fruit types
Chromatographic techniques (GC, HPLC) as fingerprint methods	Fingerprint markers (Table 9)	Addition of sugar, acids, foreign fruit
Stable isotope analyses	Isotope ratios of carbon, hydrogen, oxygen, nitrogen, sulphur, strontium (Table 10)	Various authenticity issues

5. Conclusion

Due to the complexity of authenticity control in fruit juices an analytical authenticity check is always a more or less tailor-made combination of different methods. A first establishment of an overview profile can be followed by analyses with a more precise focus and a lower limit of detection.

The high number of individual analyses to establish a meaningful overall profile is an economic and time-consuming handicap. If available, the SGF NMR-Profiling^{TM} is part of a better alternative. For the future it can be expected that this technique using modified sample preparation (e.g. fractionation, concentration) or other techniques with large data treatment like LC/HRMS [31] will successively enhance fruit juice integrity analyses.

However there will remain the necessity to confirm analytical observation by targeted methods. Isotopic techniques are likely to fulfil a major part of this need. Internal referencing methods will play an important role there.

Due to the growing number of production regions for semi-finished goods, agricultural development and the changing climate the interpretation of analytical results will become more difficult and specific reference data bases will be increasingly required in the future.

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Vinegar

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General overview of the product

Vinegar is one of the oldest fermented products in the world and its production dates back to around 2000 BC. Its acidic character (until the description of sulfuric acid, it was the strongest known acid) facilitated its use as a preservative due to its antimicrobial activity. Nowadays it is extensively used as a preservative, flavouring agent, and in some countries even as a healthy drink. Although, vinegar is mostly consumed in the food and beverage industry, it also finds applications in the healthcare and cleaning industry. The global vinegar market has reached values worth around USD 1.26 billion in 2017 growing at a rate of 2.1 % during 2010-2017 [1] and is further expected to reach a value of around USD 1.50 Billion by 2022.

As for fermented foods and beverages in general, the consumption of vinegar is a cultural trait. In Mediterranean countries, most vinegar is used directly or added to salads or to raw or cooked vegetables; thus, the appreciation of the organoleptic characteristics is straightforward. Therefore, "quality" vinegars are closely associated with these patterns of consumption. In contrast, in other countries, most vinegar is used for pickling or as part of sauces, and the impact of the organoleptic qualities, although possibly relevant for the final product, is less evident [2].

Types and major regions segment the global vinegar market. Different types of vinegar available are mostly balsamic vinegar, wine vinegar, cider vinegar, malt vinegar and rice vinegar. Geographically, Europe represents the biggest market for vinegar (more than half of the total global market share) followed by North America and the Asia Pacific region. In 2016, balsamic vinegar exhibited a clear dominance with the majority of market share. The use of vinegar is increasing in different cuisines, which results in increasing demand. Growing populations, rising disposable incomes, increasing health consciousness among consumers and the food and beverage

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industry are the main driving factors of the vinegar market. It is expected that the global vinegar market will witness growth both in terms of revenue and volume during the following years. Growth will come from changing consumer lifestyles and preferences. The interest in cooking gourmet and ethnic foods have increased among many consumers, thus prompting the sales of various dressings, most of which use vinegar as one of the key ingredients.

Some premium vinegars are being commercialised worldwide. A typical example of this trend is the increased consumption and trade of Balsamic Vinegar of Modena (Aceto Balsamico di Modena). In fact, Italy is the country that exports the most vinegar, providing twice the quantities of the other main exporters, Germany, Spain and France. Moreover, in terms of revenues, Italian vinegars are exported at much higher values than Spanish or German vinegars. The situation in Germany is different, considering that most German vinegar is sold for the pickling or sauce industry, whereas Spanish exports include also some premium vinegars such as Sherry vinegars (Vinagre de Jerez).

Sherry vinegars that are included in the European Union's Protected Designation of Origin (PDO) framework derive from Sherry wines and are necessarily aged in wood barrels for at least six months. This aging can be performed by a dynamic system (i.e., passage through different barrels containing vinegar from different vintages or different ages) or a static system. A more complex example is Aceto Balsamico, which is either Aceto Balsamico Tradizionale (ABT), regulated by two different PDO labels (ABT di Modena or ABT di Reggio Emilia), and Aceto Balsamico di Modena, which has a Protected Geographical Indication (PGI) status. The production of ABT is a long process that starts with the cooking of the grape must, which increases the sugar concentration to 400-500 g/L. Next, a partial alcoholic fermentation, which is initiated by osmophilic yeasts, produces a "sweet wine" of approximately 7 % (v/v) ethanol concentration and over 200 g/L of residual sugars. Then, some mother of vinegar is added to this "sweet wine," and it is left to be acetified. Once is acetified, the vinegar is placed in a "bateria" formed by five to seven barrels of different woods (oak, mulberry, chestnut, cherry, juniper, ash and acacia) and decreasing volumes (from 60 to 15 L), which are filled up to 2/3 of their total volume. This "bateria" is kept for at least 12 years with a yearly refilling from the previous barrel in a dynamic aging process. During this aging process, two phenomena occur: the transfer of components from the wood to the ABT and, more importantly, the concentration of the product and the integration of its components. The final product can have up to 500 g of sugar per kg of product, 7 % acetic acid (v/v) and 20 g of gluconic acid per kg. The oxidation of glucose by acetic acid bacteria yields gluconic acid. The result is a dark, concentrated and thick product sold in 100 mL bottles and with a market value that can easily reach 100 euros [3,4]. In contrast, Aceto Balsamico di Modena (ABM) is a PGI (Protected Geographical Indication) salad dressing ingredient now renowned throughout the world, obtained from cooked and/or concentrated grape must (at least 20 % of the volume), with the addition of at least 10 % of wine vinegar and a maximum 2 % of caramel for colour stability that is aged at least two months, not necessarily in barrels [5]. The geographical origin of ABM ingredients is not specified. However, some of these ABM can be aged for more than three years and are labelled "Invecchiato" (Aged). Overall, ABM is a cheaper version of ABT that has been popularized all over the world.

Some Asian vinegars, such as black vinegars from China or "kurosu" from Japan, are produced from rice and other cereals (including sorghum, wheat, and others) with a very important aging process in which concentration and thickening occur in a similar manner to ABT.

1. Product Identity

1.1. Definition of the product and manufacturing process

In general, food regulations consider vinegar the result of a double fermentation (alcoholic and acetous or acetification) of any sugar substrate, usually agricultural raw materials of plant origin with the exception of those produced from whey or honey.

In the European Union, the established limits for acidity and residual ethanol content are strictly set. Thus, the acidity of wine vinegar (acetification obtained exclusively from wine) must be at least 6 % (w/v), and the maximum residual ethanol allowed is 1.5 % (v/v) [4]. However, the variety of raw materials used in the production of vinegar is important, ranging from by-products and agricultural surpluses to high-quality substrates for the most unique and prized vinegars, such as Sherry vinegar (Spain) and Aceto Balsamico Tradizionale (Italy). There are up to ten types of vinegars, which include wine, fruit, cider, alcoholic, cereal, malt, malt distillate, honey and whey vinegars. Undoubtedly, wine vinegar is the most common type in Mediterranean countries, although the latest gastronomic trends have led to a considerable expansion of the varieties available in recent years. However, worldwide most of the vinegar produced is “white” vinegar, that is, vinegar produced directly from diluted alcohol [3]. In Asia, rice vinegar is the most common type, although other types are also found, many of them following very traditional systems of production.

Table 1: Different vinegars of the world are classified according to substrate, name and region/country of production and distribution [6]

Substrate (Raw material)	Name	Region/Country (Production & distribution)
Grape	Wine vinegar	Global
	Balsamic vinegar	Global
	Red vinegar	Global
	White vinegar	Global
	Distilled white vinegar	Global
	Sherry vinegar	Global
	Traditional Balsamic vinegar	Global
Apple	Cider vinegar	US, Canada
Different fruits (mango, kaki, berries)	Fruit vinegar	East and Southeast Asia
Date	Date vinegar	Middle East
Coconut	Coconut vinegar	Tropical Africa
Rice	Rice vinegar	China, Japan, Korea
	Kurosu	China, Japan, Korea
Malt	Malt vinegar	USA, Northern Europe
	Distilled malt vinegar	USA, Northern Europe
Whey (dairy by-products)	Whey vinegar	Europe
Honey	Honey vinegar	Global

Therefore, vinegars can be classified by their substrates of origin or by their systems of production. It is necessary to differentiate between those products derived from the double fermentation of a single fruit (most often grapes or apples) and those that are “flavored” vinegars, that is, vinegars of various origin with added concentrates of different fruits, flowers, or spices. Although these “flavoured” vinegars are not considered vinegars in some countries, lax regulations in other countries allow these products or condiments to be sold as “vinegars”.

The first fermentation is an alcoholic fermentation and transforms sugars or processed starches into ethanol. This process is performed by yeast, mostly from the species *Saccharomyces cerevisiae*, although some other species can also perform the alcoholic fermentation, partially or totally. The final result is considered the substrate of the second transformation, to convert ethanol to acetic acid. Although this second process is often referred as “acetic” or “oxidative” fermentation, it is not biochemically a fermentation but an oxidation. The proper term is thus “acetification” and involves a two-step oxidation, from ethanol into acetaldehyde and further from acetaldehyde into acetic acid, whereby the end of this process requires an electron acceptor, with molecular oxygen being the most common [2]. The microorganisms responsible for this process are acetic acid bacteria. These bacteria are found on substrates containing sugars and/or alcohol, such as fruit juice, wine, cider and beer. On these substrates, the sugars and alcohols are incompletely oxidized, leading to the accumulation of organic acids, such as the production of acetic acid from ethanol. Although more than 60 species have been described as acetic acid bacteria, only a limited number of them are involved in the production of vinegar. The species most commonly found in the production of vinegar are *Acetobacter pasteurianus, Komagataei bactereuropaeus* and *Komagataei bacterxylinus*. Several attempts have been done to have single, well-defined species of acetic acid bacteria for the production of vinegar, although it has been concluded that a mixture of at least two species (one of them as “starter” and the other as “finisher”, with different acetic acid sensitivities) is the most appropriate to be used as inoculum for the production of vinegar, especially those above 5 % (w/v) acetic acid [7,8].

Vinegars can also be differentiated by their production systems. In traditional vinegars, the transformation of ethanol into acetic acid is performed by a static culture of acetic acid bacteria at the interface between the liquid and air. Barrels are filled to 2/3 of their capacity, as to leave an air chamber, which is kept in contact with the outside air using one opening or various types of openings. This production system, which is considered to be the traditional method, is called “surface culture”. A more standardized version of this method, the “Orleans method,” includes side holes for air circulation and adds a funnel with an extension to the base of the barrel to allow wine to be added at the bottom of the barrel, preventing the alteration of the "mother of vinegar". This mother of vinegar is the biofilm formed by the transforming microorganisms, i.e. the acetic acid bacteria, which develops on the surface due to the need for oxygen. The vinegars produced by this traditional system are generally considered of high quality because of their organoleptic complexity, which is mainly due to the metabolism of the acetic acid bacteria and the overlapping vinegar production with aging. However, this process is very slow, and the production of vinegar can take from months to years.

To reduce the acetification time, other methods, such as the Schutzenbach systems with submerged cultures, have been developed. Bacteria are immobilized on wood chips or charcoal, forming a solid bed on which the vinegar spreads. After passing through the bed of chips, the vinegar is collected in a container at the bottom and pumped back to the same fixed bed. The acidity successively increases, and it is possible to obtain vinegar of reasonable quality within a week.

Submerged culture systems provide a much faster alternative. These systems rely on suitable turbines to generate a flow of air bubbles into the wine or alcoholic solution. The oxidative process occurs in the air-liquid interfaces of the air bubbles. Improvements to this process generally involve engineering (improving the maintenance and persistence of the bubbles in the liquid, the uniformity of the bubble size, the recovery of lost aromas, etc.). Vinegar is then produced at a significantly lower cost, the bacteria act as bioreactors for the transformation of alcohol into acetic acid, the airflow contributes to a considerable loss of the volatile compounds present in the original alcoholic solution, resulting in a less complex product from a sensory point of view. Although early containers for submerged culture processing were made of wood, the usual containers are stainless steel, which is more hygienic and resistant to acidic conditions. The limitations can be compensated by subsequent aging in barrels or by submerging wood fragments or wood chips, which may contribute to the recovery of some of the missing organoleptic character. Despite the loss in product quality, this methodology has two important advantages: speed (the vinegar can be produced in cycles of 24 hours, or even shorter) and acidity (the product can reach concentrations of acetic acid of up to 23-25 %, compared to the 6-13 % achieved with other systems). Higher acidity helps to reduce transportation costs by reducing water transport.

An important aspect that contributes to the organoleptic quality of vinegars is aging, which enables the integration of the different compounds in vinegars. The increase in organoleptic quality during aging is remarkable; in addition to interactions with the wood, a series of chemical reactions, evaporation, the production of esters, reactions between acids and residual alcohols, and other processes result in better integration of aromas and metabolites and a reduction in the pungency of acetic acid.

1.2. Current standards of identity or related legislation

Vinegar is regulated by different standards, and even the legal definition itself varies from country to country [3]. The regional European Codex standard for vinegar dates back to 1987 [9], and it states that vinegar is as any liquid fit for human consumption, produced exclusively from suitable products containing starch and/or sugars by the process of double fermentation, first alcoholic and then acetous. Although several attempts have been made to convert the regional standard into a world-wide standard, this conversion has so far not been addressed, especially in view of trade patterns and significant regional differences. The standard describes different kinds of vinegar, essential composition and quality criteria together with optional ingredients, contaminants, hygiene, weights and measures as well as methods of analysis. This regional standard has not taken up by all national legislations of the Member States due to the fact that in two States the name ‘vinegar’ applies to the product obtained by dilution of synthetic acetic acid.

In the USA, the FDA (Food and Drug Administration) requires that vinegar products must contain at least 4 % acids. There are no FDA standards of identity for vinegar, however the “Compliance Policy Guides” establishes the labelling requirements for cider, wine, malt, sugar, sugar and vinegar blends.

In the EU, Regulation (EC) 1493/1999 [10], there are currently established thresholds for acidity and residual alcohol. Hence vinegars are those products having a minimum 5 % (w/v) acidity and a maximum of 0.5 % (v/v) ethanol, with the exception of wine vinegar which is exclusively obtained from wine and whose acidity is 6 % /w/v) at least and has a maximum ethanol concentration of 1.5 % (v/v). More recently the European Commission published Commission Regulation (EU) 2016/263 [11] amending Annex II to Regulation (EC) No 1333/2008 [12] of the European Parliament and

Council as regards the title of the food category 12.3 Vinegars. The new title of the food category 12.3 is now: Vinegars and diluted acetic acid (diluted with water to 4-30 % by volume)**.** This category was renamed because *in some Member States only vinegars obtained from the fermentation of agricultural products are allowed to be named 'vinegars'. In other Member States, however, both products obtained from the dilution with water of acetic acid and vinegars obtained from the fermentation of agricultural products are marketed under the name 'vinegar'.*

Three EU schemes of geographical indications and traditional specialties, known as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialities Guaranteed (TSG), promote and protect names of quality agricultural products and foodstuffs. Products registered under one of the three schemes may be marked with the logo for that scheme to help identify those products. The schemes are based on the legal framework provided by EU Regulation No 1151/2012 [13] of the European Parliament and of the Council of 21 November 2012 on quality schemes for agricultural products and foodstuffs. This regulation (enforced within the EU and being gradually expanded internationally via bilateral agreements between the EU and non-EU countries) ensures that only products that originate from that particular region are allowed to be marketed as such. Regarding vinegars, there are currently five PDO registered categories and one PGI. Among PDOs: three from Spain (Vinagre de Jerez, Vinagre de Montilla-Moriles, Vinagre de El Condado de Huelva) and two from Italy (Aceto Balsamico Tradizionale di Modena, Aceto Balsamico Tradizionale di Reggio Emilia). Lastly Aceto Balsamico di Modena is registered as a PGI.

Currently there is no European trade association of vinegar producers. The Vinegar Institute is the international trade association representing the vast majority of vinegar manufacturers and bottlers, mainly those with activities in the USA.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Framework of national and international legislation

Due to the observed differences in the laws on vinegar from one country to another, it is clear that if a vinegar produced in one country is commercialized in another in which the definition of vinegar changes, it poses a problem and risk for consumers and can become an authenticity issue if its origin is not clearly declared. Thus, a number of examples exist where a vinegar from one country is commercialized in other country in which the legal definition of this kind of vinegar varies. For example, while in the European Union, the term vinegar describes 'a product of a double fermentation (alcoholic and acetic fermentation) from substances of agricultural origin', in the USA a 'synthetically-produced acetic acid diluted with water' can also be labelled as vinegar. Hence, if the latter is sold in Spain, it could be considered a fraud to the consumers. Other example of this problem occurs between Germany and Europe. The German legal definition of 'wine vinegar' permit the production of vinegar by acetic fermentation from natural ethanol, by diluting acetic acid with water or by blending fermentation vinegar with synthetic acetic acid, or with vinegar made from synthetic acetic acid [14]. However, European regulations indicate that wine vinegar can only be produced through the acetic fermentation of wine produced from fresh grapes. So commercialising some 'wine vinegars' from Germany produced with alcohol from different origins as genuine wine vinegar in a European country, could mislead the consumer.

2.1.2. Raw materials

One of the main problems in the vinegar industry lies in the difficult distinction between the use of low-quality and high-quality raw materials, between true vinegars rich in extracts from the raw materials or their blends, as well as to distinguish between highly valued, high quality wine vinegars or aged balsamic vinegars and their cheaper alternatives derived from other raw materials such as malt or alcohol and/or vinegar adulteration with diluted synthetic acid [15]. Within this section, the following issues are discussed.

2.1.2.1. Addition of chemical acetic acid

One of the first frauds in the vinegar industry, and one that has been occurring for more than eighty years, is the addition of chemical or non-biological acetic acid to different types of vinegar contrary to the vinegar industry regulations. The vinegar obtained by chemical acetic acid is called *wood vinegar* or *vinegar essence*, and it cannot be sold as *fermented vinegar* due to it contains more heavy metals per kg of pure acetic acid that the regulated permitted amount (maximum of 5 mg/kg pure acetic acid), which supposes a risk for the consumer. In this sense, European legislation indicates that authentic wine vinegar cannot contain acetic acid obtained from either petroleum derivatives or wood pyrolysis (synthetic acetic acid). These adulterated products constitute a fraud for consumers and are unfair practices to other vinegar producers. To detect the addition of chemical acetic acid to vinegar, the determination of formic acid, derived from the pyrolysis of wood, has demonstrated to be an indirect indicator of it [16], although the detection of synthetic acid added to spirit vinegar or to relevant products produced with the adulterated vinegar or synthetic acetic acid still remains difficult.

2.1.2.2. Addition of water to dried grapes or to must concentrate

The production of vinegar from dried grapes diluted with water is an unfair practice more related to the industry of wine vinegars. This so-called 'raisin vinegar' is commonly produced in some Mediterranean countries by fermenting dried grapes and rehydrating with tap water, but it cannot be regarded, or labelled, as 'wine vinegar'. Due to the fact that this method reduces the price of production, it can be considered, in some Europe countries, as a fraudulent activity. Thus, it has been noticed that some Greek vinegars produced by the above water addition method have been improperly imported into Italy as 'wine vinegar' [17].

2.1.2.3. Use of alcohol or sugar not from wine

Commercialising vinegars produced with alcohol from different origins other than grapes, as genuine wine vinegar, is one of the most common fraudulent activities in the vinegar industry. This fraudulent practice aims to reduce manufacturing costs and constitutes a fraud to consumers. Another unfair practice that is currently happening, is the addition of different proportions of alcohol vinegar to wine vinegar samples, which makes the product cheaper. This unfair economic advantage poses an important threat for this sector. These adulterations are difficult to detect because the alcohol added to the base wine prior to the commencement of the fermentation process does not always have a well-known botanical origin [18]. The alcohol added to wine vinegars should come from the fermentation of skins of grapes, but sometimes its origin is fairly diverse: molasses, sugar beet, or sugar cane. Therefore, authenticity issues arise in the ability to detect if the source of the acetic acid and the grape sugars is truly grape (wine) ethanol or wine must, or other ethanol made from fermentation of some other cheaper agricultural products (cereal, potato starch, beetroot or sugarcane), that is called synthetic acetic acid. In the case of

balsamic vinegar as Aceto Balsamico di Modena IGP, there could also be the unfair practice of adding exogenous sugars to cooked and/or concentrated grape must.

2.1.2.4. Blends of different type of vinegars

Another common fraudulent practice in the elaboration and commercialisation of vinegar is the mixture of different proportions of wine vinegar and alcohol vinegar. The authenticity issue in this case occurs when this blend is sold under the denomination of wine vinegar, as if it was a pure product. Generally, a good method for a safe differentiation between them is the identification of specific fruit acids, although this can be manipulated easily with the addition of fruit-specific acids and amino acids.

2.1.3. Authentication of geographical indications

The existence of protected origin designations or quality labels in vinegars, which is very common in Southern Europe, provides a greater guarantee to the product although, at the same time, encourages the picaresque nature of unfair producers. The basic requirements for the product to receive such protection is that it must be closely associated with a particular geographical area and with a traditional production procedure which account for the specific quality and characteristics of the vinegar, and therefore, they have higher prices. Some of these characteristics that are defined and established under the PDO Regulations and are mandatory for these vinegars are for example, total acidity, total dry extract or total ash content. Although these PDOs strictly regulate these parameters - all regularly controlled by an inspection authority - some adulteration or frauds have occurred. All too often, however, they are condoned by leading manufacturers, mainly due to the powerful argument of extra profit. Examples include the well-known case of Traditional Balsamic Vinegar of Modena PDO (Protected Designation of Origin) and the Balsamic Vinegar of Modena PGI (Protected Geographical Indication). The former is produced by a traditional, time-consuming and expensive production method obeying very strict rules of raw material provenance and production methods, ensuring a high quality. The second one is produced industrially and is a much cheaper product made from cooked must, concentrated must and wine vinegar via a complicated process [19,20]. Due to their different prices, frauds and mislabelling are frequent, and many brands of these popular vinegars commercialised in the market are in fact merely a sweetened red wine vinegar with food colouring.

Also of considerable interest is the differentiation between Spanish PDO vinegars. Good and promising results in the characterisation and classification of these PDO vinegars have been achieved using different analytical procedures [21-24], but there is still a long way to go. The need to develop methods to distinguish vinegars with this recognised label from non-authentic product is obvious, as not only will the consumer be cheated, but he or she will lose confidence in PDO/IGP labels.

2.1.4. Production process and aging

Adulteration related to production processes occur mainly in vinegars produced by traditional systems such as Sherry vinegar or Traditional Balsamic Vinegar of Modena and Reggio-Emilia. There is an increased interest in differentiating vinegars that have been produced by a traditional method from those produced by a quick production method, due to the fact that the former is associated with a higher quality but also with a longer processing time and a higher cost of production. A further authenticity issue arises when there is a specified minimum aging time for a particular vinegar, as in the case of Sherry vinegars or Traditional Balsamic Vinegar of Modena, the latter being only sold after following an ageing process of at least 12 years in a set of wooden casks

of decreasing volume [19]. The organoleptic vinegar properties developed during ageing make the finished product very appealing. Nevertheless, the production time and costs are too excessive to permit a lucrative trade. Hence, an objective of the vinegar industry is to produce these aged vinegars with the same organic characteristics related to aging, but making it in the most economic and rapid way. For these reasons, the vinegar industry has a very real interest in speeding up ageing if this can be done in a way which does not produce an inferior product or result in the consumer being misled. In this context, the use of wood chips is being investigated. Moreover, there is an increasing necessity to develop simple methods able to detect specific metabolites in vinegars as possible indicators for the ageing process and traditional procedures, in order to protect the consumers and avoid unfair competitions.

2.1.5. Adulteration by addition of grape must caramel

The colour of the vinegars is an important quality parameter as it can, for example, indicate that a wine vinegar has undergone a process of aging in wood barrels. The wine vinegar colour changes during aging from amber to mahogany due to the changes that occur, in the content of polyphenols, tannins and anthocyanins as well as an oxidation process, which are responsible for the darkening of the vinegar. In this context, although the addition of grape-must caramel is allowed by the current legislation to correct and unify the final colour of the different batches, sometimes it could be added to simulate the effect of a greater aging of wine vinegar in wood, which would be considered as an unfair practice.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

To assess the quality and authenticity of vinegars, several countries have established acceptable methods and ranges or guide values for some vinegar parameters, based on results obtained on the analysis of a large numbers of authentic samples. However, current national and international directives include more methods designed for vinegar identification and generally control than for authenticity issues. In this section officially recognised methods used on a regular basis for vinegars are described (cf. Table 2).

Table 2: Officially recognised methods to test for vinegar authenticity

Method	Reference	Technique	Objective
For wine vinegars			
Determination of total acidity content	OENO 52/2000	Neutralisation of acids in sample by alkali solution	To comply with legal requirements (definitions, PDO, PGI…)
Determination of the fixed acidity content	OENO 53/2000	Neutralisation of the (non-volatile) acids of the residue in an aqueous solution using an alkali solution	To comply with legal requirements (definitions, PDO, PGI…)
Determination of the volatile acid content	OENO 54/2000	Calculation of difference between total acidity and fixed acidity, expressed in grams of acetic acid per L	To comply with legal requirements (definitions, PDO, PGI…)
Detection and quantification of the presence of synthetic acetic acid	OENO 55/2000	After extracting the acetic acid using sodium hydroxide, complete by liquid scintillation the reactivity ^{14}C of the product converted into benzene	Authentication: Values less than the characteristic ^{14}C contents of the assumed year of production represent either a mixture with products from more recent years, or the addition of all or part of the synthetic acetic acid
Determination of the residual alcohol content	OENO 56/2000	Distillation of vinegar, oxidisation of ethanol by potassium dichromate and determination of its content by titrating the excess potassium dichromate by a solution of iron sulphate and ammonium	To comply with law requirements (legal definitions, PDO, PGI…)
Determination of total dry extract content	OENO 57/2000	Evaporation of sample and drying in oven, then weighing	Detection of frauds: the addition of water or an aqueous solution of acetic acid (very low total dry extract value) or the addition of non-volatile substances (very high total dry extract value). Database for the type and origin of the vinegar is necessary.
Determination of ash content	OENO 58/2000	Incineration of the vinegar extract between 500°C and 550°C through to complete combustion of the carbon	Detection of frauds: the addition of water or an acetic acid aqueous solution (very low ash content) or the addition of non-volatile substances (very high ash content). Database for the type and origin of the vinegar is necessary.
Determination of the non-volatile reducing substances content	OENO 59/2000	Evaporation of volatile substances, hydrochloric hydrolysis, oxidisation by a copper alkali solution in excess with titling by iodometry of copper ions	Detection of frauds: the addition of non-volatile substances.
Determination of the total sulphur dioxide content	OENO 60/2000 + OENO 13/2008	Iodometric titration direct (free SO_2) and after double alkaline hydrolysis (combined SO_2)	Control the level of SO_2 and check compliance with standards
Determination of the total ascorbic acid content	OENO 61/2000	Oxidisation of ascorbic acid by iodine with transformation into dehydroascorbic acid, precipitation with 2.4 – dinitrophenylhydrazine. Separation by thin film chromatography, solubilisation in acetic medium and colorimetric determination at 500 nm.	Detection of a fraudulent technological use.

Method	Reference	Technique	Objective
Measurement of chloride content	OENO 62/2000	Potentiometric titration of Cl ions with a solution of silver nitrate, in an acidic environment, after prior measurement of the potential equivalent point of a standard chloride solution	Detection of the fraudulent increase in the dry extract by the addition of sodium chloride
Measurement of sulphate content	OENO 63/2000	Precipitation of sulphates with barium chloride, drying, calcination and weighing	Detection of frauds (aimed at increasing the total dry extract).
Measurement of copper content	OENO 64/2000	Direct measurement by atomic absorption spectrophotometry.	Contamination from contact materials during manufacture, and the iron of the wine itself. Excessive content could cause haze or serious alterations in colour.
Measurement of zinc content	OENO 65/2000	Direct measurement by atomic absorption spectrophotometry.	Contamination from contact materials during manufacture, and excessive content could cause hazes or serious alterations in colour.
Measurement of iron content	OENO 66/2000	Direct measurement by atomic absorption spectrophotometry.	Contaminations from contact materials during their manufacture, and of course the iron of the wine itself. Excessive content could cause haze or serious alterations in the colour.
Measurement of lead content	OENO 67/2000	Direct measurement of lead content in the vinegar by atomic absorption spectrometry without flame (electrothermal atomisation).	The presence of lead in vinegars mainly has its origin in contaminations from contact materials during their manufacture, and the lead of the wine itself from which the vinegar has been made
Measurement of mercury content	OENO 68/2000	Mineralisation. Reduction by permanganate Measurement by atomic absorption spectrometry (cold vapour).	Toxicologic issue
Measurement of the acetoin content	OENO 69/2000	Neutralisation of the sample at pH 7.00 with calcium hydroxide. Direct measurement of the acetoin via gas chromatography	Authentication: Determination of quality and origin by the analysis of acetoin content in the wine vinegars (between 100 mg/L and over 400 mg/L)
Measurement of the methanol, superior alcohols and ethyl acetate	OENO 70/2000	Neutralization of the sample at pH 7.00 with a sodium hydroxide solution. Measurement, via GC, of some volatile components: methanol, propan-1-ol, butan-2-ol, 2-methylpropan-1-ol, butan-1-ol and 2-methylbutan-1-ol + 3-methylbutan-1-ol	Organoleptic and possibly toxicologic issue
Authentication by SNIF-NMR® and other isotopic methods	OENO 71/2000	Extraction of the acetic acid from the vinegar with ether. Purification using a Cadiot column. Determination of the purity of acetic acid. Measurement of the site-specific deuterium/hydrogen ratio in the resulting acetic acid, via deuterium NMR.	Detection of frauds: detection of synthetic acetic acid in vinegars and any other downgrading of vinegars. Detection of possible addition of alcohol-vinegar coming from plants whose metabolism is C_4 (sugar addition from cane) or C_3 (beet)

Method	Reference	Technique	Objective
Detection of synthetic acetic acid in wine vinegars by the determination of beta radioactivity of ^{14}C of acetic acid by liquid scintillation	OENO 12/2006	Extraction of acetic acid from the vinegar. Acetic acid of mineral origin (Control) is counted in the same way. β emission value of the ^{14}C in the sample compared with the average value of the β emissions of ^{14}C found in the ethanol in genuine late harvest wines.	Detection of fraud: detection of the addition of synthetic acetic acid (levels lower than those for a given year) or the entire content of it. Control of the year of production of the raw wines.
Method for $^{13}C/^{12}C$ isotope ratio determination of acetic acid in wine vinegar by isotopic mass spectrometry	OIV-OENO 510-2013	$^{13}C/^{12}C$ isotope ratio of acetic acid by Isotope ratio mass spectrometry (IRMS)	Detection of frauds related to the botanical origin of acetic acid and revelation of the addition of synthetic acetic acid. Determination of sugar addition (cane)
Method for $^{18}O/^{16}O$ isotope ratio determination of water in wine vinegar using isotopic mass spectrometry	OIV-OENO 511-2013	$^{18}O/^{16}O$ isotopic ratio of water by Isotopic Ratio Mass Spectrometry (IRMS)	Detection of frauds related to the production of vinegars from fresh grapes or from dried grapes with water addition
Determination of the distribution of deuterium in the acetic acid of vinegar wine by Nuclear Magnetic Resonance (NMR)	OIV-OENO-527-2015	Composite ^{1}H-NMR and ^{2}H-SNIF–NMR	Detection of frauds about botanical origin of acetic acid and revelation of the addition of synthetic acetic acid
For all vinegars			
Isotopic analysis of acetic acid and water Part 1: ^{2}H-NMR analysis of acetic acid. Part 2: ^{13}C-IRMS analysis of acetic acid. Part 3: ^{18}O-IRMS analysis of water in wine vinegar	CEN, EN 16466-1,2,3 (2012)	SNIF-NMR (D/H), $^{13}C/^{12}C$ IRMS, $^{18}O/^{16}O$ IRMS	Determination of frauds related to vinegar acetic acid, water and sugar addition (beet, cane)

3.2. Other commonly used methods

3.2.1. Sensory analysis

Sensory analysis has proven to be a simple and reliable tool for assessing the quality of vinegars [25]. The appropriate sensory methodology must be clearly defined and the attributes used in discriminant or descriptive analysis must be precise and well-recognised by the panel. The sensory characterisation of vinegars for monitoring vinegar quality has been widely performed in many studies over a number of years [26-31]. Moreover, in some vinegars, quality control is mainly based on their sensory properties, as is the case for Traditional Balsamic Vinegar of Modena. Sensory vinegar analysis can be performed by olfactive and gustative analyses, as well as by the determination of other parameters such as viscosity and colour.

3.2.1.1. Odour and taste

In order to analyse the taste and odour of the vinegars, there are different protocols such as preparing the vinegar in a way that most resembles how it is normally consumed (using lettuce suspended in the vinegar [27]or diluting with cold or hot water), or testing and smelling vinegar as is, using opaque cups to avoid colour influences, being it the usual sensory analysis for vinegar cellars [26].

Within the different types of sensory analysis, the most used are the descriptive test, that is useful for determining the sensory profile of the samples, and the discriminatory test, which include a wide range of tests such as triangular test (ISO, 2004, standard 4120) [32] and Paired Comparison tests (ISO, 1983b, standard 5495) [33], preference test, etc. These methods require a well-trained testing panel, and concrete and adequate attributes.

3.2.1.2. Viscosity

Viscosity is another important parameter in the sensorial quality of some vinegars such as in the case of the Traditional Balsamic Vinegar of Modena. Nevertheless, no procedure has yet been established to determine this objectively, as it is assessed in an empirical manner and wrongly expressed as physical density.

3.2.1.3. Colour

Colour is one of the most important parameters used by consumers to assess the quality of a food product. Some studies have described a relationship between some compounds and a darker colour such as melanoidin, and products from the degradation of sugars and Maillard reactions [3]. A darker colour is also related to a longer aging period in wine vinegars and Traditional Balsamic vinegar of Modena. Some techniques such as UV-Visible spectrophotometry or excitation-emission fluorescence or transmission colorimetric techniques are being used with promising results for this issue [34-36]. However, the colour could be easily modified with the use of grape must caramel or other additives and no methods haves been officially established to assess and control this parameter.

3.2.2. Physicochemical analysis

Notwithstanding the fact that the quality of vinegars has been traditionally evaluated by using a trained sensory panel, more rapid and objective methodologies have been tested and performed by instrumental measurements.

3.2.2.1. Chromatographic techniques

Chromatographic techniques have been widely applied, for a long time, to determine certain vinegar compounds useful for characterising, classifying or detecting adulteration in vinegars.

High-performance liquid chromatography-mass spectrometry (HPLC)

HPLC has been widely used to analyse compounds such as phenols. Phenolic compounds are present in wine vinegars due to their natural content in grapes or as a result of contact with wood during the aging process, and they have demonstrated to be important in the determination of origin and the technology involved in the production of wine vinegars [37-39].

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (GC) is the official method for the determination of acetoin content, methanol, superior alcohols and ethyl acetate (OENO 69-70/2000) [40,41], and has also been applied to determine poly-alcohols in vinegars, all of them related to quality and origin. In addition to this method, gas chromatography coupled with mass spectrometry (GC-MS) has been the most efficient and widely employed technique to date to determine the volatile composition of vinegars which is also directly related to the quality of the vinegar. This technique normally requires a prior extraction step (such as dynamic and static headspace extraction, solid phase microextraction, stir bar sorptive extraction or liquid-liquid extraction methods). Examples of the efficiency of this

methodology are the determination of volatile aldehydes as discriminant parameters in quality vinegars or the determination of the volatile profile as a classification parameter of different vinegar types or geographical indicators [42-45]. However, regardless of the fact that these sampling methods have been widely employed in the volatile analysis of vinegars, the experimental sources of variability related to GC–MS (e.g. columns, stationary phase, temperature or experimental conditions and sample preparation) still cause some variations that directly affects the final results. These problems are being recently resolved by chemometric tools such as Multiple curve resolution (MCR) or Parallel factor analysis (PARAFAC) [44].

Gas chromatography coupled with olfactometry (GC-O)

The intensity and quality of the aroma constitutes the primary quality factor in vinegars. Although the aroma of vinegars is widely studied by sensory analysis and GC-MS methodologies, all volatile compounds determined in vinegar do not have the same contribution to the overall aroma of the product. Gas chromatography-olfactometry (GCO) is the most appropriate analytical technique to determine these compounds with real impact of the aroma of a vinegar, known as impact odorants, among the whole volatile fraction. This technique provides instrumental and sensory analysis simultaneously as the eluted analytes are perceived at the same time by the human nose and a conventional detector, such as the flame ionic detector (FID) or the mass spectra detector (MSD), which turns this technique into a powerful one in food aroma characterisation. However, little research can be found in the literature regarding the application of this technique in vinegars. Thus, only a few papers deal with a comprehensive characterisation of the aroma profile of red wine vinegars [31], some Chinese vinegars [46] or with the quality perception of Sherry vinegars [47].

3.2.2.2. Spectroscopic techniques

Near infrared spectroscopy

Near-infrared spectroscopy (in the range of 5000 - 15000 cm^{-1}) is a potential spectroscopic technique that has been applied to the analysis of vinegars. Near-infrared spectroscopy (NIR) has the advantages of high speed, accuracy, simplicity, and low cost. NIR spectra can record the multifrequency and co-frequency information of organic molecules, which involves the response of molecular bonds of C–H, N–H, C–O, and O–H, being useful for determining organic acids and pH in vinegars, as in the case of MIR, mentioned below [48]. The vinegar sample is either placed in a cuvette and the spectrum collected by absorption mode or the bottles can be directly scanned in transmission mode. A multivariate analysis of the data is usually employed to develop models able to classify the different classes of vinegars, different geographical origins [23,49] or even to predict or monitor the vinegar ageing process [50,51].

Mid-infrared spectroscopy

Mid-infrared spectroscopy (MIR) (in the range of 500 - 5000 cm^{-1}) has also been shown to be able to address a wide range of issues and provide solutions for rapid analysis and on-line control of vinegars. This technique combined with chemometrics has gained wide acceptance for authenticity and classification purposes in food, being informative at the molecular level and produces a single spectral fingerprint of each sample. Moreover, the use of an accessory of Attenuated Total Reflectance (ATR) allows the direct analysis of liquids in a simple, fast, only a few minutes, and non-destructive manner, involving minimal sample preparation. This method provides a greater amount of chemical information compared to NIR spectroscopy in terms of chemical assignment of observances and allows the interpretation of the spectra without the need of complex chemometrics. Thus, Fourier transform mid infrared spectroscopy (FT-IR) coupled with

ATR has been applied to investigate its potential as a tool for characterising different categories of high-quality vinegars by a studying the differences in the spectra. FT-IR spectra have also been used to predict the sensory score of traditional balsamic vinegar of Modena by the performance of different partial least squares (PLS) regression models [52] as well obtaining a full calibration model for organic acids in vinegars [53]. Finally, the technique can also be used to control certain steps and factors of the production processes in industry, making it possible to carry out necessary corrective actions without delay [54].

Fluorescence spectroscopy

Fluorescence spectroscopy is also being investigated as an alternative quality control tool for vinegars, with the same attributes as those mentioned above. Different methods of analysis are possible, the conventional one being the measurement of the excitation or emission spectra at a single emission or excitation wavelength, respectively. However, instead of measuring a single emission spectrum at a selected excitation wavelength, the emission spectra at different excitation wavelengths can be recorded, in a technique known as excitation-emission fluorescence. The latter results in a bi-dimensional Excitation- Emission Matrix (EEM), which contains unique information of each measured sample, having the advantage of containing more information about the fluorescent species than the conventional excitation and emission spectra separately. Moreover, the potential of the EEM technique can be improved by applying multivariate methods in the analysis of the fluorescence results such as Parallel Factor Analysis (PARAFAC) and its combination with PLS discriminant analysis. PARAFAC is used to decompose fluorescence EEMs into different independent groups of fluorescence components (fluorophores), as well as their relative concentration (scores) in each sample. This method extracts the most relevant information from the data in order to build further robust calibration and/or classification models. For this reason, this technique has been more widely applied in the study of wine vinegars than the simple excitation or emission analysis. Thus, Callejón et al. [48] and Ríos-Reina et al. [16] studied fluorescence excitation–emission spectroscopy combined with suitable multivariate methods. In these studies, the fluorescence Excitation-Emission Matrices (EEMs) were obtained by varying the excitation wavelength ranging between 250 and 700 nm (every 5 nm), and recording the emission spectra from 300 to 800 (every 2 nm). For these measurements, excitation and emission slits were both set at 5 nm, and the scan rate was fixed to 1200 nm min-1. These studies demonstrated this method's ability to characterise and classify three Spanish PDO wine vinegars according to their protected designation of origin, as well as their categories (aged and sweet) [24; 55]. However, despite the promising results obtained, is not yet widely in use in this field.

Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectroscopy, which has the advantage of being a rapid and non-selective analysis without any manipulation or derivatisation, has recently achieved general acceptance as a powerful tool for vinegar quality and authenticity determination. NMR can provide information on chemical composition, concentration of soluble metabolites and their structure in the samples such as sugars, acids and flavonoids, with the advantage of providing the best combination of fast data acquisition and predictive capability. However, the large amount of data needs to be treated by multivariate methods such as principal component and discriminant analysis with the final objective of making models able to discriminate authentic and non-authentic vinegars, origins, or vinegar types.

Different nuclei to which the spectrometer is tuned have been investigated for vinegar authentication. The most commonly applied NMR technique for origin authentication, and recently recognised as an official method, is deuterium SNIF-NMR (Site-specific Natural Isotopic Fractionation studied by nuclear magnetic resonance spectrometry). However, another very used

method with promising results is proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, which, combined with multivariate statistical data analysis, has demonstrated its usefulness in the characterisation of the ageing process and the discrimination of different vinegar types [19,56]. The application of ^{13}C NMR, two-dimensional ^{1}H–^{13}C heteronuclear multiple-bond correlation (HMBC), and ^{1}H–^{13}C heteronuclear single quantum coherence (HSQC) spectra for the characterisation and discrimination of Balsamic vinegars of Modena in order to obtain an indirect indicator of authenticity and a quality control tool have also been studied, although to a lesser extent [57]. It should be also considered that as vinegar samples contain a high amount of water, optimising water suppression methods is required, since it is one of the elements that most impacts the overall quality of the spectrum [58]. Moreover, as NMR generates a complex spectrum containing information on all proton/carbon bearing compounds, multivariate data analysis such as principal component analysis or discriminant analysis is employed to develop classification/authentication models.

3.2.2.3. Other techniques

Trace metal analysis

Trace metal analysis using inductively-coupled plasma optical-emission (ICP-OES), atomic absorption spectrometer spectroscopy (AAS), flame absorption (FAAS) and emission spectrometry (FES) has been applied to determine the mineral composition and the trace metal contents in vinegars to determine geographical origin, type of raw materials and different production processes [59,60]. Since the mineral composition of the plant reflects the mineral composition of the soil where it is growing, accordingly, soil differences and differences in grape varieties could be reflected in the mineral composition of the vinegars, providing information about the geographical origin. The main parameters found in the case of Spanish PDO wine vinegars were Ca, K, Mg, Na, P and S, that are natural components of grape juice, K being the pre- dominant cation.

Isotope analysis

The analysis of the isotope ratios of the bio-elements (^{2}H/^{1}H, ^{13}C/^{12}C, ^{18}O/^{16}O or ^{3}H/^{1}H, ^{14}C/^{12}C) has also shown to be useful for providing proof of vinegar authentication and for detecting frauds such as the addition of synthetic acetic acid or water and the source of this acid [22]. In fact, isotopic methods have been recently recognised by the European Committee for Standardization (CEN) and in part by the OIV as a means of detecting the presence of exogenous acetic acid and tap water in wine vinegars.

Recently [61] it was found that the above listed OIV and CEN methods for the analysis of stable isotope ratios D/H and ^{13}C/^{12}C in ethanol and acetic acid and of ^{18}O/^{16}O in water can be applied to the ingredients of balsamic vinegar such as Aceto Balsamico di Modena IGP to evaluate their authenticity. The standard deviation of repeatability and reproducibility are indeed comparable in wine vinegar and balsamic vinegar and generally lower than those quoted in the official methods. Moreover, no changes in the isotopic values from wine to vinegar and to balsamic vinegar, and from the original must to the balsamic vinegar must were found. This provide experimental evidence that reference data from isotopic wine databanks [61] can also be used to evaluate the authenticity of the ingredients of vinegar and balsamic vinegar.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical Technique	Indicative data or Analyte	Authenticity issue or information
Colorimetric analysis	Total acidity content and fixed acidity content; total ascorbic acid	To comply with legal requirements
Gravimetric analysis	Residual alcohol content	To comply with legal requirements
	Total dry extract content; ash content; non-volatile reducing substances content; sulphate content	Detection of frauds
Iodometric analysis	Total sulphur dioxide content	To comply with legal requirements
Potentiometric analysis	Chloride content	Detection of frauds
Beta radioactivity ^{14}C	Synthetic acetic acid	Raw material and year of production
Sensory analysis	Odour and flavour attributes	Characterisation; ageing evaluation; quality certification (PGI, PDO); raw materials and production process
HPLC	Phenolic acids	Production process; Origin and technology involved
	Phenolic compounds	Ageing; production in different wood types
GC	Polyalcohol content	Origin
	Acetoin content, methanol, superior alcohols and ethyl acetate	Determination of quality and origin
GC-MS	Volatile aldehydes	Raw material and ageing
	Volatile compounds	Raw material and production process; quality certification (PGI, PDO); ageing
GC-O	Odour impact	Characterisation
NIR	Spectral profile	Raw material and production process; detection of frauds; origin; authentication (PGI, PDO)
MIR	Spectral profile	Ageing; raw material and production process; quality certification (PGI, PDO vinegars)
Fluorescence	Spectral profile	Ageing and authentication (PGI, PDO)
^{1}H-NMR	Spectral profile and vinegar metabolites	Authentication (PGI, PDO) and detection of frauds
	Organic components	Raw material and production process
^{13}C NMR, HMBC, and HSQC	Spectral profile and vinegar metabolites	Authentication (PDO, PGI…)
ICP-OES/ICP-MS	Mineral composition	Geographical origin
IRMS, SNIF-NMR	Site-specific D/H isotope ratio of acetic acid, $^{13}C/^{12}C$ ratio of Acetic acid and $^{18}O/^{16}O$ ratio of water	Detection of frauds: addition of synthetic acetic acid, water or sugar, from plants C_3 or C_4
IRMS	$^{13}C/^{12}C$ isotope ratio of acetic acid	Botanical origin, addition of sugar from C_4 sources
	$^{18}O/^{16}O$ isotopic ratio of water	Addition of water to dried grapes
SNIF-NMR	Site-specific D/H ratio of acetic acid	Botanical origin, addition of synthetic acetic acid
FES, FAAS, AAS	Metallic and trace element components	Production process
Colorimetric techniques	Volatile organic compounds	Production process
E-tongue, E-nose	Aroma and taste signals	Raw materials and ageing

5. Conclusion

The issues mentioned in the sections above are those that have already been identified and remain the most economically viable forms of adulteration at the present time. However, in the future, there could be more problems that should be kept in mind. These problems will most likely concern the growing range of new vinegar types, less common nowadays in the market or the emergence of other food ingredients that can create new, potential areas of deception when used improperly.

The diversity of vinegars in the market and the increase in demand makes it necessary to characterise them to establish quality control parameters. The characterisation of the vinegar covers different objectives including the authentication and classification of the product based on quality criteria. Consequently, there is an increasing need for investigating reliable analytical methods able to detect the possible adulterations and frauds as well as to assess the authenticity of the vinegar.

In recent years, there has been a growing need to develop fast, cheap, robust and effective analytical methods that do not require much sample manipulation such as sensors and spectroscopic techniques (e.g. MIR, NIR, Fluorescence, NMR and UV) coupled to chemometric tools. These techniques take into account both the individual contribution and the interactions of the different components presented in the vinegar, generating a global fingerprint of a food product. However, one of the main disadvantages is their ability to recognise just a limited number of molecules.

Finally, given the complexity of vinegars, and the fact that they are perceived by the consumer in a global way, they must be evaluated from a multivariate point of view. For this reason, a new trend in food authentication based on a combination of more than one of the aforementioned techniques has appeared. This promising methodology known as “data fusion” should be further studied for vinegar authentication.

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Coffee

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General overview of the product

The coffee tree is a tropical evergreen shrub classified under the genus *Coffea*, and part of the botanical family *Rubiaceae*. It grows between the Tropics of Cancer and Capricorn. Although more than 100 species exist in this genus [1], only two of them are of real economic importance for the production of the beverage coffee:

- *C. arabica*, called Arabica coffee: production area is mainly South and Central Americas, with the exception of Ethiopia, the country of origin for coffee;
- *C. canephora*, called Robusta coffee: most of the world's Robusta is grown in Central and Western Africa, parts of Southeast Asia and in Brazil.

The other species *C. liberica* (Liberian or Liberica coffee, or Excelsa coffee) is traded to a very limited extent. The share of Arabica fell from about 80 % of world production in the 1960s to around 60 % in the 2010s. Initially this was because of the strong growth of Robusta production in Brazil and parts of Africa, but more recently because of the emergence of Asia as the world's leading Robusta producing region [2].

On the world market, Arabica coffees attract the highest prices. Arabica trees are costly to cultivate because the ideal terrain tends to be steep and access is difficult. Also, because the trees are more disease-prone than Robusta, they require additional care and attention. Robusta is primarily used in blends and for instant coffees. The Robusta tree has the advantage of being able to withstand warmer climates, which enables it to grow at far lower altitudes than Arabica. Compared with Arabica, Robusta beans produce a coffee which has a distinctive taste and more caffeine.

Coffee is grown globally in around 70 coffee producing countries. In 2016/17, coffee production was 159.1 million bags (i.e. 9.5 millions of tonnes, each bag contains 60 kilograms of green coffee), from which 98.8 were Arabica and 60.4 Robusta. Brazil is the largest producer: its coffee sector contributes 35.2 % to the world's total coffee production. Vietnam is the second largest producer of coffee in the world, accounting for 16.8 % of global production. It is the main producer of Robusta. Colombia is the second-largest supplier of Arabica coffee after Brazil, with respectively 15 % and 46 % of the worldwide production. Indonesia is the world's second-largest exporter of Robusta. Ethiopia is the largest coffee producer in Africa. The European Union (EU) is the primary market, accounting for 40 % of the world's coffee bean imports, followed by the United States with 24 % [3].

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A great variety of coffee products can now be purchased. International coffee trade is conducted almost exclusively in green coffee. However, consumers are nowadays offered roasted coffee beans, roasted and ground coffee, as well as liquid and dried coffee extracts (soluble coffee). Furthermore, coffee can be mixed with coffee substitutes, and also sold as roasted and ground blends or as dried extracts. Whole-bean roasted coffee may also be soaked with liquid flavouring agents to produce flavoured coffees. Finally, dried coffee extracts already containing milk solids (café au lait, cappuccino) exist on the market. Decaffeinated forms of each of these coffee products are also available.

The different varieties of coffee bean and the region where the coffee is grown may give rise to products of different qualities that are more or less popular with the consumer. This in turns leads to price differences on the market and the potential for adulteration or misrepresentation by a dishonest trader. A popular component of the Western diet, coffee is also an important commodity in international trade upon which the economies of a number of countries are particularly dependent. In 2010 the International Coffee Organization (ICO) estimated total coffee sector employment at about 26 million persons in 52 producing countries[4]. Thus, the coffee industry itself has devoted considerable time and effort to ensuring both the quality and authenticity of its product, and to developing suitable analytical techniques for this purpose.

In the last 30 years, the coffee market has seen the emergence of an increasing number of initiatives related to fair-trade and sustainability. Often marked with a label on the coffee packaging, these labels certify the sustainability of coffee production and the respect of smallholder producers by improving their conditions of trade (e.g. more equitable and more stable prices). In the coffee market, most extended programmes are UTZ Certified and the Rainforest Alliance, which merged early 2018, and the Max Haavelar Foundation. According to Fairtrade International, fair-trade coffee farmers produced an estimated 560 900 tonnes of coffee in 2015 (approximately 6 % of the worldwide production).

1. Product Identity

1.1. Definition of the product and manufacturing process

Green coffee may be produced by either a wet or dry process. The wet process involves washing the coffee cherries and transferring them to depulping machines which remove the outer skin and most of the pulp. This process leaves some of the pulp mucilage on the parchment shells which encase the coffee bean and this remaining mucilage is fermented and washed away with clean water. The beans are then dried and the inner husk known as 'parchment' is broken by rollers and removed. Further rubbing removes the film or 'silverskin' which closely adheres to the coffee bean. The dry process involves drying the fresh ripe cherries in the sun for up to three weeks. The dried coffee cherries are dehulled mechanically to remove the outer skin, pulp, 'parchment' and the 'silverskin' to leave the clean, naked, green coffee beans.

Coffee is usually traded as green coffee beans, a state in which they can be kept without loss of quality or taste. It is roasted and further processed in the purchasing country. Roasting brings out the aroma and flavour that is locked inside the green coffee beans. The roasting process involves the heating of the green beans at about 200 °C, followed by fast cooling to stop the process. Once roasted, coffee should be used as quickly as possible before the fresh roast flavour begins to diminish.

Instant coffee (soluble coffee) is also produced in the coffee growing countries and may be traded packed ready for retail sale or in bulk for re-packaging in the country of receipt for national consumption or for further export. Instant coffee is the dried water-extract of roasted, ground coffee. Roasted, ground coffee is placed into columns known as percolators through which hot water is fed in a counter-current process. The extract is further concentrated and may be traded in bulk as such or dried to produce soluble coffee solids. Instant coffee is sold in three forms, which relate to the drying process of the soluble coffee extract. Instant coffee powder is formed by spray drying the extract; coffee granules are formed by agglomerating this powder with steam; and freeze-dried coffee is formed by removing moisture from the extract under vacuum (sublimation) at much lower temperatures than spray drying. Freeze-drying is more energy expensive but is gentler on the product as less heat is applied to evaporate the water content. Consequently, freeze-drying is used for the finer and more expensive blends of instant coffee.

Decaffeinated coffee is produced from green beans. Three different extraction processes slightly differing from each other are in use in the industry. Basically a solvent is circulated around the water soaked beans and this causes the caffeine to be released. The most widely used and less costly is extraction with an organic solvent such as methylene chloride (also known as dichloromethane) or ethyl acetate, an ester that is found naturally in fruits and vegetables. The second method is water processing: water is used as a solvent to extract the caffeine. In the third approach, carbon dioxide in supercritical state under a pressure of 250 to 300 bar circulates through a bed of green beans. At the end of the process, caffeine content is usually reduced from 1–2 g% to 0.02–0.3 g% [5].

The ICO was formed in 1962 under the auspices of the United Nations. It is a inter-government body comprising 51 coffee importing and exporting countries which aims through international co-operation on trade in coffee to achieve economic diversification and development of coffee-producing countries, increased coffee consumption, price stabilisation and improved economic relations between coffee exporting and importing countries. The ICO is well regarded for its statistical services and its role as the international forum for discussing all issues affecting the world coffee market. It also co-ordinates a number of projects (most of which deal with marketing, pest/disease/quality problems or sustainability) and holds seminars on issues such as the environmental aspects of coffee production and the use of the futures market.

The International Coffee Agreement 2007 is the legal agreement which sets out how these objectives will be met [6]. In this document, the different coffee products are defined for harmonising data collection, statistics and trade among producing and importing countries. On the other side, the International Standard Organisation has issued a standard “Coffee and coffee products – Vocabulary” (ISO 3509:2005) [7] also for setting the definitions of coffee products. The same terms, such as “Roasted coffee” or “Decaffeinated coffee” can be found in both documents, but definitions are largely consistent. Roughly ICO definitions are more statistically oriented whereas ISO focuses more on quality and process.

Table 1: Comparison of the definition of coffee and coffee products between ICO and ISO

Product	ICO Definition	ISO 3509:2005 definition
Coffee	General term for the fruits (cherries) and seeds (beans) of plants of the genus *Coffea*, as well as products from these fruits and seeds in different stages of processing, such as dry cherry, parchment, green, roasted, ground, decaffeinated, liquid and soluble coffee	–
Green coffee	All coffee in the naked bean before roasting	Commercial term designating the dried seed of the coffee plant
Roasted coffee	Green coffee roasted to any degree and includes ground coffee	Coffee obtained by roasting green coffee
Ground coffee	–	Product obtained by grinding roasted coffee
Coffee extract	–	Product obtained exclusively from roasted coffee by physical methods using water as the only carrying agent which is not derived from coffee
Soluble coffee	Dried water-soluble solids derived from roasted coffee	–
Instant coffee **Dried coffee extract**	–	Dried, water-soluble product, obtained exclusively from roasted coffee by physical methods using water as the only carrying agent which is not derived from coffee
Spray-dried instant coffee	–	Instant coffee obtained by a process in which the coffee extract in the liquid state is sprayed into a hot atmosphere and formed into dried particles by evaporation of the water
Agglomerated instant coffee	–	Instant coffee obtained by a process in which the dried particles of instant coffee are fused together to form larger particles
Freeze-dried coffee **Freeze-dried coffee extract** **Freeze-dried instant coffee** **Freeze-dried soluble coffee**	–	Instant coffee obtained by a process in which the product in the liquid state is frozen and the ice removed by sublimation
Decaffeinated coffee	Green, roasted or soluble coffee from which caffeine has been extracted	Coffee from which caffeine has been extracted

1.2. Current standards of identity or related legislation

1.2.1. Standards from ISO and the German organisation DIN

In 1980 the International Standard Organisation (ISO) created a sub-committee on coffee within its Technical Committee on Food products (TC 34 / SC 15). The scope of its work is standardisation in the field of coffee and coffee products, covering the coffee chain from green coffee to consumption. Standardisation includes terminology, sampling, test methods and analysis, product specifications and requirements for packaging, storage and transportation. About 30 standards have been written and are available on the ISO website www.iso.org.

Among these standards, two of them have a special application to instant coffee authenticity. The standard “Instant coffee - Criteria for authenticity” (ISO 24114:2011) [8] specifies criteria for authenticity of soluble (instant) coffee. Its purpose is to identify adulterated soluble coffee, defined as a “product prepared by the co-extraction or the separate extraction of roasted coffee beans and of raw or roasted materials other than coffee beans, where the product is sold as pure soluble coffee and the addition of the non-coffee bean material is not declared on the label”. The aim is to avoid incorrect declarations that adulterated products with cheaper coffee substitutes are 100 % pure soluble coffee. The standard focuses on two different parameters: total glucose and total xylose, the values of which must not exceed certain limits (respectively 2.46 % and 0.45 %) for the instant coffee sample to be declared authentic.

The standard is based on a standardised method looking at the carbohydrate content of the instant coffee, under the reference “Instant coffee - Determination of free and total carbohydrate contents - Method using high-performance anion-exchange chromatography” (ISO 11292:1995) [9]. The free and total carbohydrate profiles in soluble coffee are determined by anion exchange chromatography with pulsed amperometric detection (AE-PAD).

For roasted coffee, the German standard method “Analysis of coffee and coffee products - Determination of 16-O-methyl cafestol content of roasted coffee - HPLC-method” (DIN 10779:2011) [10] can also be used for authentication purposes. It is used to quantify the amount of 16-O-methylcafestol (16-OMC) in roasted beans originally, even if applications to green coffee beans and coffee brews have also been described in the literature [11]. It is based on the observation that 16-OMC is present exclusively in Robusta.

1.2.2. EU legislation

Beyond general regulations on food products, such as the General Food Law (Regulation EC 178/2002), the European Union has set up several regulations dealing with coffee products.

The general **EU Regulation 1169/2011** [12] on the provision of food information to consumers, combines two Directives into one legislation: 2000/13/EC - Labelling, presentation and advertising of foodstuffs, and 90/496/EEC - Nutrition labelling for foodstuffs. Among other themes, it deals with the labelling of origin. No specific rules have been set up for coffee, the general principle that “information shall not be misleading” applies. Voluntary provenance labels (i.e. indication where the green coffee was grown) can be made in relation to product claims such as ‘100 % Brazilian coffee’.

This regulation also stipulates a list of foods, including the following coffee products, which are exempted from the requirement of the mandatory nutrition declaration:

- Products covered by Directive 1999/4/EC of the European Parliament and of the Council of 22 February 1999 relating to coffee extracts and chicory extracts,
- Whole or milled coffee beans and whole or milled decaffeinated coffee beans.

Directive 1999/4/EC [13] relating to coffee extracts and chicory extracts determines which substances may be added during manufacturing of these products, lays down common rules concerning the packaging and labelling of such extracts and specifies the conditions under which particular designations may be used for some of these products. It simplifies the legislation previously regulated by Directive 77/436/EEC.

It defines coffee extracts as “the concentrated products obtained by extraction from roasted coffee beans using only water as the medium of extraction and excluding any process of hydrolysis involving the addition of an acid or a base”.

In particular it stipulates that “coffee extract must contain only the soluble and aromatic constituents of coffee”, apart from those insoluble substances which it is technically impossible to remove, and insoluble oils derived from coffee.

It controls the composition of three types of coffee extracts which differ in terms of their coffee-based dry matter content:

- Dried coffee extract: not less than 95 % by weight,
- Coffee extract paste: from 70 % to 85 % by weight,
- Liquid coffee extract: from 15 % to 55 % by weight.

Liquid coffee extract is specifically allowed to contain edible sugar provided the sugar content in the final product does not exceed 12 % by weight. The Directive does not permit coffee extract in solid or paste to contain any substance other than those derived from its extraction.

This Directive also states that the term *'decaffeinated'* can only be applied to coffee extracts which have an anhydrous caffeine content of not more than 0.3 % by weight of its coffee-based dry matter content.

The Directive does not cover roast and ground coffee.

According to **Directive 2009/32/EC** [14], solvents can be used for decaffeination of coffee in the European Union. There are maximum residue limits restrictions for the extraction solvents such as methyl acetate (20 mg/kg in the coffee), dichloromethane (2 mg/kg in the roasted coffee) and ethylmethylketone (20 mg/kg in the coffee). In the United States, according to the FDA, methylene chloride may be present in coffee as a residue from its use as a solvent at a level not to exceed 10 parts per million in decaffeinated roasted coffee and in decaffeinated soluble coffee extract (instant coffee) [15].

Directive 2002/67/EC [16] on the labelling of foodstuffs containing quinine,and caffeine sets up specific rules for protecting consumers and providing them with clear information on the presence of these compounds.

Where a beverage which is intended for consumption without modification, or after reconstitution of the concentrated or dried product, contains caffeine, from whatever source, in a proportion in excess of 150 mg/l, the following message must appear on the label in the same field of vision as the name under which the product is sold: "High caffeine content". This message shall be followed by the caffeine content expressed in mg/100 ml.

However, this obligation does not apply to beverages based on coffee, tea or coffee or tea extract where the name under which the product is sold includes the term "coffee" or "tea".

One Protected designation of origin (PDO) and one protected geographical indication (PGI) have been granted by the European Union:

- **Café de Colombia** (PGI) in Regulation (EC) 1050/2007 of 12 September 2007 [17];
- **Café de Valdesia** (PDO) in Regulation (EU) 2016/1043 of 15 June 2016 [18].

1.2.3. Further legislation and standards regulating quality

In 2004, the International Coffee Organisation (ICO) has introduced voluntary targets for minimum quality export standards for Arabica and Robusta under resolution 420 [19]. Thresholds for defects (not more than 86 defects per 300 g sample for Arabica, not more than 150 defects per 300 g for Robusta) and moisture (between 8 % and 12.5 %) are defined. The resolution aims to reduce the export of inferior beans. Coffee exporters from ICO exporting Members are advised to closely follow this resolution, except for the exports of specialty coffees which can be exempt from some of the targets as long as this is clearly mentioned in the Certificate of Origin.

Different producing countries have differing quality control systems and attach differing values to certain aspects of quality. Information is also available from coffee authorities in producing countries. Some specific coffee products are also produced in some countries with specific regulations, such as “Café torrefacto” in Spain and Portugal, which is a particular process to roast coffee beans with sugar addition.

The Coffee Quality Institute, an independent organisation founded originally in the scope of the Specialty Coffee Association of America (SCAA), has developed the Q Coffee System for Quality Control. It is an initiative to introduce international standards for coffee quality. It is based on trained and certified people in the supply chain (Q graders) who test coffee samples mainly from an olfactory and sensory point of view according to SCAA protocols. Each sample is tested by three local Q graders. Coffees that meet the standards for green, roasted, and cup quality are issued a Q Certificate. Companies who wish to promote and sell Q coffee may use the Q certification marks on their product packaging.

2. Authenticity issues

2.1. Identification of current authenticity issues

As mentioned previously the two coffee species of commercial importance are Arabica and Robusta. Producer countries and coffee traders are mainly interested in being able to recognise the country of origin of coffees, whereas food processors and regulatory authorities are interested in checking on compliance of the declared composition in commercial blends and in the detection of adulteration by addition of substitutes or other ingredients

2.1.1. Adulteration by addition of substitutes

Coffee substitutes may be added to the roasted and ground coffee if they are permitted and declared on the label. However if these substitutes are not correctly labelled or not declared at all then the consumer is being misled. In the case of roasted and ground coffee, inspection with a microscope may help to determine the presence of non-coffee material. Possible ingredients that may be found in ground coffee or coffee extracts include chicory, malt, figs, cereals such as corn and barley, caramel, starch, maltodextrins or glucose syrups as well as roasted or even unroasted coffee husks/parchment [20].

This problem is more important in soluble coffee extracts due to industrial processes which merge Arabica and Robusta beans before several steps like lyophilisation. Consequently, the detection of adulteration is no longer feasible by visual inspection, microscopy or other physical means traditionally used to identify impurities or “defects” which can be present in green or roasted bean or ground coffee.

2.1.2. Geographical origin

The taste and aroma of the coffee beverage is influenced by the country of origin, and even within a certain geographical region or "terroir" some differences are to be expected as a function of specific agro-climatic conditions. Some mild Arabicas of certain countries or regions attract high prices on the world market raising the possibility of substitution with coffee of cheaper origin or mislabelling. Geographic origin claims for middle range roasted coffees have begun to appear on supermarket shelves. Geographic origin authentication is becoming increasingly of interest.

In the majority of coffee-producing countries as well as among coffee traders there are tasters who can recognise the country of origin of the coffees they deal with, however none of them can identify reliably a large number of coffee origins. Moreover the opinions of these tasters are subjective, and in cases of arbitration, disagreements frequently occur between the tasters appointed by the parties involved. Analytical techniques for checking geographical origin allow for less subjective assessment.

2.1.3. Variety substitution and falsified proportion of inter-specific blends

Arabica are more expensive than Robusta coffees. Arabica is generally viewed as superior in cup quality to Robusta and is often sought exclusively by consumers. In these circumstances, addition of Robusta coffee to Arabica offers the possibility of commercial gain to an unscrupulous dealer and represents a fraud. Green and roasted beans normally may be recognised as Arabica or Robusta by visual inspection and specifically because of their specific organoleptic characteristics, however some washed Robusta coffees approach the taste quality of Arabica. As a result there is still room for non-declared substitution. On the other hand it is important to confirm if the proportions of Arabica and Robusta in the blend correspond to the price the consumer is paying.

Coffee breeding is still largely restricted to the two species, *Coffea arabica* and *C. canephora*, that dominate world coffee production. Efforts have been greatly intensified through breeding programmes in order to develop disease-resistant varieties, in anticipation of possible coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) epidemics that earlier in the century had devastated the *C. arabica* plantations in Asia and Africa. Serious threats of coffee berry disease (*Colletotrichum coffeanum* Noack sensu Hindorf) to Arabica coffee in the highlands of Eastern and Central Africa prompted a number of entirely new breeding programmes in the early 1980s particularly in Kenya and in Ethiopia [21].

Beyond the fight against diseases, coffee production is now threatened by climate change. Arabica coffee is highly sensitive to elevated temperatures, drought, pest, and disease. The forecasted consequences of climate change include changes in rainfall patterns, more frequent drought periods, and elevated temperatures, as well as a shift in geographical coffee growing regions, leading to environmental, economic and social threats in the coming years [22]. Since the second half of the 20th century, most breeding programmes implemented throughout the world (Brazil, Colombia, Kenya, Ethiopia, Costa Rica, Honduras, Tanzania, India, etc..) have transferred resistance to the main diseases by introgression of *C. canephora* chromosomal fragments carrying resistance genes. Today Arabica cultivars derived from *C. canephora* via the interspecific 'Timor Hybrid' (a spontaneous cross between *C. canephora* x *C. arabica*) represent more than 30-40 % of the Arabica trees cultivated around the world [23]. On the other hand, introgression via the Timor Hybrid may carry not only resistance genes but also other undesirable genes involved in a substantial drop in cup quality [24]. Consequently, complex and long term genetic selections have been performed to eliminate these undesirable organoleptic properties, while keeping the plant resistance to diseases.

Coffee buyers or roasters are paying more and more attention to the cultivar of the products they buy. If some introgressed cultivars are preferred because of specific properties, coffee buyers want to check if purchased coffee batches actually originate from the expected species. Secondly it has been shown that introgression can have a negative impact on the cup quality of cultivars derived from the Timor Hybrid. Consequently, coffee buyers or roasters may wish to assess whether the coffee they are purchasing comes from introgressed varieties [25]. Finally it has been demonstrated that the variety characteristics are not stable from one harvest to the next making it necessary to use at least two harvest dates for each variety [26]. Therefore there might be a concern about procurement quality and stability in time.

2.1.4. Counterfeiting of well-known brands of coffees

Some coffees have achieved a special reputation and notoriety based upon their rarity and overall flavour. Jamaican Blue Mountain and Tanzanian Peaberry are notable examples. As such they command a premium price [27]. Other examples are civet coffees, especially the Indonesian Kopi Luwak coffee. Kopi Luwak coffee is produced from beans processed in the digestive tract of the indigenous palm civet (Luwak) and then harvested. The action of microorganisms and enzymes gives this coffee a specific taste which is highly valued by consumers. Annual production of Kopi Luwak is estimated to be lower than 250 kg in 2004 [28] and the price is about USD 200 / lb (approximately more than EUR 500 per kg) [29]. An important concern related to the price gap between civet and regular coffees is the growing attempt of fraud involving illegal mixture of cheaper coffee into premium civet coffee. This may be even considered as counterfeit in this case.

2.2. Potential threat to public health

Coffee has been known to have both beneficial and harmful effects upon health. Coffee adulteration may therefore have harmful health consequences. In case of substitution of decaffeinated coffee by genuine coffee, people suffering from caffeine dependency (caffeinism) and who want to avoid caffeine may be misled. This is also the case of pregnant and breastfeeding women who are recommended to limit coffee consumption during pregnancy, because excessive caffeine consumption has been linked to stunted foetus development [30]. Caffeine intakes from all sources up to 200 mg per day consumed throughout the day is considered to raise no safety concern.

Another safety concern has arisen about the solvents used for decaffeination of coffee. In the early 20th century, benzene, known to cause severe illnesses when inhaled, even in small amounts, has been widely used for this application. Today, coffee manufacturers have switched to safer decaffeination methods, though many still use synthetic chemicals such as ethyl acetate (even if naturally found in some fruits) and methylene chloride (commonly used in industrial applications) to strip away caffeine. Even if authorities like the FDA or the European Commission have promulgated regulations that require solvent levels, especially methylene chloride, to be below specified thresholds in decaffeinated coffee [14,15], this question remains controversial. In organic coffee, chemical solvents (e.g. methylene chloride) are not permitted for decaffeination, but the water method or the supercritical carbon dioxide method may be used [2].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

A few standardised methods have been developed to check the authenticity of coffee.

3.1.1. Detection of adulteration with carbohydrates

In the standard ISO 11292:1995 “Instant coffee - Determination of free and total carbohydrate contents - Method using high-performance anion-exchange chromatography” [9], the free and total carbohydrate profiles in soluble coffee can be determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The HPAEC-PAD procedure enables the determination of all major carbohydrates present in soluble coffee in a single run.

Using this analytical method, fraudulent addition of cheaper coffee substitutes in commercial soluble coffee can be detected. High levels of total glucose and total xylose are good indicators of adulteration. High levels of total xylose indicate the presence of coffee husks or parchments, whereas the presence of cereals or caramelized sugar is detected by the very large amounts of total glucose. This authenticity checking procedure has been officially approved for publication as an ISO international standard ISO 24114:2011 “Instant coffee - Criteria for authenticity” [8]. Total xylose and total glucose levels of 2.46 % and 0.45 % respectively are proposed by ISO as upper limits, above which a soluble coffee should be considered as adulterated. The developed method has been tested on more than 1.000 samples [31]. The procedure has also been officially adopted as first action (Method 995.13) by the Association of Official Analytical Chemists (AOAC).

According to Thorburn Burns et al. [28], this method can also be applied to roasted ground coffee. If a specific adulterant is sought, comparison has to be made between the coffee sample and ground roasted coffee spiked with the sought adulterants.

3.1.2. Determination of substitutions with the caffeine content

The principle of the method ISO “Coffee and coffee products - Determination of the caffeine content using high performance liquid chromatography (HPLC) - Reference method” [32] is a water extraction of caffeine followed by detection and quantification by HPLC with UV detection. It can be applied to green coffee; roasted coffee; soluble coffee, regular and decaffeinated; and mixed instant coffee products (e. g. coffee/chicory mix or cappuccino-type coffee drink). The level of caffeine, usually between 1 and 2 g% and also a little bit higher in Robusta coffees than in Arabica can indicate substitution of coffee by other ingredients like husks or parchment.

3.1.3. Species determination with 16-O-methylcafestol content of roasted coffee

Roasted coffee is subject to commercial fraud, because the high-quality *C. arabica* species, described as 100 % Arabica, is often mixed with the less expensive *C. canephora* var. Robusta. A German standard DIN 10779:2011 “Analysis of coffee and coffee products – Determination of 16-O-methylcafestol content of roasted coffee – HPLC method” [10], being based on HPLC measurements for the specific determination of 16-O-methylcafestol (16-OMC), has been accepted. It is quite time expensive in sample preparation phases, even if at the end the instrument required (HPLC-UV) can be considered cheap with respect to other analytical systems. This method is tested for a mass fraction of 50 mg to 300 mg 16-OMC content per kg of roasted

coffee. It is based on the observation that 16-OMC is present exclusively in Robusta, whereas other, more abundant diterpenes, such as cafestol and kahweol, cannot be used for this discrimination.

3.2. Other commonly used methods

3.2.1. Detection of adulterations

Beyond the analysis of the carbohydrate profile (cf. § 3.1.1), other analytical techniques are commonly used for detection of adulteration with cheaper ingredients.

Real-time PCR has been shown to be an alternative to chemical methods for identification of diluents. DNA sequences corresponding to the endogenous genes for coffee, barley, corn and rice have been selected for amplification. To verify the applicability of the method, 30 commercial samples obtained in different countries were evaluated. Barley, corn and rice have been actually detected in different samples [33].

Chromatographic or spectrometric techniques followed by statistical models have been described in the literature for this application. UV-vis spectroscopy and the Successive Projections Algorithm for variable selection in association with Linear Discriminant Analysis (SPA-LDA) showed complete classification in both training and test sets (102 samples) [34]. Near Infra-Red (NIR) spectroscopy has also been widely studied in this purpose for roasted ground coffee. A study based on 165 aqueous extracts of ground roasted coffee samples employed Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). A Linear Discriminant Analysis classification model provided complete discrimination between roasted coffee, pure adulterants (corn and coffee husks) and adulterated coffee samples [35]. However these proofs of concept are not yet available in routine testing.

In the case of soluble coffee, analytical methods by NMR exist for detection of substitutions with ingredients such as chicory, or mislabelling of coffee / chicory proportions in commercial blends [36,37]. The presence of chicory in soluble coffee and conversely can be quantified at 10 % in aqueous solution by NMR.

3.2.2. Authentication of the geographic origin

Several techniques have been explored by researchers in their attempt to discover a method, or combination of methods, to authenticate the origin of any green or roasted coffee samples with the highest degree of confidence possible.

3.2.2.1. Metabolomic profile with spectroscopic methods

One potential approach to the problem of geographical origin involves the use of spectroscopic methods such as nuclear magnetic resonance (NMR) and near and mid-infrared techniques. They have been used to measure spectroscopic “fingerprints” of known samples to which spectra of unknowns are compared using a variety of statistical measurements for classification purposes. These techniques generally have the advantage of speed, relatively simple sample preparation requirements and are often non-destructive.

Multivariate data analysis of the phenolic and methylxanthine profiles obtained by liquid chromatography coupled with UV spectrophotometry provided preliminary results that showed their potential for the determination of the geographical origin of green coffees. Classification

models correctly identified all authentic Robusta green coffee beans from Cameroon and Vietnam and 94 % of those from Indonesia. Moreover, PLS-DA afforded independent models for Robusta samples from these three countries with sensitivities and specificities of classifications close to 100 % and for Arabica samples from America and Africa with sensitivities of 86 and 70 % and specificities to the other class of 90 and 97 %, respectively [38].

Using both ^{1}H-NMR and ^{13}C-NMR spectroscopy, it has been shown that metabolite levels in coffee were significantly different between the Arabica and Robusta species, and secondarily influenced by geographical origins [39]. OPLS-DA models performed on ^{1}H-NMR data led to a clear separation of samples according to their origin: fatty acids, chlorogenic acids and lactate and finally acetate and trigonelline were shown to be the main compounds characterising the American, African and Asian samples respectively. The analytical approach presented here confirmed the potential of joint NMR analysis and statistical treatment in coffee authentication [40]. Classification models were built on aqueous NMR profiles allowing the distinction of 192 coffees on countries or continents of origin [41]. More precisely, 50 samples of Colombian have been differentiated from 22 Asian, 12 African and 108 other American origins. Although the discrimination was based on the global fingerprint, fatty acids, acetate and caffeine were identified to having a particular part in the differentiation. However, some impacts of roasting processes were observed on spectral profile as well as the post-harvest processes, the ripening periods and the year of harvest.

NIR spectroscopy has also demonstrated its potential in geographical origin authentication. Fourier transform infrared spectroscopy (FTIR) following solvent extraction permits examination of molecular variation to distinguish degrees of roast and country of origin, as between Columbia, Costa Rica, Ethiopia and Kenya [42]. Near-infrared spectroscopy (NIR) has been used to distinguish geographic origin and genotype of samples grown in Brazil [43].

3.2.2.1. Isotopic ratios

The possibility of using the isotopic ratio of caffeine to distinguish between geographical origins was investigated a few decades ago. Isotope ratio mass spectrometry (IRMS) was used to determine the ^{13}C/^{12}C and ^{15}N/^{14}N isotope ratios and Site-Specific Isotopic Fractionation - Nuclear Magnetic Resonance (SNIF-NMR) for the ^{2}H/^{1}H ratio [44]. However it was not possible to discriminate within the African or the American group. Another study also reported the use of ^{13}C/^{12}C, ^{2}H/^{1}H and ^{18}O/^{16}O ratios of the caffeine to check origins [45]. In addition using the carbon and nitrogen isotopic ratios of caffeine it is possible to fully discriminate plant origins from synthetic ones.

Some studies were performed directly on green coffee beans using multi-isotope analysis by IRMS associated with elemental analysis (EA). A study applied on 68 green coffee beans has demonstrated the potential of the combination of δ^{13}C (VPDB), δ^{15}N (VAIR), δ^{18}O (VSMOW) and percentages of carbon and nitrogen in the discrimination of 20 different geographic origins distributed over Central America, Pacific, South America, Africa, Asia and Oceania [46]. Another study was applied to 54 samples of roasted coffee beans of 20 different countries of origin [47]. This second work combined stable isotope analysis by IRMS, Elemental Analysis by ICP-MS and an analysis of δ^{13}C of extracted caffeine. It has demonstrated to some extent the potential of δ^{13}C and δ^{15}N in the discrimination of coffees from Africa, Asia and Central / South America. Moreover δ^{13}C, δ^{2}H and δ^{18}O combined with 5 elements (Ca, Ti, Fe, Ni, Zn) could discriminate all the considered origins at 77 %.

As a conclusion, the direct multi-isotope analysis of green or roasted coffees (after grinding) is also possible for a routine control of declared origins, provided that suitable databases are available.

3.2.2.2. Elemental analysis

Element-specific techniques, especially inductively coupled plasma emission spectroscopy (ICP-OES), have been used to examine the trace element composition of coffee samples and have shown interesting results.

In a survey including the major growing areas worldwide (Brazil, Ethiopia, Colombia, India, Mexico, Honduras, Guatemala, Papua New Guinea, Kenya, Cuba, Timor, Mussulo and China), the variation in trace element composition has been characterised and compared [48]. These mineral profiles have also been used to differentiate coffee origins. Intercontinental and inter-country discrimination between the major world coffee producers were achieved by applying canonical discriminant analysis. Manganese and calcium were found to be the best chemical descriptors for origin. This conclusion is consistent with the results obtained on green coffee by Krivan et al., who analysed green Arabica coffees from eight different countries for twenty elements and found manganese to be the best suited element for origin discrimination [49].

Although much attention has been given to patterns of amounts of trace elements, this technique is not considered as robust enough by some authors due to the possibility of perturbations, for example from the use of fertilizers [28].

3.2.2.3. Volatile compounds

Chemical profiles of volatile compounds have been studied to determine the geographical origin of coffee. For instance changes in volatile components analysed by direct injection headspace analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TFMS) enable the distinction between beans from Ethiopia, Columbia, Brazil and India [50].

An HS-SPME–GC–TOFMS methodology was developed by an academic laboratory for the purposes of verifying its capability in terms of tracing back the coffee samples to their production area. Acquired data related to naturally volatile and semi-volatile analytes from 47 samples was submitted to principal component analysis and the corresponding geographical origin discrimination of coffee from South and Central America, Africa and Asia was successfully established [51].

However, many factors, such as the origin and the type of the coffee beans, roasting time and temperature, and the degree and method of roasting, affect the resulting volatile profile. Environmental factors like temperature during seed development also influence the sensory profile, and consequently the volatile profile [52]. The variability of volatile constituents in coffee caused by the different parameters do not appear to favour the volatile approach for the identification of origin in roasted coffee samples.

3.2.3. Determination of Arabica and Robusta blends

3.2.3.1. NMR profiling

The need related to coffee species determination is first to discriminate between Arabica and Robusta species and secondly to determine the proportion of Robusta and Arabica in commercial blends.

The verification of species authenticity was well established in NMR spectroscopy on the lipid fraction using the combination of two markers roughly specific to one species: 16-OMC for Robusta and kahweol for Arabica [23]. Up until recently, it was believed that 16-OMC is exclusively present in Robusta. This compound was therefore considered as an adequate marker in the

differentiation of Arabica and Robusta coffees. Indeed, the reference method DIN 10779:2011 [10] has proven the existing correlation between the 16-OMC concentration and the Robusta rate in Arabica roasted coffee. In parallel, kahweol was shown to be a key compound in species differentiation and was considered as a marker of Arabica species, although this compound is structurally very close to cafestol, compound present in both species.

Blend compositions were determined by ^{1}H-NMR spectral fingerprints with a high accuracy for 56 mixtures in aqueous solution using Orthogonal - Partial Least Square (OPLS) regression models [53]. This NMR method was proven to be a substitute for the official method because it requires only limited preparation, thus avoiding the loss of analytes. It was also shown that this technique could reach low limits of detection and quantification (5 and 20 mg/kg, respectively). This performance is adequate to detect the presence of Robusta at percentages lower than 0.9 % and down to 0.2 %, thus lower than the official method by HPLC (about 2 %) [11]. Furthermore, a recent paper has proven the presence of 16-OMC, a marker of Robusta, in ground roasted Arabica coffee in the order of 1-2 % [54]. Consequently the limit of quantification for Robusta content must be defined at 5 % and 10 % respectively in roasted and green Arabica coffee, in order to avoid false negative results. Moreover, this recent paper detected 2 doubtful market samples of Arabica coffee with adulterations at levels up to 30 % (w/w) in a panel of 60 retail purchased coffees using a limit of detection at 1 % and of quantification at 4 % [54].

3.2.3.2. NIR spectrometry

A near infrared spectroscopy signature, acquired over a set of harvests by keeping the most heritable zones of the spectrum, can therefore effectively characterize a coffee variety [26]. In a set of 191 roasted coffees from both pure Arabica and Robusta varieties and blends varying the final Robusta content from 0 to 60 % (w/w), classification models were built using NIR spectroscopy with Direct Orthogonal Signal Correction (DOSC) pre-processing method. It has been demonstrated that classification between pure Arabica, Arabica-Robusta blends and pure Robusta could be achieved.

3.2.3.3. Chemical compounds

The lipid content of Arabica coffee beans averages some 15 %, whilst Robusta coffees contain much less, namely around 10 % lipid. By Principal Component Analysis, oleic, linolenic, linoleic, and myristic acids used as chemical markers obtained by capillary gas chromatography were demonstrated as useful for differentiating varieties [55]. Six fatty acids were also analysed by Linear Discriminant Analysis (LDA) for a clear discrimination between Arabica and Robusta, green and roasted, coffee samples. Total monounsaturated (MUFA) and saturated fatty acids (SFA) could be used to determine amounts of Arabica and Robusta in a coffee blend [56].

Bertrand et al. compared the effectiveness of three chemical families, namely, chlorogenic acids, fatty acids, and minerals, for the discrimination of Arabica varieties (traditional *versus* modern introgressed lines) and potential *terroir* within a given coffee-growing area [57]. Although minerals provided an excellent classification of three locations under study, they were useless for Arabica variety discrimination. Chlorogenic acids gave satisfactory results, but fatty acids clearly offered the best results for the determination of both varieties and environments, with very high percentages of correct classification (79 and 90 %, respectively).

Roasted Arabica and Robusta coffees differ in their aroma as a consequence of their different chemical composition. Robustas show (due to their high content of free amino acids and chlorogenic acids) significantly higher concentrations of pyrazines, phenols and phenol ethers than Arabicas. Direct correlations were established between individual amino acids of green coffee and

aroma compounds which are formed during roasting. Arabicas contain (due to their high sucrose content) considerably higher amounts of steam-volatile furans, hydroxymethylfurfural and some aliphatic sugar degradation products than Robustas [58]. In a recent paper, a comparison between GC-C-IRMS, GC-MS, and ^{1}H-NMR was carried out to discriminate coffees from Colombia versus nearby countries (Brazil and Peru). According to the authors, results show that the quality of the classifiers depends mainly on the number of variables included in the analysis, which does not favour GC approaches [59].

3.2.3.4. DNA-based methods

Identification of Arabica and Robusta coffee species, as well as the quantification of their relative proportion in blends were performed by High Resolution Melting (HRM) analysis on green and roasted coffee products [60]. For a more sensitive detection method, chloroplastic rather than nuclear genetic variations were targeted, leading to the selection of 24 SNPs.

3.2.4. Detection of introgressed varieties

Introduction of new hydride varieties mostly induces an increase in the variability of the Arabica species, making differentiation between the Arabica and Robusta species more and more difficult for the analyst. For this purpose, chemometric approaches based on spectral profiles obtained by NMR or IR screening are being increasingly developed. They enable the extraction and combination of several species-characteristic signals from substantial datasets of coffee spectra. Consequently, coffee buyers or roasters could assess whether the coffee they are purchasing comes from traditional or introgressed Arabica varieties.

A chemometric method Independent Components - Discriminant Analysis (IC-DA) was applied to the ^{1}H-NMR fingerprints of lipophilic extracts from 272 authentic green coffees. Some signals of terminal methyl group of the fatty acid chains were identified as possible markers for the distinction between introgressed and native Arabica green coffee [61].

The NIR spectroscopy has also demonstrated its potential to be used to detect introgression in *C. arabica* cultivars on a dataset composed of 62 samples from Nicaragua and 61 from Costa Rica [25]. Moreover, particular metabolites were also identified such as fatty acids and caffeine, but also chlorogenic acids.

3.2.5. Authentication of coffee cultivars

Visual inspection can authenticate green coffee species and varieties, but after roasting, and particularly after grinding, this distinction becomes very difficult due to the morphological changes of beans induced by the high temperatures. Therefore, more sophisticated techniques have been developed, mainly based on genomics approaches, to check cultivars of roasted coffee, especially after grinding.

PCR amplification techniques are generally sensitive, reproducible and routinely available in testing laboratories. They can provide results even when testing very small amounts of degraded DNA as in the case of roasted coffee [62]. They have been effectively employed in the identification of roasted coffee species. An approach based on amplified fragment length polymorphism (AFLP) and simple-sequence repeats (SSRs) has shown to be useful for calculating genetic distance among 15 Arabica varieties from Yemen [63]. Applied to coffee species authentication, the potential of 33 SSR markers was assessed in 24 accessions of the Coffea genus [64]. The analysis included six Arabica (*C. arabica*) accessions, five Robusta accessions (*C. canephora*), three Híbrido de Timor (*C. arabica* x *C. canephora*), three Triploids (*C. arabica* x *C.*

racemosa) and one Racemosa (*C. racemosa*) accession. Six leaf rust resistant Arabica were also included. Authors concluded that that it is possible to use these SSRs for coffee variety identification. Single Nucleotide Polymorphism (SNP) has also been studied for identification of coffee germplasm with good results. A panel of 180 SNPs has been validated on 25 *C. arabica* and *C. canephora* accessions from Puerto Rico [65]. All the Robusta accessions were differentiated, as well as 10 out the 12 Arabica accessions (the 2 remaining ones were considered as synonymous).

All these tools are available for coffee players to assist in coffee germplasm management, quality control of planting material propagation, coffee cultivar authentication and protection of varietal rights in the international coffee community.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
NMR profiling	^{1}H NMR spectrum	Arabica-Robusta proportion in blends (green & roasted coffee) Geographical origin Adulteration with cheap ingredients Chicory content confirmation Identification of introgressed varieties
NIR spectroscopy and profiling		Substitution with cheaper ingredients (coffee husks, parchments) Geographical origin Arabica-Robusta proportion in blends Identification of introgressed varieties
UV-vis spectrometry	Whole spectrum	Substitution with cheaper ingredients (coffee husks, parchments) Geographical origin of green coffee
HPAEC-PAD	Carbohydrates	Substitution with cheaper ingredients (coffee husks, parchments, cereals, sugar)
HPLC	Caffeine	Substitution with cheaper ingredients (coffee husks, parchments)
HPLC	16-O-Methylcafestol	Dilution of Arabica with Robusta
HPLC	Chrologenic acids	Identification of varieties
SNIF-NMR and IRMS	^{13}C/^{12}C and ^{15}N/^{14}N, ^{2}H/^{1}H, ^{18}O/^{16}O, isotope ratios	Geographical origin Naturality of caffeine
ICP-OES, ICP-AES	Trace elements (minerals) notably Mn and Ca	Geographical origin Arabica-Robusta proportion in blends Identification of varieties
Capillary GC	Lipid content	Arabica-Robusta proportion in blends Identification of varieties
GC-MS	Volatile compounds	Geographical origin Arabica-Robusta proportion in blends Specialty coffee authentication
Real-time PCR	Endogenous genes	Dilution with barleycorn and rice
SSR fingerprinting	Genome	Varietal identification Arabica / Robusta proportion in blends
SNP fingerprinting	Genome EST transcriptome	Varietal identification

5. Conclusion

Coffee authentication is a major concern for the coffee sector. The product itself, once roasted, ground or processed as instant coffee, can be easily adulterated. Furthermore coffee is one of the most appreciated and valued food commodities. Extensive research has been carried out on coffee authentication over the last few decades with the results that robust authentications methods are now available for the industry throughout the supply chain in order to ensure that genuine products are delivered to consumers.

The problem of determining the proportion in blends or the adulteration of Arabica with Robusta has been addressed and there are techniques that provide a good estimation of mixtures. However, under pressure of changing climate conditions, new varieties are being created by breeding Arabica and Robusta cultivars, for instance. Current differentiation between these two species is becoming more and more complex. New knowledge is needed in the future to ensure accurate results and to avoid false positives.

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Tea and flavoured tea

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General overview of the product

Tea *(Camellia sinensis)* is an important agricultural crop that is grown in the tropical and sub-tropical regions of the world. The tender shoots and leaves of the plant are processed in factories and used to prepare an aromatic infusion which is consumed globally as a beverage. Tea is known to be one of the most popular beverages in the world and it is thought to be the most widely consumed non-alcoholic drink after water [1].

Tea is a global market which was worth EUR 34 billion in 2017 [2]. It is produced in more than 40 countries; mainly in Asia, Africa and Latin America. China, India, Sri Lanka, Kenya and Indonesia account for 80 % of worldwide production. China is the largest tea producer in the world which produces 2 230 000 tonnes per year. India is second with 1 191 100 tonnes and Kenya positions third with 399 210 tonnes. The Republic of Ireland, followed by Britain, is the largest per capita tea drinking nation [3]. Tea is not commercially grown in the EU and therefore, EU countries need to import all their tea for consumption. Tea has been imported into Europe for over 200 years with few, reported safety concerns and has consequently been deemed to be 'low' risk in terms of food safety. In 2016, a total of 238 224.30 tonnes of tea were imported into the EU, breaking down to 13.86 % green tea and 86.14 % black tea [4].

The tea value chain is represented in Figure 1 from producing countries to the European consumption market. A high level of vertical integration characterises this chain: few major companies control various production stages upstream and downstream, including the ownership of plantations and manufacturing operations.

Around 85 % of global tea production is sold by a few multinational companies owning plantations and buying production of smallholders [6]. Many of the larger tea companies have their own buyers based in the major tea buying centres of the world or they employ trading companies to make purchases on their behalf. Tea is exported in bulk whereby blending and packaging takes place in the importing country. Consumer markets in the EU are dominated by popular blended brands. These blends can contain 35 different types of tea that are blended in the consuming country [7]. Blending and packaging are highly added value operations and represent 80 % of the retail price.

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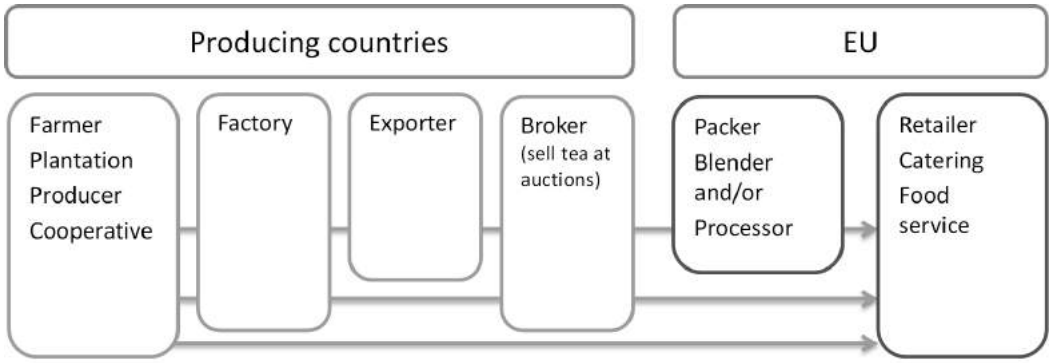


Figure 1: Tea value chain [5]

In Europe, tea is mainly sold by supermarkets and convenience stores which may sell their own-label tea as well as the major brands from tea companies. Some retailers specialise in upper-end tea blends and also pack their own products.

1. Product Identity

1.1. Definition of the product and manufacturing process

Tea is derived solely from the leaves of the plant species *Camellia sinensis*. There are many stages in tea processing which transforms the fresh shoots and leaves of the tea plant into dried leaves for brewing an infusion. Immediately after harvesting the leaves are brought to the factory for processing. It is the processing procedure that determines the type of tea produced. The main types of tea produced are; white, black, oolong and green. The manufacturing process for these types mainly differ in the degree of enzymatic oxidation or interchangeably known as the 'fermentation' process. In the processing of green and black teas, the fresh leaves are left to wither until the moisture content is reduced to a degree which depends of the type of variety of the tea [8]. The loss of water results in the concentration of polyphenols compounds and a deterioration of the leaf structural integrity. Withering is important for aroma development [9] and to prepare the leaf for rolling and/or maceration. If the leaf is still turgid when it is rolled or macerated this prevents efficient mixing of the cellular components important for initiation of oxidation or generation of aroma. The withered leaves are rolled and crushed to initiate the oxidation of tea polyphenols. Green tea leaves are dried after rolling to prevent further chemical changes. Tea leaves which have been macerated are known as 'dhool.' The preparation of the brew is simple, and involves adding hot water over the processed, dry tea leaves.

1.1.1. Major tea products

Black tea: it is produced by withering, rolling or cutting, oxidation and drying. During black tea processing, the leaves are oxidised for up to two hours to ensure they are fully oxidised. Black tea has two main process types, these are orthodox rolling and CTC (cut tear curl). India and Sri Lanka are considered the major growing regions for black tea. A brewed black tea can range in colour from amber to red to dark brown depending on the duration of oxidation, particle size, temperature and degree of aeration [10].

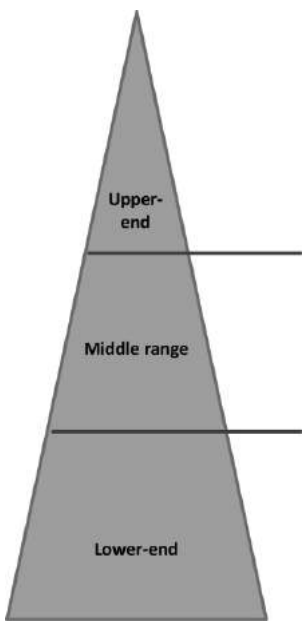
Oolong tea: traditionally from China's Fujian province and Taiwan, oolong tea is produced by withering, then partial aeration or tumbling [11]. During the tumbling process, the edges of leaf become bruised and partially oxidised. The drying process is called roasting and sometimes the tea is heated and dried repeatedly until the process is completed. The degree of fermentation, which varies according to the chosen oxidation duration, can range from 8–85 %, depending on the variety and production style. The name oolong tea came into the English language from the Chinese name which means black dragon tea or dark green teas.

Green tea: for its manufacture, the withered leaf is steamed (Japanese style) or pan fired (Chinese style) and rolled before drying. This is done to prevent the veins in the leaf breaking and thus preventing oxidation of the leaf [11]. In green tea processing, once the leaves have been dried, they can also undergo orthodox rolling or CTC. A brewed green tea is typically green, yellow or light brown in colour. Most green tea is quite light in colour and only mildly astringent.

White tea: this tea was originally established in Fujian Province, China. Authentic white tea is produced on a very limited scale, picked for only a few weeks each year in Fujian. It is made from the unopened buds of *Camellia sinensis*, which contain fine white filaments on the surface. The name is associated with these silvery white hairs on the unopened buds. For optimum quality of white tea, it is essential that shoots and leaves are gently plucked to minimise damage. The buds plucked are usually shielded from sunlight during growth which results in a reduction of chlorophyll from sunlight. The brew of white tea is usually very pale in colour. This type of tea is not as popular as black or green tea. White tea processing involves rapid drying of the freshly harvested leaves to inactivate the enzymatic reactions [12].

Pu-erh (or Pu'er) tea: it is a variety of aged dark tea which is produced in the Yunnan province of China. The tea leaves undergo microbial fermentation and oxidation after they are dried and rolled. The quality of this tea improves with maturation and time [12].

Tea products can be segmented into three different groups according to the quality of the tea. They are described in more detail in Figure 2.



Excellent quality, premium teas, handpicked, long-leaved teas. With a special story behind it or products with a sustainability label.

- Special origins such as Darjeeling, Oolong and Ceylon-Uva have the highest prices.
- Sold either by large blending and packing companies and specialty shops
- Typical retail price: € 50-55 per kg

Good quality, sold in supermarkets.

- Sustainability certification is increasingly required
- Innovation mainly in material and shape of teabags.
- Sold mainly by large blending and packing companies, to a lesser extent by specialty shops
- Typical retail price: € 10-20 per kg

Regular black tea. Low quality bulk products sold in auctions

- Extensive blending deliberately reduces differences in quality
- Many tea qualities have become interchangeable and are bought where they are cheapest
- Sold uniquely by large blending and packing companies
- Typical retail price: € 5 per kg

Figure 2: Tea products segmentation according to quality [5]

1.1.2. Other tea products

The increasing knowledge of the health benefits of tea along with the gradual increase in tea consumption has encouraged the development of other tea products on the market. Instant, ready-to-drink (RTD), flavoured, herbal and decaffeinated teas are becoming more popular in many countries.

Instant teas: these are sold as powders which require water to become reconstituted into a tea beverage. Instant teas are made using fully oxidised, partially oxidised and unoxidised dried leaves. The most common types of instant teas which are sold include green, black or jasmine tea. Other instant teas available to customers include teas which contain milk or fruit additives.

Ready-to-drink (RTD) teas: many RTD products are made from tea extract powders, similar to instant teas. The amount of tea solids is usually quite low and due to the sugar content it is difficult to make any nutritional or health benefit claims.

Flavoured teas: these are created by adding flavourings or food ingredients with flavouring properties to white, black, green and oolong teas [13]. Other food ingredients which do not lend to a specific flavour may also be added (e.g. vitamins, minerals and rice). Flavoured tea can be produced and sold as loose tea leaves, tea bags, and RTD products or as instant tea. Some of the most common flavoured teas include Jasmine and Earl Grey tea.

Herbal and fruit infusions: these are amongst the world's most popular and widely enjoyed beverages as a result of their unlimited variety and convenience. There are many types of varieties produced including, fruit, mint, sweet, and spicy. Up to 300 different plants and 400 parts of plants are used for making herbal and fruit infusions [14]. These infusions are prepared by brewing with hot water.

Decaffeinated tea: decaffeinated or decaf tea is made by processes which reduce the natural caffeine content in tea. There is no harmonised legislation in place for the maximum level caffeine content remaining in the decaffeinated product however; there is a maximum threshold of 4 mg/g in Germany, Austria and Slovakia and in some countries such as Belgium, France, Italy and Switzerland it is as low as 1 mg/g [13].

1.1.3. Health benefits

Tea is recognised for its enjoyable flavour, health benefits and stimulating effects on the human body. The growing popularity of tea coupled with the increased awareness of the potential health benefits associated with tea consumption has influenced tea chemistry to become a vibrant, developing field. Tea is composed of a range of phytochemicals that demonstrate significant physiological properties and health promoting benefits, including polyphenols, amino acids, vitamins, carbohydrates and purine alkaloids. The polyphenols determine up to 30 % of the dry weight of the leaf [15] and these compounds offer various benefits for human health, nutrition, and physiology [16,17]. Caffeine is principally valued due to its stimulatory effects [18]. It occurs naturally in the tea plant and is a central nervous system stimulant which increases alertness, stimulates metabolism and contributes to an increase in dopamine levels in the blood which improves mood. There is a considerable amount of evidence which suggests that moderate consumption of tea may protect against several forms of cancer, cardiovascular diseases, the formation of kidney stones, bacterial infections, and dental cavities [19]. Substantial studies worldwide have interpreted the role of tea in nutrition and disease [20–25].

1.2. Current standards of identity or related legislation

1.2.1. International Organization for Standardization (ISO)

ISO is a worldwide federation of national standards bodies (ISO member bodies) which has produced several standards relating to tea. The standards are produced to provide world-class specifications for tea products, services and systems, to ensure quality, safety and efficiency. The ISO methods produced mainly compromise of definition and basic requirements of black, green, white and instant tea [26–29]. ISO has also developed a range of standard methods of determining quantities of important chemical parameters of tea which include: total ash [30], water-soluble ash and water-insoluble ash [31], acid-insoluble ash [32], alkalinity of water-soluble ash [33], total polyphenols [34], catechins [35], theanine [36] and crude fibre [37].

1.2.2. European Union (EU) legislation

There is no specific regulation on tea and tea products in the European Union. All these products fall under general food regulations, such as regulation (EC) No 178/2002, regulation (EU) No 1169/2011 for labelling, regulations (EC) No 1829/2003 and No 1830/2003 for GMOs, regulation (EU) No 1169/2011 for allergens, regulation (EC) No 1334/2008 for flavourings, regulation (EC) No 1333/2008 for food additives and regulation (EC) No 1925/2006 for vitamins and minerals [13].

One protected designation of origin (PDO) and one protected geographical indication (PGI) have been granted by the European Union:

- Longjing Cha tea (PDO) in Commission Implementing Regulation (EU) No 449/2011;
- Darjeeling tea (PGI) in Commission Implementing Regulation (EU) No 1050/2011.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Adulteration of tea by dilution and substitution

Historically, tea has been prone to adulteration. The adulteration of tea was common in the 19th century to maximise profits through mixing genuine tea with leaves from other plants, or with tea leaves that had been already brewed. The most common tea adulterants are leaves of other species including bilberry, willow, elder, sloe, hawthorn and beech leaves as well as tea leaves which have already been brewed, dried and mixed with authentic tea leaves. Sometimes the resulting colour was not similar enough to tea, so anything from sheep's dung to poisonous copper carbonate was added to make it look more authentic [38]. The issue of tea adulteration with other herbs is now a less common matter as this fraudulent act can be easily detected using visual and macroscopic techniques. Tea leaves which have been already brewed can be identified by reduced quantities of extract compounds and tannins. The results from quantifying individual catechins, polyphenols, fibre, ash contents from tea extracts using ISO standards would confirm if the tea has been adulterated (cf. Section 3.1.3).

2.1.2. Geographic origin

There has been an increase in consumer interest in geographical origin of tea, which is driven by the reputation of the country or specific cultivation area [39]. The higher demand for specific regions, allows the producer to ask for significantly higher than average prices. The higher value product therefore, can become a target for food criminals. Tea which originates from the Darjeeling district in West Bengal, India has been a victim of fraudulent activities. The favourable geo-agro-climatic situation, specific soil characteristics, plantation conditions and traditional human practices results in Darjeeling tea possessing a specific flavour and a very high quality. This quality distinguishes it from tea grown elsewhere in the world. Tea products have been sold which were falsely labelled as 'Darjeeling.' This fraudulent activity is harmful to its potential market and misleading to consumers. To combat this situation, the Tea Board of India has administered the Darjeeling certification mark and logo so consumers can be assured they are purchasing an authentic Darjeeling tea.

2.1.3. Tea categories

Higher value products such as upper-end tea blends are potential targets for food criminals. The higher price of these teas attracts criminals and encourages them to falsely label and sell cheaper tea products to mislead consumers. Teas which are targets of fraud are usually less available on the market. The most widespread issues are:

- **Mislabelling of the main types of tea**: Green, oolong and black tea, and the mislabelling between white and green teas, as white tea is more expensive as it is produced on a limited scale and picked for only a few weeks each year in Fujian.
- **Mislabelling within the same tea category**: Sencha Japanese green teas processed by steaming, and Chinese green teas processed by drying.
- **Mislabelling of teas undergoing a specific process:** Smoked teas or roasted teas such as Hojicha or Matcha. Authentic Matcha tea is very expensive and there is a high risk of adulteration with teas not grown or processed in the traditional way or with other high chlorophyll containing materials. Currently there is no ISO standard for Matcha.

2.1.4. Tea grade

Tea leaf grading is a significant process required for evaluating products based on the quality and condition of the leaves. The highest grades are referred to as "orange pekoe" and the lowest as "fannings" or "dust". The characteristics which are considered in tea leaf grading mainly include, age, size and quality. Younger, smaller leaves are more valuable than larger, older leaves. The presence of buds is associated with a higher grade. The condition of the leaves, for example, ground or chopped and broken or full leaves are considered during the grading process. Like any up market product, quality grade teas face fraudulent threats.

2.1.5. Tea liquor

The current process for determining tea liquor quality is typically based on experience and subjective judgment. Human panel tests for assessing quality are carried out by trained 'tea tasters' who assess appearance, aroma and taste. Characteristics which are evaluated during this assessment include colour, physical appearance, clarity, aroma, flavour and mouth feel. Sensory scores are recorded for each batch which determines the price range. These results could be fabricated to make the buyer believe that the tea is a better quality.

2.1.6. Tea cultivar

Teas made from original cultivars tend to be of higher prices. Cultivars are made from selection and breeding of tea plants which have desired characteristics. The choice between cultivars is an important decision for tea farmers as they consider properties according to their specific needs and the demands of the market. Different cultivars produce a variety of flavour profiles and unique characteristics. Some of the most popular tea cultivars include; Yabukita (Japan), Qing Xin (Taiwan), Jin Xuan (Taiwan), Ruby #18 (Taiwan), Tie Guanyin (China) and Qi Dan (China).

2.2. Impact of climate change on authenticity

Tea cultivation depends on weather conditions for optimal growth. Global climate change therefore has a great impact on the growth of tea, quality and final tea prices. This issue could increase risk of adulteration of high value origin teas with cheaper products.

Furthermore, the increase in temperature and extreme weather events are posing a significant threat to the resilience of tea production systems, inducing social problems in impacted producing countries as well as authenticity and quality issues. Within the last few decades, the major tea producing countries (China, India, Sir Lanka and Kenya) have witnessed a significant change in climate [40]. Many growing regions may become unsuitable for tea cultivation in the future. Optimum growing regions will gradually shift to higher latitudes. Tea production and quality could benefit from the increase in temperature and CO_2 elevation. However it could be negatively affected by drought, heavy rains, and frosts, proliferation of pests and diseases and soil degradation.

2.2.1. Effects of climate change on tea production yields

The incidence and proliferation of pests and disease in tea plantations is expected to increase with climate change [41]. Warmer weather conditions will help many insects and pathogens to survive in winter, which is usually a critical time for their reduction. A higher survival rate will result in an increased rate of reproduction and will therefore increase the number of annual generations in some species. For example, one of the most threatening pests to tea plants is the Tea Geometrid *(Ectropis obliqua)* which usually has six generations per year in Hangzhou, China in normal weather conditions. This is expected to increase to seven generations, if the mean temperature rises [42]. Some studies have demonstrated that during periods of increased rainfall, yields can increase, however the overall quality of the leaves is negatively affected [43].

2.2.2. Effects of climate change on tea quality

A study [43] has shown that during the summer Monsoon in China, tea leaves grow twice as quickly than in the dry season, however the overall quality of the leaves was found to decrease. During the Monsoon, yields were 50 % higher whilst concentrations of catechin and methylxanthine secondary metabolites, major compounds that determine tea functional quality, were 50 % lower. Ultimately, this resulted in a decline of up to 50 % in household income from tea sales [44].

The formation and stability of polyphenol compounds in tea shoots depend on nitrogen and carbon metabolism and their balance in tea plants which is significantly affected by climate change. A study which has investigated the increase of CO_2 levels on tea quality found that levels of polyphenols, free amino acids and theanine concentrations increased, while the caffeine

concentration decreased [45]. Another study found that in elevated CO_2 conditions there was a decrease in caffeine, free amino acids and an increase in the concentration of the polyphenol compounds in the tea plant [46]. The gradual change in the ratio of free amino acids to polyphenols in shoots will ultimately cause deterioration of black tea quality [47]. Research has demonstrated that higher levels of amino acids can contribute to higher quality green tea and higher quantities of polyphenol compounds are positively associated with black tea quality [46].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Visual assessment and tea tasting are the initial quality control methods completed internationally. Further analysis is carried out on tea if any issues are identified during initial assessments. Sensorial, visual and analytical controls of the tea are done both by the vender and purchaser.

3.1.1. Macroscopic analyses

Macroscopic or visual examination is used for the identification of undeclared products or unwanted substances including non-vegetable materials, non-tea materials and moulds. An evaluation of the overall general appearance of the leaves is carried out using this method. Sieves can also be used to separate any foreign bodies which are amongst the tea leaves.

3.1.2. Sensory testing

The acceptability of the tea leaves processed in the factory is generally assessed by sensory evaluation and human panel tests. Skilled and fully trained personnel carry out these sensory assessments to judge the overall tea quality. These expert 'tea tasters' organoleptically determine the market value of the final product by considering the size and shape of leaf, colour and flavour. Teas are tasted after processing, on arrival at the auctions and after blending. Sensory analyses represent a high cost for the tea industry. It has been argued that this practical evaluation of tea quality is entirely subjective and may lead to inaccurate results owing to adaptation, fatigue and state of mind [48]. However, this testing method is still widely used throughout the whole tea supply chain. It is still the most efficient and cost effective way to assess tea acceptability. ISO 3110 [49] can be used for the preparation of liquor for use in sensory tests, however there is currently no ISO standard for tea sensory analyses.

3.1.3. Determination of compounds

The determination of many compounds in tea can provide information about the variety, category and geographical origin of the tea. For instance low levels of caffeine and polyphenol values will reveal substitution of tea by other material or the inclusion of already used tea leaves.

ISO 14502-1 [34] was developed for the determination of total polyphenol content of leaf tea and instant tea in a colorimetric *in vitro* assay using Folin-Ciocalteu reagent. It is applicable to both green and black tea products.

Chromatographic techniques can provide accurate, reproducible results although they are sample destructive and time consuming. High performance liquid chromatography (HPLC) has shown good separations of tea phenolic compounds [50]. HPLC is widely used for the quantification of

compounds including: tea catechins, gallic acid, purine alkaloids, theanine in tea because of its high efficiency and high resolution [51]. The quantification of catechins using HPLC has been used to detect geographical origin of tea [52–54].

ISO 14502-2 [35] specifies a HPLC method for the determination of the total catechin content of tea from the summation of 9 individual catechins. It is applicable to both leaf and instant green tea and has precision limitations to black tea. Gallic acid, theogallin and caffeine can also be determined by this method. ISO 19563 [36] specifies a HPLC method for the determination of total theanine in tea. There is currently no standard method for the quantification of theaflavins however this is under development by ISO.

3.2. Other commonly used methods

3.2.1. DNA-based methods

Molecular markers and genetic fingerprints have been studied by several research groups worldwide whose results have differentiated tea cultivars. Several sets of simple sequence repeats (SSRs) have been identified, for instance, in a recent publication using 6 SSRs markers, with a probability of identity between two random cultivars for the whole set of 6 SSR markers was estimated to be 2.22×10^{-5}, enabled full identification of 66 tested tea cultivars [75]. In another study using SNP (single nucleotide polymorphism) markers in combination with a high-throughput genotyping protocol, authors have established and verified specific DNA fingerprints using 60 SNPs for 40 tea varieties from China [76]. DNA-based methods are useful for variety authentication and quality control of premium teas for the industry as well as the management of tea genetic resources and breeding, where accurate and efficient genotype identification is essential.

3.2.2. Spectroscopic methods

During the past decades, spectroscopic methods had been investigated for their effectiveness in the quality control of teas. The most studied techniques include near-infrared spectroscopy (NIR), nuclear magnetic resonance spectroscopy (NMR) and atomic spectroscopy, such as isotope ratio mass spectrometry (IRMS). These techniques are highly repeatable, reproducible and environmentally friendly. In tea authentication, several studies have demonstrated their potential which still has to be translated in routine testing laboratories for a wide use by tea industrial organisations.

3.2.2.1. IRMS

Mass spectrometry is one of the most sensitive techniques that can be used for identification of compounds. IRMS is a rapid, reproducible technique. Stable isotope signatures of both tea leaves and tea infusions have been investigated to identify the geographical origin in several studies. Results have demonstrated the potential for IRMS to determine geographical origin in tea samples [68,69].

3.2.2.2. NMR

NMR has been widely used for metabolic profiling in medicinal plants. It provides a very fast and detailed analysis of the biomolecular composition of crude extracts. NMR spectrum is a physical characteristic of a compound and thus highly reproducible. NMR has demonstrated the ability to identify authenticity issues associated with tea including quality and geographical origin [67].

3.2.2.3. NIR

NIR spectroscopy is a fast, accurate and non-destructive analytical tool. Many studies have demonstrated the ability for NIR spectroscopy and multivariate calibration to quantify the chemical composition of teas and classify tea products into different categories [55,56], varieties [57–63], age [64] and geographical origin [39,57,65,66]. The NIR applications in tea studies show great potential for the instrument to be applied in the industry to detect authenticity parameters. Handheld portable NIR spectrometers could also be implemented online during tea production or used at tea auctions to verify authentication.

3.2.3. Other chromatographic techniques

Other chromatography methods such as high performance thin-layer chromatography (HPTLC) [70], ultra-high performance liquid chromatography (UHPLC), capillary electrophoresis (CE) [71,72] and gas chromatography (GC) with their combination with mass spectrometry (MS) have been used to determine the internal components in tea [73,74]. They usually involve expensive instrumentation and time-consuming sample preparation using solvents as well as analysis. They are therefore not suitable for quality assurance during processing for rapid analysis or online monitoring of chemical composition.

3.3. Future analytical perspectives

In order to enable food industries to rapidly respond to food adulteration, fraud and unauthentic tea products, new analytical tools are continuously being developed for instantly determining chemical composition. These techniques include computer vision technologies and electronic sensors which are easy-to-use and could potentially be used in the future if they can be developed to provide the relevant information associated with authenticity parameters.

3.3.1. Electronic sensors

The imitation of human senses using sensor arrays and pattern recognition systems has been investigated. This technique is known as electronic sensing. Within the last two decades, a considerable amount of research has focused on investigating the use of electronic sensing techniques, such as electronic nose (E-nose) and electronic tongue (E-tongue) to detect quality parameters in tea powder and infusions.

3.3.1.1. E-nose

The E-nose is designed to mimic the mammalian sense of smell by producing a composite response unique to each odorant. As an important quality factor of tea, aroma depends upon the amount of volatile organic compounds and their ratios. Compared with the conventional methods, it is an increasingly reliable, fast, and robust technology. Over the last decade, numerous applications of E-nose in tea quality detection have been reported and many studies have been dedicated to improve the capability. Preliminary results from studies have demonstrated the ability of the E-nose to be a valuable method for targeting potential and future tea authenticity issues including; tea grades [77,78], types [79], varieties [80,81], categories [82], geographical origin [80,83] and storage times [84].

3.3.1.2. E-tongue

The E-tongue is an analytical instrument that artificially reproduces the taste sensation [85]. The taste of tea infusions is the influential attribute of sensory information. It is an important factor in both assessing tea quality and classifying tea grades. E-tongue instruments have shown good precision, accuracy and reliability, but they are time-consuming, destructive and unsuitable for online monitoring. Thus, this technique opens up new avenues in taste sensing and could be successfully implemented in the near future for tea analysis. Studies have demonstrated the ability of the E-tongue to discriminate tea varieties [86,87], geographical origin [88], grades [88–94]and fermentation degree [95]. The benefits are still unclear over traditional tea tasters as it is time consuming, not practical and does not give the same resolution as a human taster.

3.3.2. Computer vision applications

Computer vision systems are becoming increasingly popular within the food industry. Computer vision provides an automated, non-destructive and cost-effective technique to analyse products. This approach is based on image analysis and has provided a great potential in tea quality assessment. Multi-spectral, hyperspectral and normal camera imaging are the most commonly studied computer vision tools. It is expected that computer vision will become an emerging platform technology in the future.

3.3.2.1. Multi-spectral imaging

Multi-spectral imaging is a technique which captures images using specific wavelength regions which are usually separated by filters. The multi-spectral imaging system applied to assess tea quality typically consists of a three-channel common aperture camera, a frame grabber, numerous tungsten halogen lights and a computer [96]. Multi-spectral imaging is developed based on hyperspectral imaging using the selected wavelengths, thus it is advantageous than hyperspectral imaging as it accumulates less data. Multi-spectral imaging techniques have been able to identify tea categories [97–99], grades [100] and brands [101].

3.3.2.2. Hyperspectral imaging

Hyperspectral imaging is a developing technique that integrates conventional spectroscopy and digital imaging to gather chemical information and visualise spatial distribution of chemical constituents within a matrix. The device system typically contains the following components: objective lens, spectrograph, camera, acquisition system, translation stage, illumination, and computer [96]. Hyperspectral images which are known as hypercubes are three-dimensional blocks of data, comprising of two spatial and one wavelength dimension. After hyperspectral image acquisition, spectral pre-processing, variables selection, image extraction and processing are completed for analysis. Some research has demonstrated that tea grade [102,103], quality [104] can be determined using this technology.

3.3.2.3. Normal camera imaging

Normal camera imaging is another commonly used computer vision tool. Tea classification studies have demonstrated the ability for normal camera imaging to discriminate; tea grades [105], varieties [106,107] and colour [108].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Sensory analysis	Visual, olfactory and taste scores	Tea grades, adulteration
Macroscopic observation	Visual assessment	Adulteration
Sieving	Foreign bodies	Adulteration
HPLC	Catechins and theaflavins	Geographical origin
HPTLC	Catechin compounds	Geographical origin
CE	Catechins, caffeine, theanine and other amino acids	Geographical origin
IRMS	Catechin and theaflavins	Geographical origin
NIR	Catechin compounds	Tea categories, geographical origin, varieties, age
NMR	Amino acids, organic acids, caffeine and catechins	Geographical origin and quality
SSR fingerprinting	Genome	Varietal identification
SNP fingerprinting	Genome EST transcriptome	Varietal identification
E-Nose	Volatile organic compounds	Tea grades, types, varieties, categories, geographical origin, storage times
E-Tongue	Catechins, amino acids and caffeine	Tea varieties, geographical origin, grades, fermentation degree
Multi-spectral imaging	Image analysis	Tea categories, grades, brands
Hyperspectral Imaging	Image analysis	Tea grades, quality
Normal Camera Imaging	Image analysis	Tea grades, varieties, colour

5. Conclusion

Tea is recognised as one of the world's most popular beverages and the authenticity of tea relies on many factors linked to the chemical composition of the final product. Global and EU legislation and standards have been developed to ensure quality and safety of tea products. The most problematic authenticity issues discovered in the industry to-date include mislabelling of tea grades and geographical origin.

Wet chemical analysis is currently used for the determination of the main quality parameters of tea, however, it would be more efficient to use alternative methods which are rapid, non-destructive and not labour intense. Looking into the future, spectroscopic, electronic or computer vision sensors would be ideal for detecting authenticity in tea products. Portable sensors could be utilised in tea factories or by purchasers to determine the chemical composition of the product. They would be beneficial at auctions to verify the tea leaf grading process and the quality of the

product. The studies which investigate the use of these rapid techniques have demonstrated early evidence that these recently developed technologies have been equally reliable as chromatographic methods which are standard methods used to test for authenticity by regulatory bodies.

It is significant that enhanced detection methods are developed and utilised in industry to ensure tea authentication. Food NIR or NMR fingerprinting approaches are expected to become very effective methods in authentication verification aiming at products with complex compositions such as teas. These approaches aim to capture as many compounds or features as possible to gain a comprehensive insight into the composition of the sample. A comparison of authentic samples may allow revealing mislabelled or adulterated products.

Global climate change will have a great impact on the growth of tea, its end quality and finally on tea prices. The increase in temperature and extreme weather events pose a major threat to the resilience of tea production systems. Significant change in climate may result in the major tea producing countries becoming less suitable for tea cultivation in the future. Therefore there is an increased risk of adulteration of high value origin teas being blended with cheaper teas.

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Fats and Oils

Olive oil

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General overview of the product

Olive oil has gained in popularity in counties where it was a relatively underused commodity in the past. Not least among the reasons for the increased popularity of olive oil are its potential health benefits, its delicious taste and aroma and its culinary and nutritional advantages over other edible oils [1]. Since countries that were only importers a few decades ago have started to produce olive oil, it has become another daily oil for cooking for consumers from these countries.

Olive oil represents only around 2 %, or even lower, of the worldwide production of oils and fats [2,3] and it is a foodstuff cherished by the consumers of the Mediterranean countries where it is of enormous economic importance for their farmers. Thus, 20 % of farms in Spain are devoted to olive cultivation compared with 25 % in Greece and 19 % in Italy. These countries produce around 70 % of the world production, Spain and Italy being the main producers (Table 1).

The International Olive Council (IOC) has clearly defined the different categories of olive oil and olive-pomace oil [4]. The most popular category is virgin olive oil. This is the oil obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. In the course of this technical procedure, olives are washed, milled, and the resulting paste is malaxed. The purpose of malaxation is to facilitate the separation of the oil and water phases. The paste is then slightly warmed in order to accelerate the merging of the oil drops. It has been found that the lower the temperature, the better the sensory quality but the lower the yield [5]. Next, a centrifugation process that produces two fractions (wet olive cake and oil) is widely implemented in olive mills all over the world. Prior to bottling, the oil is submitted to a vertical centrifuge or decanter.

Olive being a fruit, its chemical composition depends not only on the action of enzymes involved in the biochemical pathways but also on the extraction process and external parameters, such as the weather. Consequently, there are different categories of olive oil that differ significantly in their quantitative chemical composition and price. Extra-virgin olive oil commands a high price on the oils and fats market due to its sensory characteristics, the demand for it and its production costs. It is therefore the main focus of attention of fraudsters. Adulterations, which were very common in the past – like the addition of refined edible oils – are easily detected, and have been substituted by sophisticated ones, like the addition of soft-deodorized virgin olive oils or the use of oils with tailored composition to meet the legal limits. Thus, the kind of adulterant is not a cheaper edible

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oil in the market anymore but a formulation of different cheaper edible oils that can avoid detection when using trade standards. This procedure is harmful for emerging virgin olive oil markets whose local consumers buy olive oil for its potential health benefits and they would be concerned if they receive an adulterated oil instead that does not have these benefits.

Hence, effective control of olive oil adulteration requires tighter controls by exporting countries, clear definitions for olive oil products and uniform labelling regulations. As regards Analytical Chemistry, the best solution probably lies in multi-disciplinary studies involving instrumental methods of chemical and sensory analysis, and mathematical procedures.

Table 1: World production and consumption of olive oil (2015/16) of olive oil by country

Country	Production (1000 tm)	World production (%)	Consumption (1000 tm)	World consumption (%)
Spain	1403.3	44.18	494.5	16.60
Italy	474.6	14.94	598.1	20.07
Greece	320.0	10.07	140.0	4.70
Portugal	109.1	3.44	70.0	2.35
France	5.4	0.17	113.4	3.81
EU	2324.0	73.16	1660.4	55.73
Turkey	150.0	4.72	116.0	3.89
Tunisia	140.0	4.41	35.0	1.18
Syria	110.0	3.46	104.0	3.49
Morocco	130.0	4.09	120.0	4.03
Australia	20.0	0.63	42.0	1.41
USA	14.0	0.44	321.0	10.77
Chile	17.5	0.55	5.5	0.19
Argentina	24.0	0.76	7.5	0.25
China	17.5	0.56	39.0	1.31
TOTAL	3176.5		2979.5	

Source: www.internationaloliveoil.org.

1. Product Identity

1.1. Definition of the product and manufacturing process

Olive oil is the oil obtained from the fruit of the olive tree (*Olea europaea sativa* L.) to the exclusion of oils obtained by solvents or re-esterification procedures and of any mixture with oils of other kinds [4]. Olive oil is defined in three categories: virgin olive oil, refined olive oil and olive oil.

Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration of the oil. The result of the process is an oil that is chiefly a mixture of glycerides, which are esters of glycerol with fatty acids. In addition, olive oil contains small quantities of many chemical compounds (Table 2) that are commonly used in its characterisation and authenticity [6,7]. The generic concept of virgin olive oil contains four different types: extra-virgin olive oil, virgin olive oil, ordinary virgin olive oil and lampante virgin olive oil although the category ordinary virgin olive oil is not accepted by all the regulatory bodies (as for example in the EU).

Table 2: Ranges of the chemical components of virgin olive oil [8]. Information from fatty acid, triacylglycerides and squalene are given in percentages while the rest is given in mg/kg. Note: tr, traces; nd, not detected; [a], values exclusively circumscribed to some major Spanish and Italian cultivars

Component	Range	Component	Range
Fatty acids		*Triglycerides*	
Myristic	Not detected	POP	2.16 - 5.73 %
Palmitic	6.3 - 16.9 %	PXO-PLP	0.13 - 2.66 %
Palmitoleic	0.3 - 1.6 %	POS	0.39 - 2.30 %
Margaric	0.002 - 0.3 %	POO	19.54 - 30.57 %
Margaroleic	0.02 - 0.4 %	PLO-XOO	2.76 - 12.31 %
Stearic	1.02 - 3.9 %	PLL	tr - 2.43 %
Oleic	65.4 - 86.6 %	SOS	tr - 1.04 %
Linoleic	2.7 - 18.3 %	SOO	3.17 - 8.39 %
Linolenic	0.2 - 1.1 %	OOO	27.75 - 53.34 %
Arachidic	0.15 - 0.7 %	OLO	4.24 - 17.46 %
Gadoleic	0.09 -0.6 %	OLL	tr - 4.43 %
Behenic	0.01 - 0.2 %	AOO	0.25 - 1.09 %
Aliphatic alcohols and diols		GOO	tr - 1.06 %
Docosanol	0.77 – 56.27	*Hydrocarbons*	
Tetracosanol	17.79 - 60.63	α-Copaene	0.12 - 4.77
Hexacosanol	26.88 – 93.81	Calarene	tr - 0.26
Octacosanol	10.53 – 44.94	Muurolene	tr - 1.51
Phytol	35.97 – 364.58	Eremophylene	tr - 2.63
Erythrodiol +uvaol	8.07 – 112.51	Heptadecene	tr - 0.45
4,4'-dimethylsterols		Heneicosane	tr - 0.72
Taraxerol	4.14 – 12.94	Tricosane	0.65 - 16.35
Dammaradienol	5.14 – 34.94	Tetracosane	0.47 - 14.93
β-Amyrin	10.78 – 121.17	Pentacosane	2.51 - 28.8
Butyrospermol	17.7 – 80.91	Hexacosane	0.74 - 3.26
24-methylene-lanost-8-en-3-β-ol	6.33 - 20.46	Heptacosane	3.61 - 13.69
Cycloartenol	83.49 – 652.84	Octacosane	0.81 - 2.28
24-methylene-cycloartanol	144.67 – 1464.06	Nonacosane	3.07 - 9.93
4-Desmethylsterols		Triacontane	0.46 - 1.95
Campesterol	31.11 -108.37	Hentriacontane	1.89 - 8.83
Δ^5-avenasterol	52.43 – 575.04	Dotriacontane	0.16 - 1.09
β-sitosterol	681.41 – 2872.06	Tritriacontane	0.70 - 5.52
Stigmasterol	4.24 – 41.32	Pentatriacontane	0.12 - 1.33
Cholesterol	0.79 – 18.02	α -Farnesene	tr - 32.59
24-methylen cholesterol	0.63 – 7.01	Squalene	0.125 - 0.7 %
Campestanol	0.79 - 7.96	α-Tocopherol	125 – 200
Δ^7-campesterol	0.15 – 8.09	β-carotene	0.11 - 16.27 [a]
Chlerosterol	1.99 – 32.44	Lutein	1.20 - 4.49 [a]
Sistostanol	4.63 – 60.14	Violaxantin	10^{-3} - 0.77 [a]
$\Delta^{5,24}$-stigmastadienol	3.04 – 30.61	Neoxantin	70^{-3} - 0.79 [a]
Δ^7-stigmastenol	1.38 – 15.71	Antheraxanthin	nd - 0.64 [a]
Δ^7-avenasterol	2.81 – 26.93	β-cryptoxanthin	nd - 0.62 [a]
4-monomethylsterols		Luteoxanthin	90^{-3} - 0.80 [a]
Obtusifoliol	8.29 – 29.29	Mutatoxanthin	30^{-3} - 0.11 [a]
Gramisterol	6.54 – 20.71	Chlorophyll a	nd - 1.55 [a]
Cycloeucalenol	9.43 – 68.43	Chlorophyll b	nd - 0.80 [a]
24-Etillophenol	6.04 – 18.86	Pheophytin a	0.98 - 25.04 [a]
Citrostadienol	50.27 – 228.19	Pheothytin b	nd - 2.92 [a]
Oleanolic aldehyde	3.17 – 17.36	Pheophorbide a	nd - 0.57 [a]

The free acidity, expressed as oleic acid, and the organoleptic characteristics have been the parameters used to define these categories according to the trade standard of the International Olive Council [4]. Extra-virgin olive oil, a gourmet oil highly prized for its delicious flavour, tops all olive oil categories in terms of the strictest quality parameters.

Refined olive oil is obtained by refining virgin olive oil under conditions which do not lead to alteration of the initial glyceridic structure. Olive oil is the oil consisting of a blend of virgin and refined olive oil fit for consumption. Olive pomace oil is obtained by solvent extraction of the olive residue that remains after mechanical extraction of the virgin olive oil, made edible by refining methods. There are three different olive-pomace oils: olive-pomace oil, crude olive-pomace oil and refined olive-pomace oil. The first one is the oil comprising a blend of refined olive-pomace oil and virgin olive oil. The second is olive-pomace oil intended for refining while the last is the oil obtained from crude olive-pomace oil by a refining process which does not lead to alterations in the initial glyceridic structure [4].

Table 3 shows the limits of the parameters for olive oil designations according to the European Union. This information describes the characteristics of each designation that are not fully accepted by all the institutions involved in the olive oil business; in fact, there are notable disagreements [9]. Thus, Australian and South African standards propose values for palmitic, oleic and linolenic which are different from those of the IOC and the EU whereas the difference with Codex Alimentarius is in linoleic and gadoleic acids. The limit values for some 4-desmethylsterols (i.e. campesterol and stigmasterol) differ between IOC trade standards and standards from other institutions because the concentrations of those compounds are influenced by the latitude and altitude of olive tree orchards [10]. These scientific explanations have increased the debate about how olive oils from new producing regions (mostly in the Southern Hemisphere) can be classified as genuine without compromising the control of adulteration that a change in limits for these sterols would mean for the rest of world production. Thus, IOC has included decision trees for olive oils with percentages of campesterol between 4.0 and 4.5. The objective is to classify those oils as genuine oils, because they are, but without, however, comprising the fight against olive oil fraud; although no certainty value is associated to the decision tree yet. In addition, some regulations such as Australian and South African standards have even established a limit higher than 4.5 while they do not include any limit for total sterols (Australia and South Africa) and erythrodiol plus uvaol. With the aim of having a single regulation, a harmonisation program between the IOC, the EU and Codex Alimentarius is under progress.

The content of waxes is another source of disagreement between IOC/EU and the other institutions. IOC and EU assign different contents of waxes according to the olive oil designation (extra, virgin, ordinary, lampante, etc.) while the remaining institutions – Codex, USA, California, Australia and South Africa – give a value (≤ 250 mg/kg) whichever the designation. The maximum content of stigmastadienes, which can be used to determine the presence of any refined edible oil in virgin olive oil, is another source of disagreement among IOC/EU and the other institutions. Thus, the IOC and the EU recently lowered the limit from 0.10 to 0.05 due to modern analytical instruments have higher sensitivity with excellent values of precision while Codex and USA standards have values of 0.15 mg/kg.

Table 3: Limits of the chemical compounds used as parameters for protecting virgin olive oil designations against potential adulterations with edible oils [11]

Designations	(1)	(2)	(3)	(4)	(5)	(6[c])	(7)	(8)	(9[d])
Extra-virgin olive oil	≤0.05	≤0.05	≥1000	≤4.5	≤150	≤0.05	≤|0.2|	*B*	≤2.50
Virgin olive oil	≤0.05	≤0.05	≥1000	≤4.5	≤150	≤0.05	≤|0.2|	*B*	≤2.60
Lampante virgin olive oil	≤0.10	≤0.10	≥1000	≤4.5[a]	≤300[a]	≤0.50	≤|0.3|	*C*	-
Refined olive oil	≤0.20	≤0.30	≥1000	≤4.5	≤350	-	≤|0.3|	*C*	-
Olive oil	≤0.20	≤0.30	≥1000	≤4.5	≤350	-	≤|0.3|	*B*	-
Crude olive pomace oil	≤0.20	≤0.10	≥2500	>4.5[b]	>350[b]	-	≤|0.6|	≤1.4 %	-
Refined olive pomace oil	≤0.40	≤0.35	≥1800	>4.5	>350	-	≤|0.5|	≤1.4 %	-
Olive pomace oil	≤0.40	≤0.35	≥1600	>4.5	>350	-	≤|0.5|	≤1.2 %	-

Designations	(10[d])	(11[d])	(12)	(13)	(14)	(15)	(16)	(17)	(18[e])	(19)
Extra-virgin olive oil	≤0.22	≤0.01	≤0.8	≤20	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Virgin olive oil	≤0.25	≤0.01	≤2.0	≤20	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Lampante virgin olive oil	-	-	>2.0	>20	≤0.5	≤0.5	≤0.1	≤4.0	-	≥93.0
Refined olive oil	≤1.25	≤0.16	≤0.3	≤5	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Olive oil	≤1.15	≤0.15	≤1.0	≤15	≤0.5	≤0.5	≤0.1	≤4.0	<Camp	≥93.0
Crude olive pomace oil	-	-	no limit	no limit	≤0.5	≤0.5	≤0.2	≤4.0	-	≥93.0
Refined olive pomace oil	≤2.00	≤0.20	≤0.3	≤5	≤0.5	≤0.5	≤0.2	≤4.0	<Camp	≥93.0
Olive pomace oil	≤1.70	≤0.18	≤1.0	≤15	≤0.5	≤0.5	≤0.2	≤4.0	<Camp	≥93.0

Designations	(20[d])	(21[d])	(22[d])	(23)	(24)	(25)	(26)	(27)	(28)	(29)
Extra-virgin olive oil	Mf>0	Md=0	≤35	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Virgin olive oil	Mf>0	0<Md≤3.5	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Lampante virgin olive	-	Md>3.5[(f)]	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Refined olive oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Olive oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.2	≤0.2	([2])
Crude olive pomace oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])
Refined olive pomace	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])
Olive pomace oil	-	-	-	≤0.03	≤1.0	≤0.6	≤0.5	≤0.3	≤0.2	([2])

Note: (1): *trans*-oleic fatty acid (%); (2): Sum of *trans*-linoleic & linolenic fatty acids (%); (3): Total sterol content (mg/kg); (4): Erythrodiol and uvaol content (% total sterols); (5): Wax content: C42+C44+C46 for extra virgin and virgin designations and C40+C42+C44+C46 for the rest of designations (mg/kg); (6): Stigmastadiene content (mg/kg); (7): Difference between the actual and theoretical ECN42 triacylglycerol content; (8): Content of 2-glyceryl monopalmitate (2P); B, 2P≤0.9 if total C16:0≤14.0 % or 2P≤1.0 if C16:0>14.0 %; *C*, 2P≤0.9 if C16:0≤14.0 % or 2P≤1.1 if C16:0>14.0 %; (9): Absorbency in ultra-violet at K_{232}; (10): Absorbency in ultra-violet at K_{270}, if cyclohexane is used, and K_{268} if iso-octane is used; (11): Absorbency in ultra-violet (ΔK); (12): Free acidity (%m/m expressed in oleic acid); (13): Peroxide value (in milleq. peroxide oxygen per kg/oil); (14): Δ^7-Stigmastenol (%); (15): Cholesterol (%); (16): Brassicasterol (%); (17): Campesterol (%); (18): Stigmasterol (%); (19): The value of β-Sitosterol is calculated as : $\Delta^{5,23}$-Stigmastadienol + Clerosterol + β-Sitosterol + Sitostanol + Δ^5-Avenasterol + $\Delta^{5,24}$-Stigmastadienol; (20) Organoleptic assessment: median of fruity attribute (Mf); (21) Organoleptic assessment: median of defect (Md); (22) Fatty acid ethylesters (FAEEs); (23) Myristic acid (% m/m methylesters); (24) Linolenic acid (% m/m methylesters); (25) Arachidic acid (% m/m methylesters); (26) Eicosenoic acid (% m/m methylesters); (27) Behenic acid (% m/m methylesters); (28) Lignoceric acid (% m/m methylesters); (29) Other fatty acids (% m/m methylesters). [a], when the oil has a wax content of between 300 mg/kg and 350 mg/kg it is considered a lampante olive oil if the total aliphatic alcohol content is ≤ 350 mg/kg or if the erythrodiol + uvaol content is ≤ 3.5 %; [b], when the oil has a wax content of between 300 mg/kg and 350 mg/kg it is considered a crude olive pomace oil if the total aliphatic alcohol content is > 350 mg/kg and the erythrodiol + uvaol content is >3.5 %; [c], Total isomers which could (or could not) be separated by capillary column; [d], quality characteristics; [e], Camp, campesterol (%); [f], or where the median defect is less than or equal to 3,5 and the fruity median is equal to 0; ([2]), Palmitic: 7.5-20.0; Palmitoleic: 0.3-3.5; Heptadecanoic: ≤ 0.4; Heptadecenoic: ≤ 0.6; Stearic: 0.5-5.0; Oleic: 55.0-83.0; Linoleic: 2.5-21.0.

The presence of re-esterified oils in olive oils is detected by the quantification of 2-glyceryl monopalmitate, for which maximum admitted percentages depend on the designation. Values proposed by IOC/EU are lower than those described in the standards supported by Codex Alimentarius, California, Australia and South Africa. The free acidity and the peroxide value associated with olive oil designations are stricter in the standards of California than in the rest of institutions. Finally, virgin olive oil is widely regulated by IOC as regards sensory assessment by a complete set of documents, which have been copied by all the other institutions. Differences once again concern the limits for the medians of defects and fruity attribute associated to extra-virgin and virgin olive oil designations. Thus, the median of defects for VOO has been raised to 3.5 – to take into account the uncertainty in the classification of the boundaries of virgin and ordinary/lampante – in the IOC/EU trade standard/regulation whereas this value has not been changed in the other standards. Another source of disagreement is the fact that the Californian, Australian and South African standards also consider these values of medians of defects and fruity attribute for olive pomace and refined oils.

International regulatory bodies have designed their standards with the information supplied by their delegates though a high percentage of the parameters qualifying the olive oil designations and the limits for determining their genuineness were initially proposed by the IOC. The limits for some parameters are, as already described, at the core of the disagreements among international regulatory bodies because climate conditions affect the chemical and biochemical pathways that are responsible for quantitative changes in olive oil chemical composition, and today there is an increasing number of orchards that are not located at the Mediterranean basin as was the case in the past.

Harmonisation among international institutions is being developed and this activity has been identified as a priority objective for the present [9]. The harmonisation should come from the collaboration among regulatory bodies in order to achieve an agreement for some specific parameters that are currently the subject of debate. Other actions, such as reducing the number of standard parameters and methodologies for example, would be beneficial for facilitating international trade as well. Most of the methods were proposed by the IOC specifically for olive oils although there are alternatives proposed by other institutions (i.e. AOCS, ISO, IUPAC, FOSFA).

2. Authenticity issues

2.1. Identification of current authenticity issues

It has been proved that fraud has been part of commercial transactions, in one manner or another, since they were practised in the remote past, and today olive oil is still considered a vulnerable product in terms of authenticity [6,9]. Fraud can mean ruin for many actors in the olive oil market like farmers and sellers although the consumer is the ultimate one affected by this dishonest attitude. Mass media, in fact, do not usually distinguish among food actors that intentionally carry out this illegal and dishonest activity and those that are simply affected by a one-off unintentional fail in quality control. Thus, the product's authenticity of the entire food market is called into question when the mass media publicise news on fraud, with the real risk that consumers might decide not to consume olive oil any more even though the potential fraud does not pose a threat to public health. Consumer perception of the product may be affected negatively despite the strict controls that are imposed on this product today.

Authenticity has many aspects, from adulteration and mislabelling to characterisation of protected designations of origin (PDOs). With so many potential issues to be studied, the great number of olive oil designations, and the large variety that can be used in adulteration, a questionnaire was prepared in order to obtain a broad opinion of producers, wholesalers, retailers, researchers, analysts etc. A first survey was collected in 1996, inside FAIM - a European funded project -, and a second survey was launched in 2016 inside FoodIntegrity –another European funded project- with updated questions about olive oil authenticity. Table 4 shows how the priorities of olive oil actors have evolved in the last twenty years. The importance of protecting virgin olive oil designations has not decreased and there is a great interest in determining the presence of soft-deodorized virgin olive oil in extra-virgin ones, and in knowing the traceability of the extra-virgin olive oils. The authenticity of extra-virgin olive oils is still linked to the classification by means of the sensory assessment ("Panel Test") [7], the results of which are questioned by some olive oil actors up to the point that objective methods based on the quantification of volatiles responsible for sensory descriptors are being studied as a potential alternative or a complementary action to sensory assessment. Consumer interest in a reliable geographical declaration of extra virgin olive oil (EVOO) has increased over the last years but not in the expected percentage. The importance of 'Typicality' (distinctive production) is revealed in the surveys that were carried out with information from consumers. However, a PDO may show or not clear differences in characteristics compared with other PDOs or non-PDOs.

Table 4: Percentages of the importance of authenticity issues according to answers of olive oil actors to surveys launched in 1996 – European funded project FAIM – and 2016 – European funded project FoodIntegrity

Issues	Sub-issues	FAIM 1996	FoodIntegrity 2016
Authenticity	Categories of olive oil	91	95
	VOO spiked with ROO	78	28
	VOO/ROO spiked with hazelnut	83	67
	EVOO spiked with soft-deodorised VOO	-	96
	VOO/ROO spiked with genetically engineered oils	87	63
	ROO spiked with desterolised oils	64	47
	ROO spiked with refined seed oils	93	53
	ROO spiked with pomace oil	37	48
	Olive oil spiked with esterified edible oils	58	49
	VOO spiked with other vegetable oils	26	11
Mislabelling	Declared mixtures (olive oil spiked with seed oils)[1]	15	-
Characterisation	Olive oil varieties	62	58
	Designation of Origin, Countries, etc.	69	77
Miscellany	Characterisation of sensory quality of olive oil varieties	66	68
	Addition of flavour and colour to ROO	8	36
	Authentication of Organic Virgin Olive Oil	11	43
	Characterisation of extraction systems	21	-

Legend: VOO, Virgin Olive Oil; EVOO, Extra Virgin Olive Oil; ROO, Refined Olive Oil; [1], this market is banned inside producer countries but it was an increasing market in some non-producer countries, e.g. Holland, Germany; Source: FAIM, FoodIntegrity project.

2.2. Identification of potential issues

As soon as certain rough adulterations (e.g. virgin olive oil mixed with refined oils) have been practically solved with efficient methods, the fraudsters have focused on developing new adulterations that are more sophisticated and difficult to detect since they are based on selecting

oils that after being mixed cannot be detected with regular methods. However, to commit this fraud, the fraudster needs to have an advanced knowledge of olive oil chemistry [12]. On the other hand, these new adulteration issues are described in terms of feasibility from a chemical/analytical point of view. In other words, in many cases reliable information is not available about the actual incidence of these frauds and their importance in the market and they are considered, among other reasons, because they are included in those malpractices that from a theoretical point of view would pose difficulties in their detection. In order to identify potential issues in olive oil authentication, those adulteration cases that are described to prove the potential of a new method/technology but which do not exist in the real world because as they are not economically viable should be omitted to avoid confusion. That would be the case of mixtures with more expensive oils or with oils that are easily detected with existing methods (e.g. mixture of virgin with seed oils).

A new possible adulteration is the addition of soft-deodorised virgin olive oil to extra virgin olive oils, that are more difficult to be detected and new strategies are needed [13]. Thus, when soft-deodorisation at low temperatures (<100 °C) is carried out in a virgin olive oil to remove slight sensory defects, the resulting soft-deodorised oil is the so-called ‘‘deodorato” or ‘‘deodorato soft”. After undergoing this thermal process, it can no longer be considered ‘virgin’ according to the legal definition for ‘‘virgin olive oil” [4]. For that reason, any mixture of a VOO with a ‘‘deodorato” is considered to be a fraud. The proposed chemical parameters for their detection (pyropheophytins, alkyl esters) has demonstrated not to be infallible so far, so new analytical strategies are needed.

Another relevant authenticity issue that is gaining importance is the authentication of geographical provenance. Since production today is moving beyond the Mediterranean countries (USA, Australia, Argentina, Chile, South Africa) etc., and consumers are aware about commercial transactions between countries, they demand more information about geographical origin. The fact that provenance is sometimes presented as an additional value to the product (regardless of actual quality) within a marketing strategy has resulted in an authenticity issue related with mislabelling. Thus, today, if the declared origin on the label does not match with the new origin, then it is considered that the oil clearly fails in its integrity. No standard methods exist in this regard. However, building a large database with major and minor compounds and the implementation of an expert system have been suggested for geographical characterization. That was the case of the SEXIA project [10,14]. Today, new alternatives based on non-targeted techniques are being developed [15].

Despite the strict regulations in force, advanced knowledge of the chemical composition of olive oil and other edible oils has brought to the table the possibility of building tailored oils designed to pass all the controls. This possibility has led researchers to consider other authentication strategies other than those based on existing methods. Since some compounds have been studied on olive oils and are not included in the standards, they are being tested for potential authentication purposes.

Other complex authenticity issues are related to the current use of olive oil as an ingredient to be incorporated in more complex food formulations. Thus, once the olive oil is mixed with other ingredients (e.g. canned foods in olive oil), the current methods are difficult to apply since the mixing changes the natural composition of the lipid fraction. Since the addition of virgin olive oil is claimed on the label as an additional value in the food formulation to attract consumers, the authentication of the olive oil content is perceived today as an emerging authenticity issue. Sometimes, even the highest quality designation of virgin olive oil (“extra virgin”) is mentioned on the label. In this case, evaluating the quality of virgin olive oils in mixtures with other ingredients is also difficult considering the migration of compounds between ingredients.

2.3. Potential threat to public health

All the adulterations that are considered today in oils in the regular market do not pose a direct serious threat to public health. The administration actively fights against fraud because it negatively affects consumer confidence with respect to a product that has a solid cultural background and is the centre of the Mediterranean Diet. In terms of toxic effects of fraud, only hypothetical rough adulterations in clandestine oils being sold outside the regular commercial circuits are concerned. That was the case of the Toxic Oil Syndrome (TOS) in the early 80s where the oil was not distributed in the regular food supply chain. For that reason, traceability and control of the food chain is considered as an essential authenticity tool that complements the analytical methods for fraud detection. Public administration at different levels is aware of the importance of this additional control and they implement regular inspections at retail outlets and in the food service sector.

3. Analytical methods used to test for authenticity

Currently, there is a proliferation of proposals trying to demonstrate that adulterants in olive oil can be easily detected. Advances in knowledge and technology have undoubtedly led to greater success in the fight against adulteration over the years. However, it is equally true that the same techniques and knowledge have been also used by fraudsters to invalidate the usefulness of some standard methods. Such competition has required not only a considerable investment on perfecting classical techniques or developing new ones, but also that the pace of R&D for detection of malpractice has to be rapid enough to counteract the fraudster's actions.

Numerous methods have been used to detect olive oil adulteration, but most of them can detect only adulterations greater than 10 %. This scarcely represents any advantage over the standard methods, the latter being described in Table 5.

Current tests and methods can be naturally divided into two groups: those based on the determination of signals related with almost all the possible analytes in the oil sample or a large group of them - the so-called "non-targeted" methods - and those that rely on measurement of more definite information obtained from fractionation of olive oil components - the so-called "targeted" methods. The latter, which identify and quantify series of chemical compounds, analyte by analyte, ideally have the objective of looking for compounds that do not appear, or only at trace levels, in genuine olive oil but appear in adulterated oils. Since these techniques give information about how these compounds came to be present in the adulterated food, this information can also be used to remove or diminish the amount of these analytes during adulteration, e.g. the use of desterolised oils.

The other group of techniques is based on the analysis of the total chemical make-up of the oil, using a spectroscopic technique for instance. Here, fraudsters may have no clues to how to manipulate composition such that the results comply with genuine oils, but the analysts in control labs can also have problems in the interpretation of the information with plausible chemical explanations. The utility and applicability of this group of techniques can be increased by applying multivariate statistical techniques. Even then, the conclusions should be supported by chemical or biochemical explanations to rule out noise or random effects in the samples.

3.1. Officially recognised methods

The methods in the international regulations and trade standards for the detection of olive oil adulteration are mainly based on LC (liquid chromatography) and GC (gas chromatography (Table 5).

These official methods [4] have enabled the control of virgin olive oil adulteration, but have led to some particular situations in which genuine extra-virgin olive oils are classified outside their natural category applying IOC Trade Standards and other national and international regulations [12]. They are usually olive oils from certain olive tree varieties cultivated outside the Mediterranean basin that do not comply with the limits of some criteria for authenticity in official trade standards and regulations (Table 3) even when they are carefully extracted, stored and delivered. Some traditional but minor cultivars, even harvested in regions inside the Mediterranean basin, also have values of their chemical compounds that do not comply with the limits described in Table 3. The paradigm might be the Spanish cultivar *var.* Verdial de Huévar, for which limits of erythrodiol exceed those defined for the extra virgin olive oil designation simply due to its particular biochemical pathways [16]. However, if large virgin olive oil databases and multivariate statistical algorithms had been applied in the past, this and other problems would no longer exist, and *var*. Verdial de Huévar, for instance, would not have just disappeared from Andalusian olive oil orchards. The highest interest for minor cultivars today is the possibility to maintain gene diversity (Olive Germplasm Bank) and to tackle chemical singularities when establishing legal limits.

Table 5 shows the methods that can be used for the quantification of parameters for which the limits, described in Table 3, are markers for the authenticity of the different olive oil designations. The methods are provided by different institutions (AOCS, EU/EC, FOSFA, IOC, ISO, IUPAC) although those provided by the IOC (named COI/T.20) have particularly been designed to analyse olive oils. This is, for instance, the case of the method for the detection of refined oils in virgin olive oil by means of the quantification of stigmastadienes [17,18], which is still one of the most powerful methods.

The methods described in Table 5 are not exempt from required improvements, comments and useful tips. Thus, the high diversity of available chromatographic columns for the determination of fatty acid composition can produce differences in the results. Columns characterized by the highest polarity are recommended for a better separation of PUFA while lowest polarity columns are better for saturated and monoenoic compounds. A good separation of *trans* fatty acids is much better with a 50 m column with a cross-linked stationary phase of cyanopropylsiloxane [9]. The determination of sterols and triterpene dialcohols is easier with a previous HPLC separation – instead of TLC – though this kind of separation, widely used in the laboratories, is not included in some official methods. As regards the determination of actual and theoretical ECN42, the IOC recommends a method based on the use of propionitrile solvent in the determination of triacylglycerides which adds a supplementary complication with no clear advantage. The determination of the content in stigmastadienes should be implemented by determining the concentration of sterenes (campestadienes and stigmastadienes) if the concentration of stigmastadienes is higher than 4 mg/kg. The presence of re-esterified oils in olive oils is detected by the quantification of 2-glyceryl monopalmitate, a lengthy and tedious method that requires previous knowledge such as neutralising the sample if its acidity is higher than 3 %, the readjustment of the pH to 8.3, and a strict control of pancreatic lipase that may lose activity easily.

Table 5: Summary of the relevant methods proposed in the international regulations supported by the International Olive Council (IOC), Codex Alimentarius, EU, USDA, California State (USA), Australia, and South Africa (Source: [9])

Determination	Method
Fatty acid composition	(EU) 1833/2015 Annex IV; COI/T20/Doc No 33; AOCS Ce 1f-96 Methyl ester preparation: ISO 5509:2000; AOCS Cc 2-66; COI/T20/Doc No 24 Gas Chromatography: ISO 5508:1990; AOCS Ch 2-91
Trans fatty acid content	(EU) 1833/2015 Annex IV; COI/T20/Doc No 33; COI/ T20/Doc No 17 Rev 1; ISO 15304:2002; AOCS Ce 1f-96; AOCS Ch 2a-94 (Rev 2002)
Sterol and triterpene dialcohols composition	(EU) 1348/2013 Annex IV; COI/T20/Doc No 30; COI//T20/ Doc No 10 Rev 1; ISO 12228:1999; AOCS Ch 6-91 -Erythrodiol + uvaol: COI/T20/ Doc No 30 ; IUPAC 2431
Wax content	COI/T20/Doc No 18 ; AOCS Ch 8-02; (EC) 702/2007 Annex IV
Aliphatic and triterpenic alcohol content	COI/T20/Doc No 26 Rev1; (EU) 2015/1833 Annex VI
Difference between the actual and theoretical ECN 42 triacylglycerol content	COI/T20/Doc No 20 rev 3; COI/T20/ Doc No 23; AOCS Ch 5b-89; (CE) 2472/97 Annex XVIII
Stigmastadiene content	COI/T20/Doc No 11/Rev2; COI/T20/Doc No 16/Rev1; ISO 15788-1:1999; AOCS Cd 26-96; ISO 15788-2:2003(EC) 656/95 Annex XVII
Content of 2-glyceryl monopalmitate	COI/T20/Doc No 23; (EC) 702/2007 Annex VII
Unsaponifiable matter	ISO 3596:2000; ISO 18069:2000; AOCS Ca 6b-53
Organoleptic characteristics	(EU) 1348/2013 Annex V; Amended by (EU) 2016/1227; COI/T20/Doc No 15
α-tocopherol	ISO 9936
Waxes and alkyl esters	COI/T20/Doc No 28; COI/T.20/Doc. No 33; (EU) No 61/2011 Annex II
Biophenols	COI/T20/Doc No 29
Free acidity	COI/T20/Doc No 34; (EU) 2016/1227 Annex I; ISO 660(03); AOCS Cd 3d-63; AOCS Ca 5140
Peroxide value	COI/T20/Doc No 35; ISO 3960; (EU) 2016/1784 Annex III; AOCS Cd 8b-90
Absorbency in ultra-violet	COI/T20/Doc No 19 Rev 3/Rev 2; ISO 3656; AOCS Ch 5-91; (EU) 2015/1833 Annex III
Moisture and volatile matter	ISO 662; AOCS Ca 2c-25
Pyropheophytins	ISO 29841:2009
Insoluble impurities in light petroleum	ISO 663; AOCS Ca 3a-46
Flash point	FOSFA Int. method; ISO 15267:1998
Trace metals copper, iron and nickel	ISO 8294
Traces of heavy metals	Lead ISO 12193; AOCS Ca 18c-91; AOAC 994.02 Arsenic AOAC 952.13; AOAC 942.17; AOAC 985.16
Traces of halogenated solvents	COI/T20/Doc No 8; (EEC) 2568/91 Annex XI
Waxes fatty acid methyl esters and fatty acid ethyl esters by GC using 3g of silica gel	COI/T20/Doc No 31 *provisional*
Composition of triaclyglycerols and diacylglycerols by GC in vegetable oils	COI/T20/Doc No 32 *provisional;* ISO 29822
Refractive Index	ISO6320:2000; AOCS Cc 7-25
Iodine value	(EEC) 2568/91 Annex XVI
Saponifiable value	ISO 3657:2002; AOCS Cd 3-25
Fatty acid in the 2-position of triglycerides	ISO 6800:1997; AOCS Ch 3-91
Relative density	IUPAC 2101[1]
Oxidative stability index	AOCS Cd 12b-92

Note: [1], with the appropriate conversion factor; EU, European Union; EC, European Commission; AOCS, American Oil Chemists Society; ISO, International Organization for Standardization; COI, International Olive Council; FOSFA, Federation of Oils, Seeds and Fats Associations Ltd; IUPAC, International of Union of Pure and Applied Chemistry.

Sources: IOC: www.internationaloliveoil.org, Codex: www.fao.org/fao-who-codexalimentarius/codex-home/es/, EU: ec.europa/agriculture/olive-oil_en, USDA: www.ams.usda.gov/grades-standards/olive-oil-andolive-pomace-oil-grades-and-standards, California State (USA):www.cdfa.ca.gov, Australia: www.aph.gov.au, and South Africa (SANS) www.sabs.co.za/.

The determination of pyropheophytins (PPP) is another major point of discrepancy between the IOC and the associations of new olive oil producing countries (Australia, California, South Africa, New Zealand). The increment in PPP is associated with the presence of energy in terms of light and/or temperature during the extra virgin olive oil (EVOO) shelf-life, which provides information on EVOO freshness, a concept that is not accepted for producer countries structured around the IOC. The analytical method based on the reverse-phase solid-phase extraction (RP-SPE) has a critical point in the collection of the analytes in 0.2-0.3 mL of acetone because of its high volatility which suggests that the injection in the HPLC instrument should be as rapid as possible. The method allows two kinds of elution, with petroleum ether (40-60 °C) or with petroleum ether (40-60 °C): ethyl ether (9:1) for removing the lipids.

The concentration of ethyl esters of fatty acids (FAEEs) is among the parameters that have been recently approved by IOC/EU for determining EVOO quality though there is no causal relationship between the concentration of these compounds and the sensory assessment, which is the official method for determining whether a virgin olive oil is or is not extra-virgin. The role of FAEEs is not accepted by international associations other than the IOC.

The development of standard methods is normally a consequence of industrial or commercial needs and they are established as standards after their validations by collaborative studies. The lobbying that groups of chemists carry out in the implementation of methods is becoming increasingly irrational, so the usefulness of some new methods gets more and more preposterous. However, the requirement of validation as a prerequisite may prevent standard methods providing unsatisfactory results being put forward.

3.2. Other used methods

Most of the methods described in Table 5 are based on chromatography, which is a time-consuming technique that needs several steps to carry out quantification, uses polluting solvents and is impracticable for on-line control, the latter being a common demand from farmers and co-operative societies in the fight against adulteration. Alternatives must come from techniques that have simple or no sample preparation or pre-treatment as those described in Table 6. Such techniques have been thought most likely to be spectroscopic though unfortunately their methods have not been widely applied in olive oil authentication yet. There have been numerous attempts, however, such as the procedure that combined artificial neural networks and Curie-point PyMS (Pyrolysis Mass Spectrometry) [19] for a rapid assessment of adulteration of extra-virgin olive oil or the application of ^{13}C-NMR to distinguish virgin olive oil from refined olive oils and olive-pomace oil [20]. The comparison of these, and other techniques, with methods based on the detection of stigmastadienes by gas chromatography showed the superior behaviour of chromatographic methods in terms of time of analysis and false positives, which has led to the delayed implementation of spectroscopy in olive oil authenticity. Table 7 shows the application of some alternative methods in authenticity issues, mostly based on these spectroscopic techniques.

Table 6: Main characteristics of alternative techniques proposed for the authentication of olive oils

Characteristics	Techniques
Structural & Pattern Recognition	NMR, MS, NIR, FTIR, FT-Raman, DSC, TG, SF.
Stable Isotope Analysis	IRMS.
Trace Element Analysis	ICP-AES, AAS, FAAS, ETA-AAS.
In-tandem	GC-MS, HPLC-MS, ICP-MS, CG×GC, LC×LC, SFC, δ^2H-EA-Py-IRMS, δ^2H-GC-Py-IRMS.

Note: Nuclear magnetic resonance (NMR); near infrared spectroscopy (NIR), Fourier transform infrared spectroscopy (FTIR) and Fourier transform Raman spectroscopy (FT-Raman); isotope ratio mass spectrometry (IRMS); inductive coupled plasma-atomic emission spectroscopy (ICP-AES); atomic absorption spectroscopy (AAS); flame absorption spectroscopy (FAAS); electrothermal atomization-AAS; mass spectrometry (MS), GC-MS, LC-MS and ICP-MS; elemental analyser-pyrolysis-isotope ratio mass spectrometry (δ^2-H-EA-Py-IRMS) and δ^2-H-GC-Py-IRMS; bidimensional chromatography (GC×GC, LC×LC); supercritical fluid chromatography (SFC); synchronous fluorescence (SF); differential scanning calorimetry (DSC) and simultaneous thermogravimetry (TG).

3.2.1. Vibrational Spectroscopy *

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Vibrational spectroscopy methods, based on NIR, MIR or Raman spectroscopy technique, are part of the fingerprinting methods used in authenticity, which regroup all the analytical protocols that provide a full physical or chemical pattern of the samples [21]. Spectroscopic techniques have been considered as promising tools for rapid sample screening over a number of years. However, the fact that they need large datasets in order to calibrate any given instrument and to provide a chemical interpretation of spectra has limited their application in olive oil authentication beyond the determination of classical values and oil indices; i.e. *trans*/*cis* double bonds, free fatty acids, unsaturation degree, oxidation state and moisture content among others [22].

Considering near infrared spectroscopy (NIR), in the last years, more and more applications have been developed in at-line and on-line quality control. Regarding specifically the authentication of olive oil, NIR spectroscopy has been used to detect the hypothetical adulteration of olive oil with vegetables oils like sunflower seed, corn, walnut, soya and hazelnut [23,24].

The application of Mid Infrared Spectroscopy (MIR) to the detection of extraneous edible oils in olive oil in 1990's [22] has led to a period where infrared spectroscopy was used in olive oil traceability with success [25,26]. Thus, for example, MIR has been used to detect the adulteration of extra virgin olive oil with a corn-sunflower binary mixture (5 % (v/v)), cottonseed and rapeseed oils (5 % (v/v)) [27,28]. Baeten and collaborators [29] also proposed the use of MIR spectroscopy in combination with Raman spectroscopy to determine the presence of hazelnut oil in olive oil.

Several studies have also described the use of Raman spectroscopy for detecting and quantifying the adulteration of olive oil [30-32]. The method is suitable for the analysis of compounds rich in unsaturated functional groups and has proved to be useful in studies involving olive oil. Based on the intensity ratio of the cis (=C-H) and cis (C=C) bonds normalised by the band at 1 441 cm-1 (CH_2), Zou and collaborators [33] demonstrates the interest of Raman spectroscopy for the authentication or the detection of fake olive oil. El-Abassy and collaborators [34] tested a dispersive Raman system using a 514 nm laser to discriminate olive oils from different types of sunflower oils in only a few seconds.

Fatty acids are the most abundant biomolecules in olive oil, and they do not allow vibrational spectroscopy to get information from minor compounds due to the barrier effect that is exerted by saponifiable matter in the spectra acquisition [30]. Thus, one of the problems in vibrational spectroscopy is caused by the interferences of the saponifiable matter (fatty acids and TAGs) when determining unsaponifiable compounds (i.e. sterols) [9]. One solution is to perform a previous transesterification of the oil [30]. Unfortunately, those minor compounds are the most informative compounds for detecting habitual and sophisticated adulterations of oils nowadays.

3.2.2. ^{1}H and ^{13}C -NMR spectroscopy

The first application of high resolution proton nuclear magnetic resonance (^{1}H-NMR) in the field of oils and fats was the determination of global unsaturation (corresponding to the classical iodine number value) made on the basis of the integral of olefinic protons at 5.3-5.4 ppm. In addition to this application, several researchers have proposed NMR as a suitable technique for analysing different components in olive oil [35]. Thus, ^{1}H-NMR methods can be applied to obtain structural and quantitative information on a wide range of organic metabolites. NMR can be applied to quantitate fatty acids, although the determination of individual fatty acids is not possible. Thus, saturated fatty acids (SFAs), monounsaturated oleic acid (MUFA), and polyunsaturated linoleic and linolenic acids (PUFAs) can be obtained by determining several signal intensities. With respect to ^{13}C-NMR, it enables almost all analyses performed by ^{1}H-NMR and it is the preferred technique to obtain information about the positional distribution of the saturated, oleyl, linoleyl, and linolenyl chains on the glycerol moiety [35]. Whatever the kind of NMR used, this technique requires the application of multivariate statistical analysis of ^{1}H or ^{13}C signal intensities of the oil samples or suitable chemical parameters determined by NMR. Assuming that a fraudulent addition of an extraneous oil changes slightly the chemical composition of the oil, NMR spectra can point out changes in the profile that can be highlighted with statistical analysis. Thus, the appearance of a resonance in the carbonyl region ascribed to saturated fatty acids at the *sn* -2 position of glycerol and slight differences in the chemical shifts of the saturated and unsaturated acids is associated with fraudulent oils [35]. However, in the case of real adulterated oils, where low adulteration percentages and oils with similar composition are used, it is more difficult to highlight slight differences in the NMR spectra.

Table 7: Basic characteristics of non-standard (in-house) methods proposed for some analytical challenges of the olive oil authenticity issues

Issue	**Addition of cheaper oils to olive oils**
Objective:	Detection of the presence of any edible oil (crude or refined) in virgin or refined olive oil.
Analyte/Signal:	Selected ^{13}C- & ^{1}H-NMR bands of the spectrum.
Technique:	^{13}C-NMR and ^{1}H-NMR spectroscopies.
Level of applicability:	Universal although has been checked with only a few adulterants.
Official method?:	No, but the adulteration with hazelnut oils have been validated with blind trials.
Time of analysis[a]:	Pre-treatment: No; measurements: 4 h for ^{1}H-NMR and 1.45 h for ^{13}C-NMR; data analysis: 20 min applying procedures of Artificial Neural Networks (ANN).
Limit of detection[b]:	>10 % using bands from ^{13}C-NMR and ^{1}H-NMR for adulterations with hazelnut oils. ~15 % using bands from ^{13}C-NMR or from ^{1}H-NMR for adulterations with hazelnut oils.
Advantages:	Good repeatability.
Disadvantages:	Time-consuming. Poor reproducibility. False positives. Hyper-optimist models.
References:	[36-38]
Objective:	Detection of the presence of any edible oil (crude or refined) in virgin or refined olive oil.
Analyte/Signal:	Infrared or Raman bands.
Technique:	FTIR or FT-Raman.
Level of applicability:	Universal although has been checked with only a few adulterants.
Official method?:	No, some kinds of adulteration have been validated with blind samples.
Time of analysis[a]:	FTIR: Pre-treatment: 5min[c]; measurement: 5min; data analysis: 5min applying ANN. FT-Raman: Pre-treatment: nil[c]; measurement: 10min; data analysis: 5min applying ANN.
Limit of detection[b]:	>10 %
Advantages:	Rapid and easily implementable method.
Disadvantages:	Full checked with hazelnut oils only. A large set of spectra is required. Unstable mathematical equations.
References:	[22,29,39-40]
Issue	**Addition of refined oils to virgin olive oils**
Objective:	Detection of the presence of any refined edible oil in virgin olive oils.
Analyte/Signal:	*cis*/*trans* FTIR or FT-Raman bands.
Technique:	Spectroscopy by FTIR or FT-Raman.
Level of applicability:	Universal.
Official method?:	No, but the method has been validated with blind samples.
Time of analysis[a]:	Pre-treatment: Nil; measurement: 10min; data analysis: 10min.
Limit of detection[b]:	>8 %
Advantages:	Rapid method.
Disadvantages:	Limit of detection. The method does not work properly with less unsaturated oils.
References:	[25,29,31]
Issue	**Geographical traceability of VOOs**
Objective:	Determination of the geographical provenance (country, region, county, PDO, PGI) of VOOs.
Analyte/Signal:	Several: fatty acids, alcohols, sterols, hydrocarbons etc.
Technique:	Gas chromatography for chemical analysis and expert system (SEXIA®) for data analysis[e].
Level of applicability:	Whole Spain and partially the other EU producer countries.
Official method?:	No, but SEXIA® has been validated with hundreds of samples for years.
Time of analysis[a]:	Pre-treatment: 180min; measurement: 300min; Data analysis: 10min using expert system.
Correct classification[b] (%):	Average certainty factors (CF): 92 % for Andalusian PDOs, 95 % for Spanish regions, and 96 % for the identification of major EU producing countries/varieties among others.
Advantages:	Results are associated to high CFs. It based on the largest VOO database.
Disadvantages:	Time-consuming. Several different chemical analyses. It constantly needs to be updated.
References:	[14,41,42]

Table 7 (follow-up)

Objective:	Determination of the geographical provenance of VOOs.
Analyte/Signal:	δ^2H, $\delta^{13}C$ or $\delta^{18}O$.
Technique:	EA-IRMS or NMR.
Level of applicability:	Universal.
Official method?:	No.
Time of analysis[a]:	Pre-treatment: nil; measurement: few minutes; data analysis: 5 min.
Correct classification[b] (%):	Not reported by authors.
Advantages:	Rapid method.
Disadvantages:	Reproducibility. Need of a previous large database. Harmonisation of calibration procedure.
References:	[43-45]
Objective:	Determination of the geographical provenance of VOOs.
Analyte/Signal:	Multi-elements.
Technique:	ICP-MS or ICP-AES.
Level of applicability:	Universal.
Official method?:	No.
Time of analysis[a]:	Digestion (in microwave): 75-90 min; measurement: 3-5min; data analysis: 15 min using ANN.
Correct classification[b] (%):	Not reported by authors.
Advantages:	Causal relationship between soil and oil. A large number of variables (elements). Repeatability.
Disadvantages:	Low concentration of elements in the oils. Need of information of soils for training the model. Interference of fertilizers and fungicides[d].
References:	[46-49]

Note: [a], checked by the authors at their labs and in the course of collaborative analyses of European funded projects. [b], the best figure reached in the course of collaborative analyses with blind samples. [c], the measurement is carried with the entire oil but if the measurement is of the unsaponifiable matter, 60 min has to be added to the total analytical procedure. [d], foliar fertilizers can contain K, Fe, Mg, Mn, P and Zn in different proportions, together with other elements (i.e. B, Ca), which can be presented complexed with amino acids such in the cases of Ca, Fe, Mg, Mn and Zn. Fungicides can contain Cu among other elements. [e], other authors have proposed the study of particular geographical production zones by diverse series of compounds, and data are analysed by an umpteen different number of statistical procedures, either unsupervised (e.g. PCA, MDS) or supervised (e.g. LDA, PLS).

3.3. Looking to the future

Today both the production and the consumption of olive oil are moving slowly but inexorably beyond the Mediterranean countries, and olive trees are being planted in countries as far from the Mediterranean basin as New Zealand, Australia, Argentina and Chile with an agricultural technology that has increased up to 16 MT of olives per hectares with adequate sensory and nutritional properties. These practices overcome the negative benefit balance of traditional agriculture while maintaining the prestige of olive oil as a tasteful and health promoting oil.

This revolution in agricultural techniques is not, however, exempt from challenges and even problems from the chemical viewpoint. Traditional orchards were planted with diverse and autochthonous cultivars and used rainfed water supply, but the new orchards demand large quantities of water and the diversity of their cultivars is fewer than one dozen. Questions emerge beyond the classical issues concerning olive oil purity and nutritional benefits [58]. How does the water demand of the new orchards fit into sustainable agriculture? How does the water quality (i.e., salinity) influence olive oil chemical composition? Are the current techniques ready to treat and make use of the increasing tons of by-products? How much is the olive oil chemical composition affected by the latitude of new orchards? Are we going to lose the great diversity of olive tree germplasm with the unstoppable new monocultivar plantations? Are the numerous virgin olive oil Protected Designations of Origin (PDOs) and Protected Geographical Indications

(PGIs) safeguarded from fraudulent labelling? Is the authentication of the geographical origin of olive oil the great forthcoming challenge? How are the proposed non-targeted techniques managed in a legal framework (in court proceedings)? How can olive oil authenticity benefit from the new approaches on big data and data management? Should the olive oil market move toward a common commercialization as daily oil instead of delicatessen marketing?

These are some questions that highlight the current problems in the field of olive oil research and compel food scientists to bring continue their efforts to solve them and to find new methods. The solutions to the current problems of olive oil may come from a high level of chemical characterization. International institutions, led by the IOC, are tackling the influence of climate and geographical provenance in the chemical composition of some genuine olive oils by means of a mathematical algorithm so-called Decision Trees. Although the already accepted Decision Trees [4] do not have mathematical support to their conclusions yet - it is a matter of time that they have a statistical probability associated to their conclusions -, the results seem to be acceptable.

4. Overview of methods for authenticity testing

The combined action of methods and trade standards that regulate the limit values of some analytical parameters results in a procedure that allows determining the presence of extraneous edible oils in olive oils. Figure 1 shows the minimum detectable percentage of some edible oils when they are mixed with olive oil. On the other hand, Table 8 shows the chemical parameters that are used for detecting these oils and the information on authenticity that is derived from them. Thus, some vegetable oils are characterized by relatively high concentration of some compounds, so the latter may indicate their presence in olive oil. However, it is advisable to check the concentration of all the compounds to extract conclusions. Tables 9 and 10 show the methods used to quantify these chemical parameters and the basic characteristics of the analytical procedures.

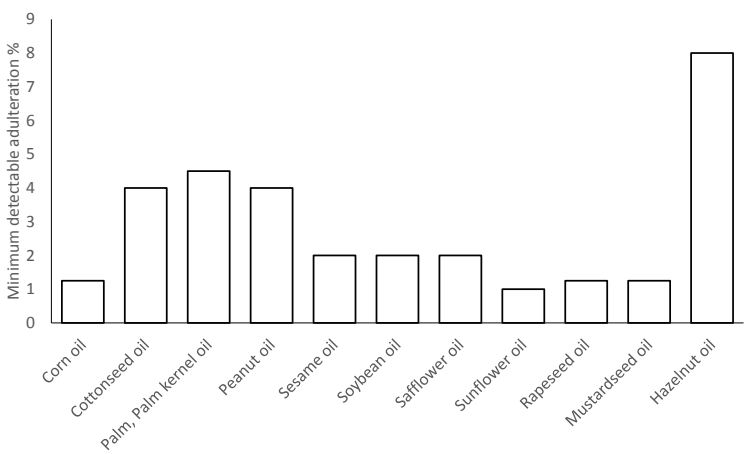

Figure 1: Minimum detectable percentage of some edible oils from different vegetable origins when they are mixed with virgin olive oil by applying the methods described in Table 8

Table 8: Methods and their analytical parameters to be quantified with the objective of detecting the presence of extraneous edible oils in olive oils.

Parameter	Compounds	Authenticity information (presence of…)
Sterols	Brassicasterol Brassicasterol; β-sitosterol Campesterol; β-sitosterol Cholesterol Stigmasterol	Brassicaceae oils Rapeseed oils Mustards seed oils Fractionated palm oils Palm kernel oil Peanut oils
ECN42	ΔECN42 + apparent-β-sitosterol + campesterol + stigmasterol ΔECN42 + Apparent-β-sitosterol ΔECN42 + Campesterol + Stigmasterol ΔECN42 + Apparent-β-sitosterol+ Δ7-stigmastenol	Corn oils Safflower, sesame and soybean oils Cotton oils Sunflower oils
Fatty acid methyl esters	Myristic acid Linolenic, eicosanoic and behenic acids Lignoceric acids	Fractionated palm oils Soybean and canola oils Peanut oils
Trans isomers of fatty acids	tC18:1 & t(C18:2+C18:3)	Refined oils
Stigmastadienes	Stigma-3,5-diene Campestadiene and stigmastadiene	Refined seed oils Desterolised oils
Triterpene dialcohols	Erythrodiol + Uvaol	Olive-pomace oil Seed oils (e.g. grapeseed oil)
Waxes	C40+C42+C44+C46	Olive-pomace oils
2-Glyceryl monopalmitate	Palmitic acid at the 2-position of the triacylglycerols	Oils synthesized by means of FFA esterification with glycerol

Note: The minimum detectable percentage of adulteration with some of these oils are shown in Figure 1.

Table 9: The standard methods for quantifying acyl lipids and fatty acids

Compounds	Technique	Sample preparation	Chromatographic characteristics
Triacylglycerols	HPLC-RI	0.12 g oil in 0.5 mL hexane is charged into SPE-cartridge (1 g of Si) and solution pulled through and, then, eluted with 10mL hexane-diethylether (87:13 v/v).	Mobile phase flow-rate (0.6 to 1.0 mL/min) Oven temperature: 20 °C Mobile phase: propionitrile Column: RP-18 (4 µm) Detector: RI
	HPLC-RI	0.5 g oil in 10 mL acetone or acetone/chloroform (1:1 v/v).	Mobile phase flow-rate (0.6 to 1.0 mL/min) Oven temperature: 25 °C Mobile phase: acetone/acetonitrile (1:1 v/v) Column: RP-18 (4 µm) Detector: RI
2-glyceryl monopalmitate (%)	GC-FID	Hydrolysis with pancreatic lipase. Separation by LC or SPE. Require silanisation.	Column: Capillary (12m×0.32mm×0.10-0.30 µm) Phase: methylpolysiloxane or 5 % phenylmethylpolysiloxane. Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: on-column
Fatty acids	GC-FID	Total Fatty acids: Methylation with cold methanolic solution of KOH or double methylation in a methanolic medium with alkaline and acid catalysis.	Column: Capillary (25-100m×0.2-0.8mm×0.1-0.2µm) Stationary phase: polyglycol, polyester or cyanopropylsilicone Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split
	GC-FID	*Trans* fatty acids: Methylation with cold methanolic solution of KOH.	
	GC-FID	Fatty acid in the 2-position: Hydrolysis with pancreatic lipase previously and methylation in a methanolic medium with alkaline and acid catalysis.	
Waxes	GC-FID	Isolation on LC Si-column.	Column: Capillary (12-15m×0.25-0.32mm×0.1-0.3µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split or on-column

Note: GC, Gas Chromatography; FID, Flame Ionisation Detector; HPLC, High Performance Liquid Chromatography; RI, Refractive Index detector; SPE, Solid Phase Extraction.

Table 10: The standard methods for determining minor compounds

Chemical series	Technique	Sample preparation	Chromatographic characteristics
Sterols	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Isothermal Injection mode: split
Erythrodiol+uvaol	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Isothermal Injection mode: split
Aliphatic alcohols	GC-FID	Unsaponifiable-matter isolation TLC or HPLC. Requires silylation.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split
Aliphatic hydrocarbons and sterenes	GC-FID	Unsaponifiable-matter isolation on LC Si-column.	Column: Capillary (25-30m×0.25-0.32mm×0.15-0.30µm) Stationary phase: 5 % phenylmethylpolysiloxane Carrier gas: Hydrogen Operation conditions: Temperature gradient Injection mode: split

Note: GC, Gas Chromatography; FID, Flame Ionisation Detector; HPLC, High Performance Liquid Chromatography; TLC, Thin Layer chromatography.

5. Conclusion

The criteria defining the authenticity, purity or genuineness of a food product are numerous and vary from one foodstuff to another although many generic definitions have been proposed. Concerning virgin olive oil, authenticity issues may be associated with adulteration with other edible oils but also with designation of origin, olive varieties and with oils that do not meet the requirements of integrity and good practices in labelling. Trade standards, either at national or international level, define the chemical characteristics of a genuine oil with much more detail compared to other vegetable oils. However, considering that consumers expect olive oil to be a foodstuff endowed with reputed sensory and healthy properties, today the authenticity issues of extra virgin olive oil also reach the sensory properties of the oil, which would be inside the declared designation.

The great interest of researchers in virgin olive oil authentication shown in the last few years, mostly analysing the chemical/sensory results by mathematical procedures, has led to an improvement in the control of virgin olive oil adulteration although the new adulterations - e.g. oils with similar chemical composition (hazelnut oil) - represent a new challenge for researchers. Regardless the endless discussion on olive oil authenticity over the decades, the continuous achievement of solutions from analytical chemistry has posed serious problems to the fraudster to commit adulteration and it can be concluded that only the most sophisticated authenticity issues are challenges for the future (olive oils spiked with soft-deodorised oils or tailored oils).

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Vegetable oils

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General overview of the products

Oilseeds are among the most important agricultural commodities worldwide. Edible oils are obtained from the crushing of oilseeds from species that belong to various botanical groups, some of which are perennials (e.g. argan, avocado, olive) with a majority being herbaceous plants (e.g. maize, sunflower, soya). Although these species have been traditionally cultivated for centuries, some of them have been recently modified by conventional breeding programs or by genetic modification. The development of a worldwide market has led to the fact that 80 % of oilseed traded has been soya in the past years (Table 1), whereas it was mainly used in East Asia (China) only two centuries ago. Today the United States is the largest producer of soybean (together with Brazil), peanut and maize. The Russian Federation and Ukraine are major producers of sunflower while India, China and Canada are the largest producers of rapeseed. However, new species have been modified to adapt to diverse environmental conditions and they are progressively colonising geographical areas that were unthinkable only a few decades ago; i.e. olive trees planted in Australia or Argentina. One exception is the argan tree, which is well adapted to the extremely dry climate of Southwestern Morocco.

Agricultural practices have revolutionized vegetable oil production by increasing yields per hectare to incredible figures and molecular biology has been able to modify chemical composition up to a point that many of the major oilseeds have varieties with a profile of fatty acids (e.g., high oleic acid) in line with the consumers' appeal for healthier foods. The increase in oilseed world production has also contributed to a diversification of their use beyond the manufacture of oils; well thought-out, the oil is not the only by-product of oilseeds. Thus, olive products include table olives, skin care products and olive oil soap among others. Varnish, leather and furniture polish, paint, insecticides and lubricating oil are among the uses of peanut oil while rapeseed oil is used in the manufacture of biodiesel for powering motor vehicles and just as many other vegetable oils are used in bio-gas production. The use of soya has become a primary ingredient in dairy products. In fact, infant formulas based on soya are used for lactose-intolerant babies and for babies that are allergic to cow milk proteins. The corn flour is among the most preferred for human nutrition and for feeding farm animals (i.e. chicken and pigs). Likewise, sunflower plants have been used in phytoremediation to remove chemical pollutant is soils such as lead, arsenic and uranium [1].

The production of oilseeds is strongly linked to geographical areas where the farms are evolving towards monoculture cropping. There is not only the well-known link between olive and the Mediterranean basin, or peanut and Georgia (US) or argan and Morocco but also the great productions of oilseeds like soybean and the Central States of the USA and Amazonia (Brazil) or

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sunflower and the Ukrainian steppes [2]. Geographical provenance and the characterization of oils produced in particular geographical areas are not aspects of minor importance as this information can help in health warnings (e.g. Ukrainian sunflower oil contamination).

This chapter does not deal with all the vegetable oils but a set of them: arachis, cottonseed, evening primrose, maize, palm, palm kernel, rapeseed, safflower, soya, sunflower and argan; olive oil is studied in an independent chapter.

Table 1: World production of selected edible oils (harvest 2016/17) in 1 000 mT

Oilseed	Production	1st producer	2nd producer
Arachis[1]	5.910	China (2.896)	India (1.238)
Argan	0.005	Morocco (0.005)	-
Cottonseed[1]	4.419	India (1.160)	China (1.115)
Maize	3.189	USA (1.818)	China (0.267)
Palm[1]	65.068	Indonesia (36.000)	Malaysia (18.858)
Palm kernel[1]	7.596	Indonesia (4.400)	Malaysia (2.300)
Rapeseed[1]	28.841	EU (10.119)	China (7.059)
Safflower	0.674	Kazakhstan (0.175)	India (0.109)
Soya bean[1]	53.861	China (15.770)	United States (10.035)
Sunflower[1]	18.220	Ukraine (5.590)	Russia (4.089)

Note: [1] figures from USA Oilseeds: World Markets and Trade, April 2018 (www.fas.usda.gov). In the case of evening primrose, China accounts for approximately 90 % of world production.

Sources: USDA Foreign Vegetable Oils (www.fas.usda.gov), indexmundi (www.indexmundi.com) and world atlas (www.worldatlas.com).

1. Product Identity

1.1. Definition of the product and manufacturing process

Codex Alimentarius (henceforth, Codex) defines edible vegetable oils as foodstuffs which are composed primarily of glycerides of fatty acids being obtained only from vegetable sources. They may contain small amounts of other lipids, such as phosphatides, of unsaponifiable constituents and of free fatty acids naturally present in the fat or oil. These kinds of oils are labelled as virgin oils, cold pressed oils or refined oils according to their manufacturing process. Codex also defines these designations. Thus, virgin oils are obtained, without altering the nature of the oil, by mechanical procedures (e.g. expelling or pressing) and the application of heat only; they may have been purified by washing with water, settling, filtering and centrifuging only. Cold pressed oils, according to Codex, are obtained, without altering the oil, by mechanical procedures only (e.g. expelling or pressing) without the application of heat; they may have been purified by washing with water, settling, filtering and centrifuging only. Refined edible vegetable oils result from oilseeds or solvent-extracted oils which have undergone a comprehensive processing to be deacified in one of the following ways: a) with alkali; b) by physical refining or both; c) by miscella refining using a permitted food grade solvent, followed by bleaching with absorbent earth and/or

activated carbon or both of them and deodorised with steam without using any other chemical agent; and d) also including the process of degumming using phosphoric/citric acid. Applying appropriate quality management systems, the refining process produces oils with consistent quality.

Oilseeds for producing edible vegetable oils selected for this chapter can have different synonyms for the same name or various species can be associated to the same name. Following definitions of oilseeds help to oil identity (Codex):

Arachis oil (synonyms: peanut oil; groundnut oil) is derived from groundnuts (seeds of *Arachis hypogaea* L.). More than 70 % of this oil is produced in China (50.4 %) and India (20.3 %).

Cottonseed oil is derived from the seeds of various cultivated species of *Gossypium spp*. China (26.8 %) and India (25.2 %) produce more than 50 % of this oil.

Evening primrose oil is derived from the seeds of the evening primrose (*Oenothera biennis*) plant.

Maize oil (synonym: corn oil) is derived from maize germ (the embryos of *Zea mays* L.). It is produced all over the world but USA (55.2 %), China (8.2 %) and Turkey (5.9 %) are major producer countries.

Palm oil is derived from the fleshy mesocarp of the fruit of the oil palm (*Elaeis guineensis*). Note: Palm olein is the liquid fraction derived from the fractionation of palm oil while palm stearin is the high-melting fraction derived from the fractionation of palm oil. Two countries supply more than 80 % of palm oil: Indonesia (55.2 %) and Malaysia (29.4 %).

Palm kernel oil is derived from the kernel of the fruit of the oil palm (*Elaeis guineensis*). The production of this oil in Indonesia (54.2 %) and Malaysia (28.3 %) accounts for more than 80 % of world production.

Rapeseed oil - low erucic acid (synonyms: low erucic acid turnip rape oil; low erucic acid colza oil; canola oil) is produced from low erucic acid oil-bearing seeds of varieties derived from the *Brassica napus* L., *Brassica campestris* L. and *Brassica juncea* L. species. The European Union is the major producer of this oil (40.4 %) followed by China (26.4 %) and Canada (15.5 %).

Safflower seed oil (synonyms: safflower oil; carthamus oil; kurdee oil) is derived from safflower seeds (seeds of *Carthamus tinctorius* L.). Safflower seed oil - high oleic acid (synonyms: high oleic acid safflower oil; high oleic acid carthamus oil; high oleic acid kurdee oil) is produced from high oleic acid oil-bearing seeds of varieties derived from *Carthamus tinctorius*. The main producer of these oils is the USA followed by India and Mexico.

Soya bean oil (synonym: soybean oil) is derived from soya beans (seeds of *Glycine max* (L.) Merr.). USA has a dominant position in soybean (39 %) followed by Brazil (23.8 %) and Argentina (17.9 %).

Sunflower seed oil (synonym: sunflower oil) is derived from sunflower seeds (seeds of *Helianthus annuus* L.). Sunflower seed oil - high oleic acid (synonym: high oleic acid sunflower oil) is produced from high oleic acid oil-bearing seeds of varieties derived from sunflower seeds (seeds of *Helianthus annuus* L.). Russia is the largest producer of sunflower oil (17.8 %) followed by Ukraine (16.7 %) and Argentina (14.8 %).

Argan oil is derived from the kernel of the fruit of the spiny argan tree (*Argania spinosa*). Morocco is the exclusive producer country of argan oil.

Edible vegetable oils are mainly consumed after being submitted to a refining process although the market for crude oils - either virgin or cold pressed oils - has recently increased. Refining is a homogenous well-established process that has peculiarities for some of the selected oils. Thus, arachis oil cannot be winterised because of its high melting point – it solidifies at 3°C. Besides, peanut oil may contain aflatoxin B1 that can be removed in the refining process whether the standard alkali refining and the washing of the oil are used – detoxifying can then lower the aflatoxin content to 10-14 ppb – so that a subsequent bleaching operation is essential to reduce it to less than 1 ppb. The refining of safflower oil has the peculiarity of increasing the content of free sterols – due to the acid-catalysed hydrolysis of steryl esters in the degumming and bleaching processes – and a significant reduction of the content of total sterols during the bleaching process because of reduction of esterified sterol fraction. Crude maize oil has a high content of phosphorous and a wet degumming process at low temperature is recommended; degumming at 10-30 °C results in the removal of more phosphorous than at 70 °C.

The identity of edible vegetable oils usually requires its characterization with information of the most defining/characteristic physical-chemical parameters. As the refining process may modify the original chemical composition (e.g. tocopherols, sterols), the changes of which depend on the refining process used, the following tables show the values related to crude vegetable oils.

The distribution of fatty acid methyl esters (Table 2), which account for more than 95 % of edible oil chemical composition, is used most frequently to characterize oils and confirm their authenticity. The second source of information is the composition of sterols, which is the major series of the unsaponifiable matter of vegetable oils and as important as fatty acids in food authentication. In fact, some sterols may be unique to an oil (i.e. brassicasterol). Sterol composition as shown in Table 3 covers 4-desmethylsterols or also so-called phytosterols or simply sterols. Table 3 also displays the composition of methyl tocols (tocopherols and tocotrienols) not only because they are powerful lipid-soluble antioxidants and a major dietary component but also because their profiles can be used to distinguish vegetable oils; e.g., sunflower seed oil is a good source of α-tocopherol and palm oil of the tocotrienols. In addition to the detailed chemical composition specification, essential physical-chemical characteristics of selected crude vegetable oils (relative density, refractive index, saponification value, iodine value, unsaponifiable matter content) are given in Table 4, which may be also used as identity criteria. Finally, the stable carbon isotope ratio is also included but only for maize oil as it is believed that this measurement provides a better means of detecting foreign oils in this type of oil than other more traditional techniques.

1.2. Current standards of identity or related legislation

1.2.1. EU Legislation

Unlike olive oil, which is extensively covered in EU regulations, there is no specific EU legislation for other edible vegetable oils. The only vertical legislation relevant in this area, is Directive 76/621/EEC which limits the level of erucic acid permitted in oils and fats intended for human consumption, and Directive 80/891/EEC that describes the method for the analysis of erucic acid. National legislation, in particular in the producing countries, defines permitted processing conditions such as neutralization, bleaching, hydrogenation, deodorization, and so on. In fact, EU laws do not provide for a “generally acknowledged definition of food fraud” but there is an extensive EU legislative framework focused on food safety. Only a general guideline is found in EU regulations requiring that food labelling, advertising, presentation and packaging “shall not mislead consumers” [3].

1.2.2. Codex Alimentarius

A number of Codex standards for edible fats and oils was published individually as Recommended Standards until 1981 but unified since then as: Codex Alimentarius. Volume 8. Fats, oils and related products. Information here reported corresponds to: Standards for Named Vegetable Oils Codex Stan 210-1999. Adopted in 1999. Revision: 2001, 2003, 2009. Amendment: 2005, 2011, 2013 and 2015. Last modified: 2017.

This standard contains identity and quality characteristics together with provisions relating to food additives, contaminants, hygiene and labelling. Methods of analysis are also specified. The identity characteristics, which include fatty acid composition, iodine value and relative density etc., essentially define the product and can be used as the basis for determining purity. The standard contains only those provisions essential for public health and safety and consumer protection, as well as other elements needed to ensure fair trade and to prevent fraud.

Tables 2-4 include information of physical-characteristics of the selected crude vegetable oils; evening primrose and argan oils are not described by Codex Stan 210-1999 yet. Thus, those crude vegetable oils for which characteristics fall within the appropriate ranges of the Standard are in compliance with Stan 210-1999 [6]. However, Codex also includes particular values of some indicators, tests and chemical compounds that have to be fulfilled for some of the selected crude edible vegetable oils. Thus, (i) the ranges of the Reichert and Polenske values for palm kernel should lie between 4-7 and 8-12 respectively; (ii) the Halphen test for cottonseed oil should be positive; (iii) arachidic and higher fatty acid content of arachis oil should not exceed 48 g/kg; (iv) total carotenoids (as beta-carotene) for unbleached palm oil should be in the range 500-2000 mg/kg; (v) the Crismer value for low erucic acid rapeseed oil should be in the range 67-70; (vi) the concentration of brassicasterol in low erucic acid rapeseed oil should be greater than 5 % of total sterols; (vii) low-erucic acid rapeseed oil must not contain more than 2 % erucic acid (as % of total fatty acids); (viii) high oleic acid safflower oil must contain not less than 70 % oleic acid (as a % of total fatty acids); and (ix) high oleic acid sunflower oil must contain not less than 75 % oleic acid (as % of total fatty acids).

Codex, however, keeps the door open to consider supplementary criteria based on geographical provenance of crude edible vegetable oils because of climatic variations. Ranges of chemical compounds and physical-parameters vary according to the geographical provenance of crude vegetable oils as shown in the Annex Table of the Chapter of Fats and Oils of the first edition of this book [7]. Geographical traceability is still an important issue of the authenticity of crude vegetable oils.

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Adulteration by addition of other products

While olive oil is one of the most expensive oils and therefore a prime target for adulteration or misrepresentation, other less expensive oils and fats are also at times fraudulently adulterated. This obviously involves adding a cheaper oil. There is not usually any problem of food safety but there is one of misrepresentation and false labelling if the resulting blend is offered or traded as a pure or genuine oil. An individual analysis of authenticity problems of these oils is analysed next

although the number of documented incidents may be a small fraction of the actual number since they usually do not result in a food safety risk and consumers often do not notice them.

Groundnut and sunflower seed oils.

The contamination or adulteration of groundnut and sunflower seed oils with cheaper soya bean oil has been identified for several years in traded oils containing low concentrations of linolenic acid whereas according to chemical information pure sunflower seed and groundnut oils should be free of this acid (≤0.3), unlike the high content of this fatty acid in soya bean oils (4.5-10 %). More recently (November 2015) the adulteration of groundnut oil was reported in India, the second major producer of this oil, because the demand for this oil had increased leading to an abrupt spike in its average price. The Consumer Association of India found that a majority of a large set of groundnut oil samples was adulterated with palmolein and cottonseed oils among other cheaper oils. Thus, 7 % of the samples contained less than 10 % of groundnut oil and 43 % contained less than 20 %. Furthermore, the Food and Drug Administration (FDA) of India reported that traders blended sunflower seed, groundnut and soybean oil with cheaper cottonseed oil. As regards groundnut, this is one of the major edible oils in China, besides soybean oil and rapeseed oil, but it is more expensive than the other two making it prone to adulteration. In January 2012 it was reported that unscrupulous dealers had mixed cottonseed oil and flavour-enhanced soybean oil and marked it as peanut oil. In addition, oils such as soybean oil, sunflower oil, canola oil, and palm oil are also blended into peanut oil in several proportions.

Safflower oil

Safflower oil is a high-priced oil, favoured as a result of its high content of linoleic (C18:2) acid and almost zero content of the easily oxidised linolenic (C18:3) acid. Sunflower seed oil is closely related to it, with a high content of linoleic acid but a very low content of linolenic acid. The similarity of the fatty acid composition and other properties of the two oils has meant that it is difficult to detect the adulteration of safflower seed oil with sunflower seed oil. The main producers of sunflower seed oil are adjacent to the main producer of safflower oils, which might not help end this fraudulent practice.

Other adulterations have been described using safflower oils as an adulterant of virgin olive oil in scientific papers. However, it is unlikely since safflower oil should be refined first, and then the determination of stigmastadienes would make that adulteration unprofitable.

Palm oil

A major problem in the early 1980s was the so-called Singapore Cocktail. This was a reconstituted palm oil made by blending unrelated palm stearin and olein fractions. The blend was much more severely oxidised than unprocessed whole palm oil. Unfortunately, as both the fractions had originated from Malaysian palm oil and were usually blended back in something approaching the right ratio, it was almost impossible to prove by conventional analytical methods that the manipulation had taken place. Another adulteration with a toxic effect is the addition of the artificial dye Sudan IV to some palm oil brands from Ghana; Sudan IV is known to cause cancer. The addition of artificial colouring to palm oil is so widely used that it can be rare to buy any palm oil that has not artificially coloured. In fact, Solvent red 24, which is used in colouring plastics, or Anatol dye are added to palm oil by the nefarious traders to improve its redness.

The Roundtable on Sustainable Palm Oil has reported that palm oil made with sustainable and ethical sourcing claims is at high risk of food fraud; it has been claimed that some manufactures of palm oil use child labour in harvesting or refining processes.

Recently, oleic-enhanced palm oil interesterified with high oleic acid components, followed by fractionation using a patented MPOB (Malaysian Palm Oil Board) process, has been product. However name of “high oleic acid palm oil” is still under consideration for Codex (Codex Alimentarius, REP17/FO-Rev, 3 March 2017) as are value ranges for its chemical compounds and physical-chemical parameters.

Palm kernel oil

Another concern is the co-mingling of lauric oils, usually palm kernel and coconut oils. These two oils have closely related chemical compositions (both contain about 47 % lauric acid) and low levels of unsaturation. Coconut oil usually trades at a higher price, however, so there is a temptation to adulterate it with small amounts of palm kernel oil. An equally worrying, if less prevalent, problem is associated with an oil called babassu. This oil is produced mainly in Brazil and has no international market. However, its chemical and physical properties are similar to those of palm kernel oil. There has therefore been a temptation to bring babassu oil into the edible oil trade by blending it with palm kernel oil.

Palm kernel oil can be fractionated into hard and soft fractions in much the same way as palm oil. In this case, however, it is the hard fraction that is valuable, as a confectionery butter or cocoa butter substitute. The by-product is palm kernel olein, which, as in the case of palm stearin, has only a limited number of outlets and it therefore trades at a lower price than whole unfractionated palm kernel oil. There is a temptation to dispose of the palm kernel olein by blending it into palm kernel oil. Levels of about 10 % are difficult to detect. If the palm kernel oil is then used for the production of hydrogenated palm kernel oil, another useful confectionery fat, the addition of up to 40 % prior to hydrogenation is difficult to detect.

Cottonseed oil

A related case of adulteration occurred in 1983, when cottonseed oil was diluted with palm olein [8]. The incident was widely publicised by the Malaysian Palm Oil Processing Industry at the time [8,9] since, although the Malaysians had supplied the palm olein, they had done so as part of an honest transaction and had not been part of the deception. Cotton is one of the top four GMO crops produced in the world (83 %) - along with soybean (89 %), canola (75 %), and corn (61 %) - and approx. 90 % of all US cotton is genetically engineered. GM products are not labelled in some countries since manufacturers are not required by National Safety Authorities to list the existence nor the quantity of GM food in a producer’s products (i.e. cottonseed oil) on their labels. Thus, it is a potential, if not actual, authenticity problem for consumers concerned about consuming GM foods.

Table 2: Composition of fatty acids of the selected crude edible vegetable oils from authentic samples. Sources: [4-6]

FAME	1	2	3	4	5	6	7a	7b	8	9a	9b	10	11
C6:0	nd	nd	nd	nd	≤0.8	nd	nd	nd	nd	nd	nd	nd	nd
C8:0	nd	nd	nd	nd	2.4-6.2	nd	nd	nd	nd	nd	nd	nd	nd
C10:0	nd	nd	nd	nd	2.6-5.0	nd	nd	nd	nd	nd	nd	nd	nd
C12:0	≤0.1	≤0.2	≤0.3	≤0.5	45.0-55.0	nd	nd	≤0.2	≤0.1	≤0.1	nd	nd	nd
C14:0	≤0.1	0.6-1.0	≤0.3	0.5-2.0	14.0-18.0	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2	≤0.1	≤0.2	≤0.1
C16:0	8.0-14.0	21.4-26.4	8.6-16.5	39.3-47.5	6.5-10.0	2.5-7.0	5.3-8.0	3.6-6.0	8.0-13.5	5.0-7.6	2.6-5.0	11.5-15.0	6.0-10.2
C16:1	≤0.2	≤1.2	≤0.5	≤0.6	≤0.2	≤0.6	≤0.2	≤0.2	≤0.2	≤0.3	≤0.1	≤0.2	nd
C17:0	≤0.1	≤0.1	≤0.1	≤0.2	nd	≤0.3	≤0.1	≤0.1	≤0.1	≤0.2	≤0.1	≤0.1	nr
C17:1	≤0.1	≤0.1	≤0.1	nd	nd	≤0.3	≤0.1	≤0.1	≤0.1	≤0.1	≤0.1	nd	nr
C18:0	1.0-4.5	2.1-3.3	≤3.3	3.5-6.0	1.0-3.0	0.8-3.0	1.9-2.9	1.5-2.4	2.0-5.4	2.7-6.5	2.9-6.2	4.3-7.2	1.5-3.5
C18:1	35.0-69	14.7-21.7	20.0-42.2	36.0-44.0	12.0-19.0	51.0-70.0	8.4-21.3	70.0-83.7	17-30	14.0-39.4	75-90.7	43.0-50.1	5.0-12.1
C18:2	12.0-43.0	46.7-58.2	34.0-65.6	9.0-12.0	1.0-3.5	15.0-30.0	67.8-83.2	9.0-19.9	48.0-59.0	48.3-74.0	2.1-17	23.3-36.0	65.0-85.4
C18:3	≤0.3	≤0.4	≤2.0	≤0.5	≤0.2	5.0-14.0	≤0.1	≤1.2	4.5-11.0	≤0.3	≤0.3	≤0.3	8.0-14.1
C20:0	1.0-2.0	0.2-0.5	0.3-1.0	≤1.0	≤0.2	0.2-1.2	0.2-0.4	0.3-0.6	0.1-0.6	0.1-0.5	0.2-0.5	≤0.5	≤0.3
C20:1	0.7-1.7	≤0.1	0.2-0.6	≤0.4	≤0.2	0.1-4.3	0.1-0.3	0.1-0.5	≤0.5	≤0.3	0.1-0.5	≤0.6	nr
C20:2	nd	≤0.1	≤0.1	nd	nd	≤0.1	nd	nd	≤0.1	nd	nd	nd	nr
C22:0	1.5-4.5	≤0.6	≤0.5	≤0.2	≤0.2	≤0.6	≤1.0	≤0.4	≤0.7	0.3-1.5	0.5-1.6	≤0.2	nd
C22:1	≤0.3	≤0.3	≤0.3	nd	nd	≤2.0	≤1.8	≤0.3	≤0.3	≤0.3	≤0.3	nd	nd
C22:2	nd	≤0.1	nd	nd	nd	≤0.1	nd	nd	nd	≤0.3	nd	nd	nr
C24:0	0.5-2.5	≤0.1	≤0.5	nd	nd	≤0.3	≤0.2	≤0.3	≤0.5	≤0.5	≤0.5	nd	nd
C24:1	≤0.3	nd	nd	nd	nd	≤0.4	≤0.2	≤0.3	nd	nd	nd	nd	nr

Note: FAME, fatty acid methyl esters. 1, Arachis oil; 2, Cottonseed oil, 3, Maize oil; 4, Palm oil; 5, Palm kernel oil; 6, Rapeseed (Canola) oil; 7a, Safflower oil; 7b, Safflower high oleic oil; 8, Soya oil; 9a, Sunflower oil; 9b, Sunflower high oleic oil; 10, argan oil; 11, evening primrose oil; nd, non-detectable, defined as <0.05 %; nr, not reported.

Table 3: Levels of 4-desmethylsterols as a percentage of total sterols (in mg/kg), tocopherols and tocotrienols in mg/kg in the selected crude edible vegetable oils from authentic samples. Sources: [4-6]

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	11
Cholesterol	≤3.8	0.7-2.3	0.2-0.6	2.6-6.7	0.6-3.7	≤1.3	≤0.7	≤0.5	0.2-1.4	≤0.7	≤0.5	nd	nd
Brassicasterol	≤0.2	0.1-0.3	≤0.2	nd	≤0.8	5.0-13.0	≤0.4	≤2.2	≤0.3	≤0.2	≤0.3	nd	nd
Campesterol	12.0-19.8	6.4-14.5	16.0-24.1	18.7-27.5	8.4-12.7	24.7-38.6	9.2-13.3	8.9-19.9	15.8-24.2	6.5-13.0	5.0-13.0	≤0.4	8-9
Stigmasterol	5.4-13.2	2.1-6.8	4.3-8.0	8.5-13.9	12.0-16.6	0.2-1.0	4.5-9.6	2.9-8.9	14.9-19.1	6.0-13.0	4.5-13.0	nd	nd
β-sitosterol	47.4-69.0	76.0-87.1	54.8-66.6	50.2-62.1	62.6-73.1	45.1-57.9	40.2-50.6	40.1-66.9	47.0-60	50-70	42.0-70	nd	87-90
Δ^5-avenasterol	5.0-18.8	1.8-7.3	1.5-8.2	≤2.8	1.4-9.0	2.5-6.6	0.8-4.8	0.2-8.9	1.5-3.7	≤6.9	1.5-6.9	nd	≤4
Δ^7-stigmastenol	≤5.1	≤1.4	0.2-4.2	0.2-2.4	≤2.1	≤1.3	13.7-24.6	3.4-16.4	1.4-5.2	6.5-24.0	6.5-24.0	20.5-35.9	≤2
Δ^7-avenasterol	≤5.5	0.8-3.3	0.3-2.7	≤5.1	≤1.4	≤0.8	2.2-6.3	≤8.3	1.0-4.6	3.0-7.5	≤9.0	1.2-4.4	nd
Others	≤1.4	≤1.5	≤2.4	nd	≤2.7	≤4.2	0.5-6.4	4.4-11.9	≤1.8	≤5.3	3.5-9.5	59.0-78.0[a]	nr
Total sterols	900-2900	2700-6400	7000-22100	300-700	700-1400	4500-11300	2100-4600	2000-4100	1800-4500	2400-5000	1700-5200	1300-3190	nr
α-tocopherol	49-373	136-674	23-573	4-193	≤44	100-386	234-660	234-660	9-352	403-935	400-1090	14-78	76-356
β-tocopherol	≤41	≤29	≤356	≤234	≤248	≤140	≤17	≤13	≤36	≤45	10-35	≤5.2	nd
γ-tocopherol	88-389	138-746	268-2468	≤526	≤257	189-753	≤12	≤44	89-2307	≤34	3-30	322-810	187-358
Δ-tocopherol	≤22	0-21	23-75	≤123	nd	≤22	nd	≤6	154-932	≤7.0	≤17	28-113	≤19
α-tocotrienol	nd	nd	≤239	4-336	nd	nd	nd	nd	≤69	nd	nd	nr	nr
γ-tocotrienol	nd	nd	≤450	14-710	≤60	nd	≤12	≤10	≤103	nd	nd	nr	nr
Δ-tocotrienol	nd	nd	≤20	≤377	nd	nd	nd	nd	nd	nd	nd	nr	nr
Total (mg/kg)	170-1300	380-1200	330-3720	150-1500	≤260	430-2680	240-670	250-700	600-3370	440-1520	450-1120	597-880	263-661

Note: 1, Arachis oil; 2, Cottonseed oil, 3, Maize oil; 4, Palm oil; 5, Palm kernel oil; 6, Rapeseed (Canola) oil; 7a, Safflower oil; 7b, Safflower high oleic oil; 8, Soya oil; 9a, Sunflower oil; 9b, Sunflower high oleic oil; 10, argan oil; 11, evening primrose oil; nd, non-detectable, defined as <0.05 %; nr, not reported. Maize oil also contains ≤52 mg/kg β-tocotrienol; [a], stigmasta-8,22-diene-3-ol (3-6 %), spinasterol (34-44 %), schottenol (44-49 %) and sigmasta-7,24-diene-3-ol (4-7 %).

Table 4: Physical-chemical characteristics of the selected crude edible vegetable oils from selected samples. Sources: [4-6]

	1	2	3	4	5	6	7a	7b	8	9a	9b	10	11
RD	0.912-0.920 x=20 °C	0.918-0.926 x=20 °C	0.917-0.925 x=20 °C	0.891-0.899 x=50 °C	0.899-0.914 x=40 °C	0.914-0.920 x=20 °C	0.922-0.927 x=20 °C	0.913-0.919 x=20°C[a]	0.919-0.925 x=20 °C	0.918-0.923 x=20 °C	0.909-0.915 x=25°C	0.906-0.919 x=20 °C	nr
RI	1.460-1.465	1.458-1.466	1.465-1.468	1.454-1.456 at 50 °C	1.448-1.452	1.465-1.467	1.467-1.470	1.466-1.470 at 25 °C[b]	1.466-1.470	1.461-1.468	1.467-1.471 at 25 °C	1.463-1.472 at 25 °C	14791 at 20 °C
SV	187-196	189-198	187-195	190-209	230-254	182-193	186-198	186-194	189-195	188-194	182-194	189.1-199.1	192-198
IV	86-107	100-123	103-135	50.0-55.0	14.1-21.0	105-126	136-148	80-100	124-139	118-141	78-90	91-110	147-155
UM	≤10	≤15	≤28	≤12	≤10	≤20	≤15	≤10	≤15	≤15	≤15	≤11	≤2

Note: 1, Arachis oil; 2, Cottonseed oil, 3, Maize oil; 4, Palm oil; 5, Palm kernel oil; 6, Rapeseed (Canola) oil; 7a, Safflower oil; 7b, Safflower high oleic oil; 8, Soya oil; 9a, Sunflower oil; 9b, Sunflower high oleic oil; 10, argan oil; 11, evening primrose oil; nd, non-detectable, defined as <0.05 %; nr, not reported. RD, relative density (x °C/water at 20 °C); RI, refractive index (ND 40 °C); SV, saponification value (mg KOH/g oil); IV, iodine value; UM, unsaponifiable matter (g/kg). [a], 0.910-0.916 x=25°C; [b], 1.460-1.464 at 40°C. Stable carbon isotope ratio for maize oil should oscillate between -13.71 and -16.26.

Maize oil

Maize oil is a premium oil which may also be adulterated but, because its fatty acid composition overlaps that of other vegetable oils, blending is difficult to detect. Similarly, although maize oil has a much higher level of sterols than other oils, the ratios of the concentrations of the individual sterols (useful in resolving purity issues with most other oils) hardly change at all in blends of maize oil with minor amounts of other vegetable oils. For these reasons, it is difficult to detect adulteration of maize oil by conventional analytical techniques. This is the case for the reported addition of cheaper rapeseed oil to maize oil at high percentages which is an economically motivated adulteration. There are, however, many scientific papers that describe the addition of maize oil to virgin olive oil which would, in fact, be easily detected by only tasting the hypothetical mix if crude maize oil is added, or by the quantification of stigmastadienes if refined maize oil is added to virgin olive oil. The fraud of adding refined maize oil to refined olive oil has been detected at very low percentages using stable carbon isotope ratio analysis since the beginning of 1990's. Although any kind of adulteration is always possible if there is a profit for fraudsters, some types of adulteration put forward by some scientists may not be supported by facts and may be far from the actual oil market.

Rapeseed and soya-bean oil

Rapeseed and soya-bean oils have similar fatty acid compositions. Although they are among the cheaper vegetable oils, they are traded at slightly different prices, soya-bean oil usually being the more expensive. Blends of the two may alleviate a temporary shortage of one or other of the oils or provide a small commercial advantage but such blends have been difficult to identify when the levels of addition are below 20 %. Conversely, soya bean oil can be adulterated with rapeseed oil. The type of adulteration depends on the market demands for one or the other edible oil, and the tariffs to be paid for the declared edible oil in the destination market. Whenever large tariffs are payable in the destination country, there is a high risk of cross-border smuggling operations, including various proportions of the adulterated mixture.

Evening primrose oil (EPO)

EPO is produced, at a high cost, because of its moderate content of gamma-linolenic acid (GLA, i.e. all *cis*-6,9,12 octadecatrienoic acid), which is distinguished from normal or alpha-linolenic acid (all *cis*-9,12,15 octadecatrienoic acid). GLA is normally formed in the human body by the metabolism of linoleic acid (all *cis*-9,12 octadecadienoic acid). However, some people have a deficiency in their delta-6 desaturase enzyme system leading to a deficiency of GLA and subsequent metabolic products. It is claimed that this results in a number of human aliments that can be alleviated by consuming preformed GLA. Thus, evening primrose is produced commercially as a source of GLA [10]. Borage and blackcurrant seed oils are also considered as valid sources of GLA [11]. As an unofficial standard requires that EPO should contain at least 10 % GLA, these other oils have been considered as possible adulterants of evening primrose in order to reach the required concentration of GLA. Although consumers thus receive the required amount of GLA, it is fraudulent to describe the oil as pure EPO.

Sunflower seed oil

Although sunflower seed oils are produced at a low price, they have not been free from adulteration with other food products. In 2017, for example, the Security Service of Ukraine carried out inspections of sunflower seed oil market operators because it had been alleged that producers adulterated this oil with chicken fat. In the past, the enzymatic interesterification of lard and high-oleic sunflower oil was used for the legal development of new products [12], which

increased the stability of the vegetable oil and its bland flavour in fried foods [13]. During winter/spring 2007/2008, in Ukraine, nearly 100 000 t of sunflower oil were contaminated with mineral oil at concentrations often above 1 000 mg/kg. Fortunately, the European Food Safety Authority (EFSA) concluded that "exposure to such oil, although undesirable, would not be a public health concern" since no additives for lubricating oils or pesticides were detected – the risk assessment was exclusively based on the hydrocarbons - but the complete absence of n-alkanes suggested that the contaminant consisted of a base oil for the manufacture of lubricants or hydraulic oils [14].

Argan oil

Argan oil is expensive, its current price in Europe is above 100 euros per litre. Thus, such a price is likely to incite unscrupulous behaviour and illegal practices are not uncommon (i.e. dilution with olive oil coloured with paprika or other substances). In addition, the detection of its adulteration is sometimes a complex problem [15]. Historically, detecting such fraud has been difficult because of the small databases establishing appropriate purity criteria. Today, argan oil is widely sold in Western-Europe, North-America and Japan, and the set of potential adulterants include now soya bean and sunflower seed oils among others.

Coconut oil

The current edible oil market shows a fast-rising demand for coconut oil in developed countries (e.g., USA), whereas at the same time coconut production is falling in producer countries, mostly the Caribbean and Central America, because of a lethal yellowing disease which is threatening coconut crops [16]. An immediate consequence has been widespread adulteration and counterfeiting of retail coconut oil brands, alleged to be occurring in large amounts (50 %) in India [17]. The expected fraudulent activity could not be circumscribed to the dilution of coconut oils with cheaper edible oils but also includes aspects such as the misrepresentation of the organic coconut oil status and country of origin (geographical provenance) or the addition of undeclared ingredients for flavouring or colouring possibly resulting in an allergen risk.

Oil processing

Oils prepared by mechanical means alone, without the application of extra heat and in the absence of further processing, are described as cold-pressed. These normally have a fine flavour, depending on the quality of the seeds used as raw material. They are produced in relatively low yields and are therefore more expensive. Oils obtained by hot-pressing and/or solvent extraction are obtained in higher yields and are therefore cheaper. Furthermore, oils can be solvent-extracted from inferior seeds and then refined and deodorised to give bland, manufactured oils which are even cheaper than those produced, for example, by hot-pressing from high grade seeds.

Processing of poor-quality oilseeds or poor storage conditions may lead to an unwelcome increase of free fatty acids (FFA) in the oil. Crude oils are often purchased on contracts that specify a maximum FFA content. If, as a result of some mishap, an oil has an FFA above the contractual maximum, there is a temptation to refine part of the oil to remove the FFA and then to blend back unprocessed oil to give a supposed crude oil that is now within the contractual limit.

As a further consideration, partially hydrogenated oils (PHOs) are going to be banned in several countries in 2018 (e.g. Final Determination Regarding Partially Hydrogenated Oils, FDA- 80 FR 34650). The demand for PHOs will fall and the price of replacement oils expected to rise, with a medium risk that unscrupulous suppliers may use PHOs in edible oils and other food materials that are claimed to be free of such compounds.

2.1.2. Geographical origin

Values of the physical-chemical parameters characterising crude vegetable oils are influenced by the variety of the harvested plant and the pedoclimatic characteristics of their orchards; for example, West African groundnut oil has a low iodine value, which makes it preferred by buyers. In fact, the existence of some discrepancies in the authenticity of edible oils and errors among laboratories are mostly due to the scarcity of information on the samples analysed (e.g. cultivar and geographical origin) that can be remedied by databases that store information for most of the chemical compounds and physical-chemical parameters relevant to authenticity-characterisation. Thus, Leatherhead Food RA (UK) put a lot of effort into collecting a set of authentic vegetable oilseed samples that were representative of world trade. Annex of Oils and Fats Chapter in Food Authenticity: Issues and Methodologies [7] displays the ranges of a series of crude edible vegetable oils (coconut, cottonseed, maize, groundnut, palm kernel sesame, rapeseed as canola, sunflower seed, soya bean, safflower and palm) from their different geographical origins that were characterised by their fatty acids, main phytosterols, triglyceride carbon number, tocopherols, iodine value, slip melt point, fatty acids at the 2-position and enrichment factors.

The importance of characterization of crude edible oils by their geographical origin is becoming more and more important as some oilseeds and edible oils are being smuggled along “drug” routes between origin and destination countries to avoid the high tariffs payable on original destination. Although the ratio between domestic production and export volumes is the main parameter to imply that an imported food might be fraudulent, it is not always easy to get this information and the values of analytical parameters can be of help.

2.1.3. Organic edible oils

It is well-known that the demand for organic edible oils has grown rapidly and now outstrips the figures of production for some edible oils (e.g., corn and soybeans), up to the point that accredited certifying agents for organic foodstuffs have increased surveillance of organic edible oils in order to detect possible fraudulent organic certifications. American commentators have claimed that the volumes of imports from certain producer countries exceed the legitimate oilseed organic production volumes that could be produced by those countries. In developed countries, paradoxically an organic edible oil may be qualified as conventional oil just because the organic verification process is too expensive (> 1 000 EUR) whereas, at the same time, an imported edible oil can be qualified as organic using a fraudulent certification. Sometimes, the problem of organic oils arises from pesticides from neighbouring farms or from polluted soil and water (e.g. China organic farms). Conversely, after oilseeds or crude vegetable oils are washed, the concentration of pesticide residues can reach similar values to those of organic food products.

2.2. Identification of potential issues

Food fraud, or the act of defrauding buyers of food has vexed the food industry throughout history, and edible oils are not an exception. Thus, the adulteration of edible vegetable oils goes beyond the label on their bottles as many of the oils described in this chapter are very common ingredients present in many food products including chips, margarine, mayonnaise, salad oils and dressings, pasta sauces, packed foods, baked and many more. Thus, the adulteration of edible vegetable oils for direct consumption could be just the visible part of an iceberg of the adulteration if the Control Authorities for Food Fraud turn a blind eye to the adulteration of edible oils as ingredients in food formulations, only reacting when risks to public health are detected in the food chain.

The combined action of databases with information on genuine crude vegetable oils with analytical methodologies that are able to detect additions of cheaper edible oils to expensive ones at low concentrations is making classical adulteration less profitable, at least in developed countries (e.g., EU, USA, Canada). However, it does not guarantee that the adulteration of seed oils does not exist if international and national control organisms lift legal barriers.

There are, however, relatively new adulterations like the addition of “gutter oils” to edible oils [18]. Gutter oils are used edible oils or waste cooking oils, which are collected from restaurant fryers, grease traps, slaughterhouse waste etc. and re-labelled as normal edible oils. Unfortunately, the very diverse sources (e.g., processes, kind of oils, mixtures) of the gutter oils mean that the identification of a good marker for their detection in adulterated edible oils is an analytical challenge. In addition, the detection of the toxic substances might not be reliable if one carefully analyses the variability of the origin of the oil and also the fact that these oils are treated with chemicals prior to being sold back to restaurants in Asia. In September 2014, a scandal was reported involving 240 tons of gutter oil in Taiwan, some of which may have been exported overseas. Although, this kind of adulteration is currently limited to Asia, finding it in developed countries in the next years cannot be ruled out.

Another adulteration that is expected to continue is the illegal colouring of palm oils in developing nations which remains a challenge for the enforcement authorities. The Migration of populations often leads to an increase in the imports of food products that were common in the home countries of the migrants, and these are then bought in markets used by foreign traders. There have been recent reports (4 March 2018) of palm oil that is been adulterated with artificial colouring [19] in such markets.

Other consumer concerns that are likely to increase include the demand for sustainably-grown palm oil, with the high risk that this sustainability status is fraudulently misrepresented. Consumers in some countries are also worried about consuming edible oils extracted from GM oilseeds because of possible mislabelling not only of their containers but also as they are ingredients in a number of food products. The high complexity of national and international regulations dealing with genetically modified organisms adds furthers difficulties to effective authentication in this regard.

An attempt at identifying potential issues in authentication should also focus on the abrupt changes in price and a break in the value balance between edible oils. A common rule is that larger harvests of an oilseed often lead to lower prices, which usually means a decreased risk of fraudulent activity for this particular oil; whereas the availability of cheap and abundant amounts of the oil make it attractive as an adulterant, a diluent or filler. For example, a large harvest of hazelnuts makes refined hazelnut oil a potential adulterant of olive oil but, on the contrary, a large harvest of olives may make roasted hazelnut oil the object of adulteration with virgin olive oil.

Food fraud can include economic adulteration, economically motivated adulteration, intentional adulteration, or food counterfeiting, according to the United States Pharmacopeial Convention (USP). Given the diversity of possible types of fraud, it is necessary to register real cases of adulteration to understand the actual incidence of a fraud type and go beyond hypothetical cases described in analytical studies. Today, the coordination between institutions for registering these cases is assumed as a critical tool for detecting new fraud types.

2.3. Potential threat to public health

Vegetable oil adulteration involves the substitution of a high-value product with a less expensive or lower quality alternative that deprives the food buyer of the product they think they are getting. However, the vast majority of fraud incidents does not pose a public health risk or food safety crisis although some journals have published alarmist headlines such as “deep-frying with sunflower oil and corn oil releases toxic chemicals linked to cancer”.

The consumption of edible oils either adulterated with gutter oils or even 100 % gutter oil is a source of potential health concerns. As gutter oil contains many kinds of toxic waste material, arsenic being one of them, it can have a serious impact on gastrointestinal diseases and digestive disorders. Gutter oil also contains a large variety of detergents and chemical cleaning substances (e.g., lead content is surprisingly high), which can cause abdominal cramps and anaemia and even liver function damage. Besides, gutter oil is usually extracted from sewers, but also refining the rotten animal meat and rotten animal offal, and the immediate consequence in the consumers is diarrhoea [20].

Today's rising food prices and the global nature of the food chain offer the opportunity for criminals to sell counterfeit and substandard food in a multi-billion criminal industry, and sometimes it has the consequence of health risk. These health risks, when happening, are of several degrees of importance. Thus, for example, cottonseed oil, if partially hydrogenated, as found in margarines or solid shortenings, contains high amounts of trans-fats, which are considered dangerous for health. When consumed in regular small amounts, the effect on health is negligible. However, consumer preference is for non-hydrogenated oils. In other cases, when the oil is adulterated, and it is out of the regular market chain, the health risk may be more important. Sometimes this importance is justified by a toxic effect of an unexpected compound. In other cases, allergic reactions are involved, sometimes due to minor compounds present in the oil. Thus, allergic reactions to cotton as food, may also involve physiological responses to the presence of harmful pesticides, herbicides, fungicides, and GMOs. Gossypol may be another factor related to cottonseed food allergies.

It is essential, in order to protect public health, to consider also the contaminants at levels which are toxicologically acceptable [21]. In regard to authenticity, contaminants can be related with additions of other oils or with processes that are not commonly applied. It is true that the chemical 3-monochloropropane diol (3-MCPD) and its esters are formed unintentionally during the refining process of vegetable oils, mainly palm oil, as high temperatures are applied in order to achieve quality and safety specifications. These substances are genotoxic and carcinogenic (i.e. they can damage DNA and cause cancer) although the consumption levels of 3-MCPD in food are considered safe for most consumers. However, EFSA has determined the maximum 3-MCPD tolerable daily intake at 2.0 *micrograms per kilogram of body weight* to prevent high consumers in younger age groups from a potential health concern (e.g., male fertility).

The progress in analytical techniques has provided advanced knowledge of chemical composition, focusing attention on particular some compounds for their health implications. The detection of the presence of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in foods in general, and in edible oils in particular, has started a debate about which is the best method to control their presence and the best and realistic limits to be included in regulation. The European Food Safety Authority (EFSA) has published a scientific opinion on this topic [22] in which it was stated that the major sources of MOH in food are food packaging and additives, processing aids, and lubricants. The health concern on these compounds is explained by the observation that these compounds may accumulate and cause micro-

granulomas in several tissues. Currently modifications in the production process are under discussion to avoid the sources of this contamination. Similar problems can be identified with other migrating contaminants, such as phthalates [23].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Edible vegetable oils that are misdescribed, insufficiently described or adulterated or deceptive by any combination of these factors can all be detected, in theory, by analysis and comparison of the resulting data with those from an authentic population. This means that these oils are characterised by their most relevant chemical compounds and physical-chemical parameters in regards to each authenticity issue. The information from those variables requires a set of standardised methods to be implemented in control laboratories. Table 5 summarises the determinations that are relevant to edible oil authenticity and characterisation and possible methods validated by international institutions.

3.2. Other used methods

With such a variety of oils and an extensive scientific literature, identifying alternative methods require selecting those approaches that permit to tackle real cases and adulteration percentages. A high proportion of literature reports, understandably perhaps, relate to obscure varieties of oilseeds that had been analysed simply because they were unusual. In other cases, samples for analysis had been "picked" and were superior examples of the variety under study. The full stock of literature information is, as a result, not always related to the vegetable oils traded for use as food on world markets. The alternative methods that are proposed beyond those described in trade standards are mostly based on non-targeted techniques, such as vibrational spectroscopy, nuclear magnetic resonance and isotopic techniques. The fundamentals and methods are very similar to those described for olive oil.

Infrared absorption and Raman scattering give complementary information about molecular vibrations, yielding a vibrational fingerprint of the molecules [24]. Both the mid-infrared (MIR) (4000-400 cm^{-1}, 2.5-25 µm) and near-infrared (NIR) (15000-4000 cm^{-1}, 0.72-2.5 µm) regions have been used in the study of fats and oils.

The IR spectroscopy and multivariate approach [25] has been applied to authentication of non-edible oils [26,27], and today the combination of non-targeted techniques and Chemometrics seem to be an alternative to solve sophisticated cases of adulteration [28]. Thus, this approach has been used to detect contaminants and additives from lubricating oils and diesel in edible oils resulting from the use of diesel tankers and lubricating oil drums for transportation [29]. Such contaminants have serious health implications.

Further developments in the analysis of the spectral data through computer-aided techniques have opened up new opportunities for use in the on-line analysis of industrial processes. Thus, these analytical approaches can be used to measure several properties, which are described below.

Table 5: Summary of the relevant methods proposed in Codex Alimentarius [6]

Determination	Method
Fatty acid composition	IUPAC 2.301, 2.302 and 2.304 or ISO 5508: 1990 and 5509: 2000 or AOCS Ce 2-66, Ce 1e-91 or Ce 1f-96
Sterol content	ISO 12228:1999, or IUPAC 2.403
Tocopherol content	IUPAC 2.432 or ISO 9936: 1997 or AOCS Ce 8-89
Total carotenoids	BS 684 Section 2.20
Acidity	IUPAC 2.201 or ISO 660: 1996 or AOCS Cd 3d-63
Unsaponifiable matter	IUPAC 2.401 (part 1-5) or ISO 3596: 2000 or ISO 18609: 2000
Peroxide value	IUPAC 2.501 (as amended), AOCS Cd 8b - 90 (97) or ISO 3961: 1998
Matter Volatile at 105°C	IUPAC 2.601 or ISO 662: 1998
Arsenic content	AOAC 952.13, IUPAC 3.136, AOAC 942.17, or AOAC 985.16
Insoluble impurity	IUPAC 2.604 or ISO 663: 2000
Trace metals of copper and iron	ISO 8294: 1994, IUPAC 2.631 or AOAC 990.05 or AOCS Ca 18b-91
Determination of traces of heavy metals	Lead: IUPAC 2.632, AOAC 994.02 or ISO 12193: 1994 or AOCS Ca 18c-91 Arsenic: AOAC 952.13; AOAC 942.17; AOAC 985.16
Slip point	ISO 6321: 1991 and Amendment 1: 1998 for all the edible oils, or AOCS Cc 3b-92 or Ce 3-25 (97) for palm oils only
Crismer value	AOCS Cb 4-35 (97) and AOCS Ca 5a-40 (97)
Badoiun test[1]	AOCS Cb 2-40 (97)
Halphen test	AOCS Cb 1-25 (97)
Reichert and Polenske values	AOCS Cd 5-40 (97)
Refractive Index	IUPAC 2.102 or ISO 6320: 2000 or AOCS Ce 7-25
Iodine value	Wijs - according to IUPAC 2.205/1, ISO 3961: 1996, AOAC 993.20, or AOCS Cd 1d-92 (97), or by calculation - AOCS Cd 1b-87 (97)
Saponifiable value	IUPAC 2.202 or ISO 3657: 1988
Soap content	BS 684 Section 2.5
Relative density	IUPAC 2.101[a]
Apparent density	ISO 6883: 2000[a] or AOCS Cc 10c-95

Note: [a], with the appropriate conversion factor; [1], modified Villavecchia test or sesame seed oil test; AOCS, American Oil Chemists Society; ISO, International Organization for Standardization; FOSFA, Federation of Oils, Seeds and Fats Associations Ltd; IUPAC, International of Union of Pure and Applied Chemistry.

The majority of the unsaturated fatty acids that make up edible oils are found in the *cis* form. When oils are hardened by hydrogenation (to formulate margarine), or partially hydrogenated to stabilise against oxidation, there is a conversion of some *cis* to *trans* double bonds. FTIR can be used to determine the *trans* isomer content of oils and fats with a good agreement with GC results [24]. Actually, FTIR has demonstrated good performance in this determination and a IUPAC method was developed with this objective [30]. Raman spectroscopy has also been used to determine the *cis/trans* isomer content of edible vegetable oils, as well as to determine the total unsaturation of oils and margarines. Furthermore, Fourier transform mid infrared (FT-MIR) has

been used in the detection of adulteration of virgin coconut oil [31], the mixtures of sesame and corn oils [32], and the presence of lard in some vegetable oils [33] also analysed by differential scanning calorimetry [34]; a large set of applications is described by [7].

FTIR analysis also provides a rapid means of evaluating the oxidative state of an oil or of monitoring changes in oils undergoing thermal stress [35]. Rapid methods based on FTIR have also been developed for the quantitative determinations of the iodine value and saponification number, free fatty acids, peroxide value and solid fat index.

In terms of oil authenticity, FTIR, NIR and Raman spectroscopy coupled with multivariate analysis techniques have been used to characterise edible oils, according to their degree of unsaturation and other characteristics [25,27]. The basis for the discrimination between fats is often the concentration of unsaturated fatty acids, and different concentrations of linoleic acid in the case of oils (sunflower, olive and peanut oils) [25,36].

^{1}H-NMR has also been applied to the study of the triacylglycerol structure of palm oils, seed oils, some hydrogenated fats and vegetable margarines as, for instance, the adulteration of peanut oil [37].

Quantitative ^{13}C-NMR data of the acyl profile have been reported to be in good agreement with GC for other edible vegetable oils, fats and lipids. Thus, a profiling strategy with ^{13}C-NMR, ^{1}H-NMR and the analysis of results by chemometrics [38,39] has shown satisfactory results in detecting the presence of different vegetable oils, although always with the involvement of a database that can affect these results.

Isotopic analysis has already been carried out with respect to the isotope ratio of individual fatty acids in an oil and it has been shown that there are slight differences [40]. Any contamination of an oil will upset these slight differences, the nature of the distortion from the established pattern revealing the cause of the impurity. Thus, for example, carbon isotope ratio was used, in combination with GC-FID, to detect the presence of corn oil in sesame oil [41].

The technique of site-specific isotope fractionation studied by NMR (SNIF-NMR) has been applied to alcoholic beverages and fruit juices. There is every possibility that it may also show advantages in the evaluation of edible seed oils.

The main applications of spectroscopic techniques in authenticating edible oils has been focused on detecting their presence in olive oil categories as described in the chapter on olive oil. Sometimes, however, authors imagine a virtual world in which any kind of adulteration can be possible, some ones even being unprofitable for fraudsters, and they study cases that do not exist in the real world even being published in reputed scientific journals.

4. Overview of methods for authenticity testing

In the investigation of suspect oils, it is usual that fatty acid compositions are studied first as they are easy to determine and are sufficiently different to clarify the majority of uncertainties of food authenticity. However, many other methods can provide chemical or physical-chemical information that can be also useful in authentication. The following table provides an overview of common methods for the detection of seed oil adulteration. The reader is referred to previous tables provided for chemical differences among edible oils (Tables 2-3) and analytical methods suggested by Codex (Table 5).

Analytical Method	Analyte - Indicative data	Applicability
Fatty acid profile by GC	Linolenic acid (C18:3) Fatty acid concentration at the triglyceride-2 position	Contamination of groundnut and sunflower seed oils with soybean and rapeseed oils
Sterol profile by GC	Brassicasterol	Detection of rapeseed oil in sunflower seed or groundnut oils
Tocopherol content by HPLC	Gamma-tocopherol	Detection of soybean oil in sunflower seed oil
Tocopherol content by HPLC	Delta-tocopherol	Detection of soybean oil in groundnut oil
Carbon number triglyceride composition by HPLC	C60/C58 ratio	Detection of sunflower seed oil in safflower seed oil
Slip melting point/Iodine values		Detection of stearins or oleins in palm oil
Carbon number triglyceride composition by HPLC	C48 concentration * palmitic acid enrichment factor	Detection of stearins or oleins in palm oil
ICP-OES	Elemental content	Detection of olive oil and soybean oil in argan oil
Sterol profile by GC	Campesterol	Detection of olive oil in argan oil
HPLC	Triacylglycerols	Detection of sunflower seed, soybean and olive oils in argan oil
1H LF-NMR	Ratios	Detection of soybean, palm and rapeseed oils in peanut oil
Fatty acid profile by GC	Palmitic acid (C16 :0)	Adulteration of cottonseed by palm olein
Carbon number triglyceride composition by HPLC	C50 and C54	Detection of palm olein oil in cottonseed oil
Carbon number triglyceride composition by HPLC	Various purity criteria	Mixtures of palm kernel and coconut oils
Fatty acid profile by GC and Iodine values	Oleic acid (C18 :1)	Detection of palm kernel olein in palm kernel oil
Differential Scanning Calorimetry	Thermogram profiles	Detection of animal fat in sunflower seed oil
Stable isotope analysis	$^{13}C/^{12}C$ ratios	Detection of commercial oils in maize oils and vice versa
Stable isotope analysis	$^{13}C/^{12}C$ ratios	Detection of maize oil in olive, sesame and soybean oils
Fatty acid profile by GC	Oleic and linoleic acids	Detection of rapeseed and soybean blends
Fatty acid profile by GC	Linoleic and erucic acids	Detection of borage oil in evening primrose oil
Fatty acid profile by GC	Linoleic and stearidonic acids	Detection of blackcurrant seed oil in evening primrose oil

5. Conclusion

A large number of the problems in identifying more than 10 % adulteration or contamination of bulk edible oils have been clarified. However, changes in commercial trade patterns and in consumer eating habits, together with increasing application of genetic engineering to improve oil crops mean that tomorrow's problems may not be the same as those encountered today. Thus, using genetic modification it is possible to obtain oils from different botanical sources with chemical characteristics that are similar to those oils that are targets for adulteration. In consequence, authentication strategies should consider this fact to be efficient in detecting frauds.

As regards analytical developments, the difficulty is that some of the methods, such as sterol analysis, are long, tedious and therefore expensive. Others, such as some spectroscopic analysis (e.g. NMR, isotopic analysis), require sophisticated equipment which are only available in a limited number of laboratories.

The challenge for the future is still the identification and detection of adulteration, but at lower levels of impurity, and by simpler routine methods. In global terms, the analysts predict that the greatest increase in vegetable oil consumption will take place in South East Asia and South America. North America, Australia and Japan will experience a moderate increase in the demand for vegetable oils mostly as a result of health concerns. Thus, abrupt changes in demand may also bring some new chances for adulteration that will need to be controlled with coordinated and efficient tools that combine analytical enhancement and data management. Since vegetable oils are essential in the human diet and they form part of many food formulations, they cannot be omitted or substituted by other ingredients. Thus, any problem of fraud is magnified and can have a significant impact on health and consumer concern. Therefore, an oriented strategy on vegetable oils authentication is always necessary and it should be on the table of food safety authorities, without forgetting the news on food frauds reported on media (https://ec.europa.eu/jrc/en/food-fraud-and-quality/monthly-summary-articles).

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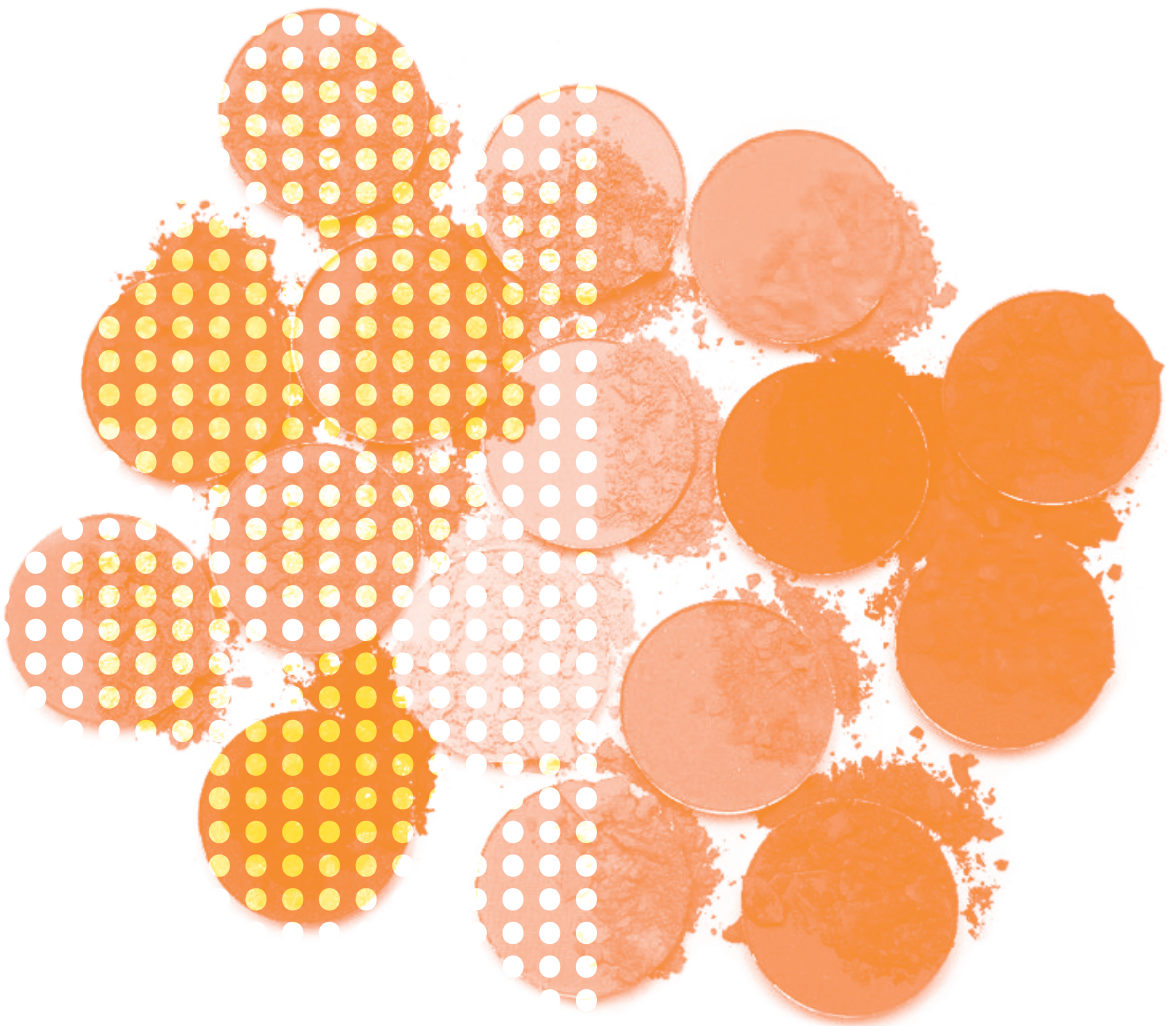
Food Additives



Food flavourings

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General overview of the product

The roots of the flavourings industry date back to early Egyptian history, when simple methods for the distillation and extraction of essentials oils and resins were first elaborated. In medieval times, monks pioneered the production of food flavourings. Then at the beginning of the 19th century, the industrial production of essential oil started, quickly followed by the first syntheses of single aroma chemicals such as vanillin. In the 20th century, the flavour industry then expanded from Europe to the USA. Today's leading companies are usually those early European ones that are now based around the world [1].

The total flavour and fragrance market, valued at USD 9.6 billion in 1995, has nearly doubled in the ensuing decade, with the global share of food flavouring being approximately 50 % [1].

While the overall consumption of food flavourings has been growing over the last 100 years, consumer demand has changed dramatically since the 1970s, moving towards more "natural", "fresh", and "functional" ingredients.

There have also been changes in processing with a major trend towards the use of biotechnology (fermentation) and "soft chemistry" processes which aim to combine "naturalness" with economic value, as the traditional processes are too expensive and artificial flavours are less attractive to consumers.

Establishing analytical criteria to control naturalness is therefore of the utmost importance to ensure fair trade in food flavourings. Quality Assurance managers in the food industry should also be aware of the definition, regulations and analytical methods that are used to monitor the authenticity of these very specific and high value ingredients.

1. Product Identity

1.1. Definition of the product and manufacturing process

The three main categories of flavourings are:

- Essential oils and natural extracts are obtained from natural sources such as flowers, fruits, etc. The processes used included solvent extraction, steam distillation, etc.

https://doi.org/10.32741/fihb.20.flavourings

- Aroma chemicals are single compounds, either produced by chemical synthesis or biotechnology processes.
- Formulated flavours are complex blends of aromatic materials.

1.2. Current standards of identity or related legislation

1.2.1. European Union (EU) regulations

The EU Regulation 1334/2008 [2] defines flavourings as "products: (i) not intended to be consumed as such, which are added to food in order to impart or modify odour and/or taste." Their use is only permitted provided that "they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer; and their use does not mislead the consumer".

The EU regulation then differentiates several types of flavouring types and components. A simplified version of the definitions is given below:

- "'**flavouring substance**' shall mean a defined chemical substance with flavouring properties": these are pure chemical compounds, which can be produced from any source by any process".
- "'**natural flavouring substance**' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II": these correspond to substances that are "naturally present and have been identified in nature".
- "'**flavouring preparation**' shall mean a product, other than a flavouring substance, obtained from: (i) food […] and/or (ii) material of vegetable, animal or microbiological origin, other than food […] and/or (ii) material of vegetable, animal or microbiological origin, other than food". These are usually mixtures of many compounds, either "taken as such" or "prepared by one or more of the traditional food preparation processes listed in Annex II".
- "'**thermal process flavouring**' shall mean a product obtained after heat treatment from a mixture of ingredients not necessarily having flavouring properties themselves, of which at least one contains nitrogen (amino) and another is a reducing sugar; the ingredients for the production of thermal process flavourings may be: food; and/or (ii) source material other than food". This is typically caramel!
- "'**smoke flavouring**' shall mean a product obtained by fractionation and purification of a condensed smoke yielding primary smoke condensates, primary tar fractions and/or derived smoke flavourings[…]".
- "'**flavour precursor**' shall mean a product, not necessarily having flavouring properties itself, intentionally added to food for the sole purpose of producing flavour by breaking down or reacting with other components during food processing; it may be obtained from: (i) food; and/or (ii) source material other than food".

The above mentioned 'appropriate physical process' "shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve, inter alia, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation."

Besides Europe, national regulations exist in other parts of the world, but they will not be covered here. A complete review can be found in [1].

1.2.2. IOFI guidelines

IOFI is the International Organisation of the Flavour Industry, based in Geneva, Switzerland. The IOFI Code of practice [3] provides additional comments and interpretation of the EU regulation. In particular it includes guidelines on the IOFI Interpretation of the Term "Natural".

1.2.3. Fruit juice directive

The EU fruit juice directive 2012/12 [4] is the only case of a vertical directive for food including specific requirements for the flavour component of the juice. Above all, it requires the use of "suitable processes, which maintain the essential physical, chemical, organoleptic and nutritional characteristics of an average type of juice of the fruit from which it comes." The flavour lost during processing may be restored. But in any case the flavour profile must not deviate from typical compositions.

Interpretation therefore requires expertise and reference knowledge regarding typical values found in juices, for all types of fruits and processes used by this industry.

2. Authenticity issues

2.1. Identification of current authenticity issues

Thanks to Mother Nature's gifts on one hand, and human creativity on the other hand, a wide range of natural sources and processes can be used to produce food flavourings, which are usually complex mixtures of chemical compounds. Many organic compounds have a flavouring impact, which is not correlated to their concentration. What makes it even more subtle is that the final sensory impact depends on all flavour compounds present, their proportions, and even the effect of the food matrix itself on the perception. Synthetic mixtures may smell as beautiful and natural as extracts, and conversely some natural sources may produce poor quality aromas. Therefore using one's nose is not sufficient for judging the authenticity of an aroma.

Natural flavourings are among high risk ingredients regarding economic food fraud, because of their high price and the availability of cheaper substitutes. The most commonly encountered fraud is the addition of synthetic compounds which are chemically identical to the main component(s) of a given natural flavouring. Typical examples are the addition of synthetic vanillin or para-hydroxybenzaldehyde to vanilla extracts / aromas, or the addition of synthetic benzaldehyde to bitter almond oil.

2.2. Potential threat to public health

Some flavouring substances may have negative health impacts: some have been classified as allergens, and must be labelled on perfumes and cosmetic products, and some others have been defined as “biologically active compounds”, for which a maximum concentration threshold is defined in the Annexe III of the EC Regulation 1334/2008 [2]. Typical examples are pulegone and menthofuran in mint products (confectionery, drinks).

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Application of GC-MS methods

Due to the volatile nature of flavour components, gas chromatography (GC) is the most common way of isolating them. The most widely used type of detector is Mass Spectrometry (MS), which enables each compound to be precisely identified.

A first approach is to screen all or almost all compounds present, identify and quantify them, in order to compare the obtained flavour profile with reference data, which may be a database or literature data. This can reveal the use of source material other than the ones declared, or the presence of artificial compounds.

Direct GC-MS analysis can be performed directly on matrices such as essential oils and aroma. On the other hand, the analysis of flavoured food products usually requires a prior step of extracting the volatile fraction.

3.1.1.1. Common extraction methods

Simultaneous Distillation Extraction (SDE) is the technique used in the German official method, and used in most expert laboratories, especially for testing fruit juices and fruit products. The main advantage is that this technique enables the extraction of a wide range of compounds, thus covering the main aroma compounds of fruit flavours: esters, lactones, alcohols, aldehydes, etc. It is also applicable to mint flavours, either in drinks or in confectionery, and even works for fruit-flavoured dairy products.

For matrices containing a significant amount of alcohol (wines, spirits, liquors, alcohol-based flavours), the above approach is no longer applicable, and a liquid-liquid extraction usually offers a better recovery of the compounds of interest.

Modern instrumentation enables an on-line extraction, followed by direct injection to the GC-MS instrument. This allows for a better productivity in the laboratory, but should be handled with care to avoid losing information or getting some artefacts. The main ones are:

- Headspace injection systems: various systems exist to introduce the headspace gas above a sample into the chromatograph.
- Solid-Phase Micro Extraction (SPME): the compounds of interest are adsorbed on a fibre coated with a specific material, and successively desorbed in the GC injector.
- Stir-Bar Sorptive Extraction (SBSE): the compounds of interest are adsorbed on a stir bar with a specific material, and successively desorbed in the GC injector.

3.1.1.2. Flavour profiles

In full scan mode, GC-MS chromatograms usually show around one hundred identifiable and quantifiable compounds. Most of them can be identified through the NIST MS database. An accurate quantification then requires determining individual response factors of each compound, taking into account both the extraction rate and the chromatographic response.

Absolute concentration values are not essential for the authenticity assessment. The presence of foreign molecules such as undeclared solvents or artificial / untypical components is the first type of deviation to be considered. However, the relative proportions and orders of magnitude must also match with the product type: unbalanced proportions tend to indicate the use of specific compounds instead of full extracts. Some guidelines have been published regarding key-components, for example in apple juices [5,6].

Finally some regulatory limits are defined for biologically active compounds. When judging the compliance regarding those thresholds, the analytical uncertainty should be taken into account.

3.1.1.3. Chiral analysis

Using the enantioselectivity of chiral chromatography stationary phases, the R and S spatial conformations (i.e. enantiomers) of chiral molecules can be appropriately separated and quantified.

Most industrial chemical synthesis processes use mineral catalysers, leading to racemic mixtures of R and S forms. Some fine chemistry methods might also produce pure enantiomers. On the other hand, natural biosynthesis in the plants implies enzymatic catalysers, which usually lead to an enantiomeric excess of one of the forms R or S, depending on the compound and the plant. Therefore chiral analysis is an effective means of detecting synthetically produced compounds, based on appropriate reference knowledge about enantiomeric distributions.

This also requires expert interpretation, as some natural compounds may undergo natural racemisation processes.

3.1.2. Application of carbon 14 activity measurement

Due to their age, fossil organic molecules do not possess any carbon 14 activity. On the other hand the activity of natural molecules reflects the current ^{14}C activity of CO_2 in the atmosphere. Measuring ^{14}C activity therefore can be used to detect compounds made partly or totally from fossil precursors [1]. This can be applied to pure compounds or mixtures containing some major compounds, such as some essential oils.

Nevertheless it should be emphasised that synthetic compounds produced from natural precursors are not detected by ^{14}C activity measurements.

3.1.3. Application of stable isotope ratio analysis

3.1.3.1. Isotope Ratio Mass Spectrometry (IRMS)

The global isotopic ratios of stable isotopes of carbon, hydrogen, oxygen, nitrogen or sulphur can be used, usually discriminating between extractive, synthetic and biosynthetic sources.

When coupled to gas chromatography (GC), such measurements can be applied to individual compounds from a mixture, as recently reviewed for aromas in general and essential oils in particular [7]. A typical example is the analysis of the ^{13}C deviation of vanillin, to check a declared

vanilla beans origin. Not only can this method be applied to flavouring ingredients such as beans, extracts, and aromas, but also to finished products such as vanilla-flavoured ice-cream, cakes, etc.

After a solvent extraction, applied to the vanilla-flavoured food or ingredient, the volatile components are separated by GC and the peak of vanillin is selectively submitted to combustion and $^{13}C/^{12}C$ isotopic ratio measurement. Since the ^{13}C deviation of agricultural vanillin is less negative than most of its artificial counterparts, it is possible to detect blending or substitution. However this approach is not sufficient for precisely identifying the artificial sources.

3.1.3.2. Site-Specific Natural Isotopic Fractionation – Nuclear Magnetic Resonance (SNIF-NMR)

This method introduced in the 1980s makes it possible to go one step further by determining isotopic ratios at the different positions within a molecule, thus providing more precise information [8]. Initially applied to deuterium, SNIF-NMR has been used to authenticate key-flavour molecules such as vanillin [9–11], benzaldehyde [12], anethole [13], raspberry ketone [14], etc.

More recently, suitable conditions have been developed for the quantitative NMR measurement of isotopic ratios of carbon, opening the path to new authentication possibilities [15–18]. Indeed the higher natural abundance of ^{13}C versus deuterium and the possibility to use polarisation transfer dramatically reduces the amount of pure compound required for the measurement, while keeping a satisfactory level of discrimination between sources [19].

3.2. Other commonly used methods

Liquid chromatography is the most suitable technique for analysing thermally instable or non-volatile compounds. A typical example is the use of limonin, a key compound causing bitterness in citrus drinks. This can also be used especially for “salty” aroma in which the impact compounds are usually less volatile.

Multi-dimensional chromatography using several columns in series can allow higher selectivity. They also make the analytical process more complex, so their use is usually restricted to specific cases.

4. Overview of methods for authenticity testing

Analytical technique	Indicative data or analyte	Authenticity issue / information
GC-MS (apolar phase)	Composition	Essential oils
SDE, GC-MS (polar phase)	Flavour profile	Fruit products, mint products
SDE, GC-MS (chiral phase)	Enantiomeric profile	Fruit products, mint products
Liquid Scintillation, Accelerator Mass Spectrometry	Carbon 14 activity	Flavouring substances
GC-Combustion-IRMS	^{13}C deviations (of isolated compound)	Vanilla products, Fruit products
GC-Pyrolysis-IRMS	^{2}H deviations (of isolated compound)	Vanilla products, Fruit products
IRMS	^{13}C, ^{2}H, ^{18}O, ^{15}N, ^{34}S deviations	Flavouring substances
^{2}H-SNIF-NMR	Site-specific D/H ratios	Flavouring substances
^{13}C-SNIF-NMR	Site-specific $^{13}C/^{12}C$ ratios	Flavouring substances

5. Conclusion

The world of food flavourings is extremely rich in terms of sources, compounds and sensorial impacts. Their high commercial value and scarce sources make them prone to economic adulteration risks. Climatic and political incidents might be aggravating factors to the fraud risk. The price increase of vanilla beans following the “Enawo” hurricane in Madagascar in 2017 can be taken as an example: the price, that had already increased over the last decades then suddenly doubled from USD 200 per kg to USD 425 per kg, causing a lot of trouble in the market. Similarly, unstable political situations can influence the risk level of supplies for many aroma sources.

When performing authenticity controls, the first question to ask is the precise definition of the flavouring being used, which leads to some expectations based on legal definitions. Then suitable analytical method(s) performed by laboratories having access to appropriate reference knowledge bases should be selected to check whether the composition of the aroma matches with these expectations.

In many cases also the choice of the appropriate method is governed by technical feasibility, and R&D work is still on-going to cover unsolved issues. The identification of precursors has made considerable progress thanks to the use of isotopic methods. One of the most difficult challenges remains the characterisation of processes used for manufacturing these high value ingredients, as a given precursor may be transformed into the final flavouring substance through different ways.

The large amount of information generated by the above-mentioned methods can be exploited in an optimal way using multivariate statistics. And finally, instead of considering only known signals, one can imagine to use the aroma screening as a non-targeted screening, which could enhance the possibility to detect unexpected manipulations of flavours.

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Determination of species origin of gelatine in foods

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General overview of the product

Gelatine is widely used in the food industry. With excellent gelling properties, it is used in a broad range of food products, including confectioneries, desserts and pies, often as a binder or to enhance texture. Gelatine is also an important product in the pharmaceutical, medical, cosmetic, adhesive and photographic industries. It is prepared from the partial hydrolysis of skin and bone material, usually of bovine or porcine source, and the correct labelling of foods regarding species origin is therefore important for those with religious or ethical preferences to avoid these species.

After starch, gelatine has the second greatest proportion of the global market share in terms of value, representing over a quarter of the market share for food hydrocolloids (Gelatine Manufacturers of Europe[1]). In 2008, approximately 326 000 tons of gelatine were produced. In a growing market, it is believed that around 400 000 tons of gelatine were consumed in 2017, according to Global Industry Analysts, in a market worth USD 1.77 billion [1]. Gelatine appeals to the food industry since it is often (depending on species and tissue source) free of colour, odour and taste and thus can be added to products without affecting perceived quality. As a by-product of the meat and fish industries, gelatine is often sold as a natural ingredient and may appeal to customers since it is sold as a sustainable by-product[2].

Most gelatine manufactured globally is of bovine or porcine origin although piscine and poultry gelatines are also available. Many consumers abstain from the consumption of bovine products (e.g. those adhering to Hinduism) or porcine products (e.g. those adhering to Islamic law seek Halal products and followers of Judaism seek Kosher products), or indeed any animal tissues (e.g. vegetarian and vegan consumers). Since porcine gelatine is cheaper than bovine gelatine, many producers prefer using this gelatine in their products for profit gain [2]. There have been instances of products being incorrectly labelled, either due to deliberate fraud or contamination with an alternative species of gelatine (e.g. [2] and UK Food Standards Agency, 2009). In terms of food authenticity and food integrity, it is therefore important that analytical methods are available to determine and verify the species origin(s) of any gelatine present in foods. The high levels of sequence homology between collagens of different species, particularly of bovine and porcine origins, present a significant challenge in distinguishing gelatine species origin.

[1] https://www.gelatine.org/gelatine/comparison-hydrocolloids.html

[2] https://www.gelatine.org/ and https://www.gelatine.org/gme/sustainability.html

https://doi.org/10.32741/fihb.21.gelatine

1. Product Identity

1.1. Definition of the product and manufacturing process

1.1.1. Gelatine composition and properties

Collagen is an abundant structural protein in animals. Prepared from the collagen of bone, skin and other connective tissue, collagen consists of three amino acid chains wound together as a triple helix, stabilised by interlinking bridges between adjacent collagen chains. It is a robust protein which survives high levels of processing, albeit in an altered state. Concerning collagen and gelatine composition, proline constitutes around 18 % of the amino acid composition of collagen and is often subject to hydroxylation during collagen synthesis. Asparaginyl and glutaminyl residues within collagen undergo deamidation during gelatine manufacture. Approximately one third of the amino acid composition of bovine and porcine collagen is glycine. In addition to collagen protein, gelatines also contain low levels of minerals and water.

Gelatine is often labelled on foods according to the animal of origin (usually beef, pork, fish or chicken). As discussed below, gelatine is a highly processed product prepared by the aggressive processing of collagen causing partial hydrolysis of the protein and degradation of the DNA. The final product, especially for bovine and porcine gelatines, is a powder or granules with no apparent indicator of animal origin. When gelatine is manufactured, the animal origin of each batch is denoted by a paper trail. Certificates are issued and used to distinguish bovine gelatine from porcine or indeed from any animal origin of gelatine. However, gelatine can potentially be prepared from the collagen of any animal species. This is especially true for bone material but less so for hide material, since hide cutting instruments are animal-specific due to the variations in hide thickness between species. There is therefore potential for unscrupulous manufacturers to adulterate such highly processed products leaving no visible trace of animal origin for purchasers to consider. There is also the chance of accidental contamination of one gelatine species with another with no visible indication.

Gelatines can differ in terms of their strength. The Bloom test (1925) determines the weight in grams needed by a specified plunger to depress the surface of the gel at a specified temperature 4 mm without breaking it [3]. The result is expressed in Bloom (grades), e.g. Bloom 50 or Bloom 325. The higher a Bloom value, the higher the melting and gelling points of a gel, and the shorter its gelling times. Bloom strength depends on a number of factors including the age of the starting material and the processing method used. The Bloom can therefore not be predicted accurately from the starting material at the factory but can be predicted with in a range. Once manufacture is complete, the final gelatine product requires testing in order to accurately determine the Bloom. The higher the Bloom, the higher the financial value of a gelatine. In general, the lower the extraction temperature, the higher the Bloom, although this is also influenced by other factors including pH and processing time.

1.1.2. Gelatine Manufacturing Process

Gelatines which are commercially available globally tend to be prepared from bone and hide of cows older than 18 months, calf hide, pig hide, chicken skin or feet and fish skin or swim bladder. The raw materials used to produce gelatines in Europe are shown in Figure 1. The process for the industrial preparation of each gelatine depends on the starting material as is summarised below. Gelatines are often prepared by incubation in an acid or alkali followed by high temperature extraction and sterilisation and many gelatine manufacturing plants handle only one type of

gelatine, although some plants handle more than one species. Since gelatine is prepared from bone or hide material, there is opportunity for unscrupulous manufacturers to prepare a batch of gelatine from any animal species if the raw materials were available and label it with an alternative species origin. This batch could then enter the food chain.

Approximately 80 % of the gelatine prepared in Europe is derived from porcine skins and 15 % from cattle split. The remaining 5 % is prepared from porcine and bovine bones and fish[3]. Globally, 46 % is prepared from porcine hide, 29.4 % from bovine split, 23.1 % from bones and 1.5 % from other sources including chicken [4]. There is also interest in increasing the amount of fish gelatine which is manufactured, due partly to its abundance and biodegradability [1,5].

The processes used to prepare gelatine in industry differ depending on the starting material and examples of the acid, alkali and enzymatic processes used to prepare gelatines are discussed below. The gelatine yield from the raw starting materials tends to be approximately 10 %.

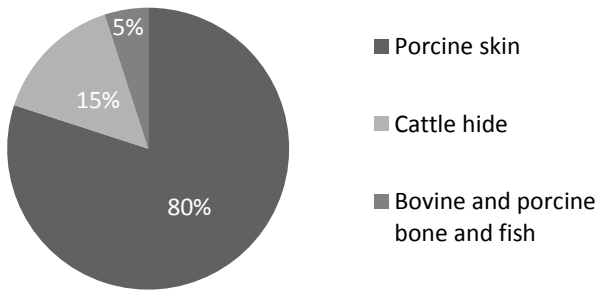

Figure 1. Production of edible gelatines in Europe, by raw material.
Information taken from http://www.gelatine.org/gelatine/manufacturing.html

1.1.2.1. Acid process

Pork hide, fish, poultry (chicken) and calf hide gelatines are produced by an acid incubation process which is quicker and less aggressive than alkali processes. Younger collagens (pig and calf) which have softer cross links to stabilise the helical collagen structure respond better to acid treatment which is a relatively gentle treatment of the cross links. The acid cleaves the protein and results in branched chains of protein. Gelatine prepared from hide is cheap and often used in food. Amongst other effects caused during this aggressive manufacturing process, incubation in acid is known to cause depurination degradation of DNA [6]. The manufacture of calf hide uses an acid process or acid followed by the alkali process.

Chicken skin and feet are used for gelatine preparation. Fish gelatine is prepared from the skin and swim bladders of farmed, warm-water fish since cold-water fish gelatine has poor quality with a very low melting temperature. Chicken and fish gelatines are prepared by incubation in acid at 5°C. Citric, lactic, acetic or phosphoric, or a blend of some or all of these acids, are used. The process then uses a filtration treatment to remove the oil before drying gently in drying tunnels.

[3] https://www.gelatine.org/gelatine/manufacturing.html

1.1.2.2. Alkali process

Bovine bone and hide of cows older than 18 months tend to be prepared by an alkali incubation process. The alkali process is a more time-intensive process than the acid process. The only bovine bone from which gelatine is prepared is the thigh bone as it has the correct sinew level required for gelatine preparation. The bone is demineralised prior to further treatment.

Cows tend to be 18 months old at slaughter and thus have more established cross links in the hide compared to pigs which are slaughtered at a younger age. Cow hide gelatine is prepared by the alkali process which is a relatively more aggressive process and results in longer, straighter chains. Material is soaked in lime, sometimes along with sodium hydroxide, calcium chloride and/or sodium sulphide over an incubation period of several months with an aim of yielding only pure gelatine which is used by the pharmaceutical, plasma replacement and photographic film industries.

1.1.2.3. Enzymatic production

Enzymatic production of gelatine is cheaper than acid or alkali treatments. Enzymes such as Alcalase® and Neutrase® are used followed by incubation in lime which is used as a preservative. Enzymatic methods involve reduced levels of processing and tend to result in gelatines of a darker colour which command a lower financial value.

1.2. Current standards of identity or related legislation

Processed foods and other consumer products require accurate labelling according to the species they contain to enable consumers to make informed decisions about the food they buy. The commercial pressure for suppliers to provide gelatines of known species is driven largely by due diligence. Issues surrounding the discovery of horse meat in beef products in Europe in 2013 highlighted consumer interest in food labelling and authenticity and the subsequent Elliott Review (2014) recommends that managing the food supply chain must involve more than maintaining a paper trail [7]. Given the above, there are benefits in developing a reliable test method to aid with labelling in terms of the species origin of added gelatine.

Gelatine falls under the European Commission's scope of 'other products of animal origin.' Therefore the rules governing both imports and intra-community trade of other products of animal origin for human consumption, laid down in Council Directive 2002/99/EC, apply to gelatine. This Directive, which has been amended several times, harmonises the rules and establishes the animal and public health rules for the import and trade in the Community for animal products where specific Community rules have not been laid down elsewhere. The EU Health and Consumer Protection Directorate publish opinions regarding the use of gelatine in food, feed, cosmetics, pharmaceutical and medical products with respect to risks from Transmissible Spongiform Encephalopathies (TSEs).

The Gelatine Manufacturers of Europe (GME) is an organization involving gelatine and collagen peptide manufacturers in Europe since 1974. According to its mission and objectives, GME's primary focus is on setting the highest standards for quality, safety and sustainability amongst its member companies[4].

[4] https://www.gelatine.org/gme/mission-and-objectives.html

There is no legislation requiring that the animal origin of gelatines is included on food labels, but many suppliers choose to include this information to better-inform consumers. A paper trail is the method used to determine the origin of batches of gelatine. Supplier premises may be inspected by accreditation and certification bodies, including Halal food certification bodies.

2. Authenticity issues

2.1. Identification of current authenticity issues

Regarding authenticity issues, as previously mentioned, due to the high levels of processing involved in the preparation of gelatines, resulting in gelatine granules or powders with little or no apparent indicators as to origin, there is potential for deliberate adulteration or accidental contamination to occur using raw material from animals other than those on the product label. Since many consumers choose to abstain from certain species due to religious rules or ethical preferences it is important that methods are available to ensure correct labelling.

Since the physical appearance of gelatines provides little or no significant indicator of animal origin, it is perceivable that batches of gelatine can be mislabelled or mixed, either fraudulently or accidentally. Also, certain gelatine manufacturers use the same factory establishments to process gelatine of different origins and therefore mixing of species can occur at the point of manufacture.

The widespread adulteration of processed beef products with horse in 2013 highlighted that species-related fraud is present in the food chain. The subsequent Elliott Review (2014) into the integrity and assurance of food supply networks investigated, amongst other aspects, the ‘causes of the systemic failure that enabled the horsemeat fraud’. Further highlighting the issue in terms of gelatine adulteration, highly processed gelatine (hydrolysed collagen) of bovine origin has been found by the UK Food Standards Agency as a plumping agent in chicken breasts labelled as containing chicken only (UK Food Standards Agency, 2009). Therefore, methods to determine species origin of processed products such as gelatine would support the food chain and consumers by aiding policing against known potential threats. Further, given the religious and ethical sensitivities regarding the species origin of gelatine, it is important that analytical methods are available to authenticate the animal origin of gelatines in foods and capsules. While gelatine manufacturers are audited to support the species authenticity of gelatine, there is still opportunity for the accidental and deliberate mislabelling, particularly since porcine gelatine is cheaper in terms of cost than bovine gelatine [2]. Analytical methods which can determine the presence of an adulterating gelatine present at low levels when mixed with an alternative gelatine are required with a high level of sensitivity to support food integrity.

2.2. Potential threat to public health

Following the incidence of Bovine Spongiform Encephalopathy (BSE) in cattle in the EU in 1986, strict restrictions were imposed regarding the food use of certain animal tissues where the BSE-inducing prions can be present at high levels. These regulations are slowly being relaxed in terms of which tissues can enter the animal and human food chains based on on-going risk assessments in the light of other controls that are now in place.

Due to concerns linked to BSE in 1997, the TSE Advisory Body, in collaboration with the US Food and Drug Administration, began monitoring the potential risk of transmitting BSE. The disease was mainly associated with consumption of tissue of the nervous system including skull, brain and vertebrae. It was recognized that the heat, alkali and filtration treatments used during gelatine manufacture could be effective in reducing the level of contaminating transmissible spongiform encephalopathies. In 2002, the Scientific Steering Committee of the European Union (SSC) stated that the risk associated with bovine gelatine is very low or zero and, in 2004, a team determined that the acid and alkali processes of gelatine manufacture from bovine bone reduce infectivity to undetectable levels [8].

Regarding alternative threats to health, from a nutritional point of view, although gelatine is composed of around 98 % protein (dry weight), it does not contain all essential amino acids and therefore must be consumed only as part of a balanced diet.

3. Analytical methods used to test for authenticity

While there are no officially recognised methods, researchers in the past have investigated technologies to determine the species origin of gelatine. As discussed above, due to the high levels of homology in structure, properties and amino acid sequence between bovine and porcine gelatines, conventional physicochemical methods cannot be applied.

Gelatines are manufactured by an aggressive processing causing partial hydrolysis of the collagen. As detailed above, the raw material is incubated either in acid or in alkali, followed by extraction at high temperature, filtration and further high temperature sterilization. Under such conditions, most of the DNA is denatured and some protein molecules show signs of denaturation. Conventional methods used in species determination of foods such as Polymerase Chain (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), which rely on high quality DNA and protein respectively, are not generally applicable. Indeed, ELISA assays designed for species determination of processed meat, such as the Biokits Cooked Species Identification Test Kit (Neogen Europe Limited) carry notices confirming that they are not necessarily applicable to gelatine determination.

Since bovine and porcine gelatines share around 95 % amino acid sequence homology [9], and much homology in structural and physicochemical properties, they are difficult to differentiate by conventional physicochemical methods such as calcium phosphate precipitation [10] and HPLC [11]. Further, methods such as Fourier-transform infrared spectroscopy (FTIR), one-dimensional and two-dimensional gel profiling have shown low sensitivity and cannot be used to determine gelatines or products containing a mixture of species [12,13].

Similarly, conventional PCR can often not be applied due to high levels of denaturation of the DNA [14]. In a study investigating the gelatine origin of 36 foods, in only twelve of the foods was any DNA detected [15]. There has been some success regarding the ability to apply PCR to determine gelatine species origin with one team reporting the ability of PCR targeting the Mitochondrial Cytochrome b gene to differentiate bovine and porcine DNA at the 0.1 % level [12] while others gained false negative results when evaluating their Real-Time PCR methods [16]. Recent work comparing real-time PCR with liquid chromatography mass spectrometry methods demonstrated that the PCR technique could not always be applied to correctly assign gelatine species of origin in all samples due to no traces of DNA remaining in some products. Therefore, while a small amount of success has been reported for the DNA-based methods investigated by the scientific

community, alternative methodology capable of accurate, specific and precise determination with high sensitivity across a wide range of food types, which also addresses false positive and false negative issues in gelatine species determination, is required.

There are emerging technologies which focus on the screening of collagen peptides present in a food sample. Peptides are often more robust to degradation compared to DNA and whole proteins which become fragmented and denatured during manufacture. The peptide complement tends to be comparatively intact for the food material. Peptide mass spectrometry can be used to determine species-specific peptides. Matrix Assisted Laser Desorption Ionisation Time-of-flight Mass spectrometry (MALDI-ToF MS) has been used to compare and contrast the collagen peptide fingerprint of species. Indeed, this technology has been used in research to determine species markers in ancient archaeological collagen samples, so robust is the collagen protein [17].

In terms of using MALDI-ToF technology to support food integrity, issues regarding sensitivity have been reported. Pork gelatine could only be determined in bovine gelatine when present at 20 % (w/w) [18] whereas significantly greater sensitivity is required in the food chain with the UK Food Standards Agency requiring sensitivity at the 1 % (w/w) level. The early stages of work have also been carried out using low resolution liquid chromatography mass spectrometry (LC-MS) instruments using multivariate analysis to compare the mass spectral data for bovine, porcine and fish gelatine with success on a limited number of samples and sample types tested to date [2]. However, development of methods with integrated confirmatory techniques, such as tandem mass spectrometry (MS/MS) would prove valuable as a single test to determine origin.

One successful emerging technology, in the form of high-resolution accurate mass liquid chromatography mass spectrometry (HR LC-MS/MS), can be applied to compare and contrast samples of high sequence homology to determine species differences. The peptides in a food sample are separated by nano-flow liquid chromatography. Each peptide is fragmented and then further fragmented in the mass spectrometer and the accurate mass of each fragment ion is measured. Algorithms are used to determine the amino acid composition of each peptide and to ultimately determine species origin, screening for marker peptide.

A full scan (untargeted) high resolution, high accuracy mass spectrometry (HR LC-MS/MS) method is available for the qualitative determination of the animal origin of gelatine extracted from foods [19]. Despite the high levels of collagen amino acid sequence homology between bovine and porcine gelatine, the method can differentiate a wide range of species using a suite of peptides in a proprietary database which contains species-marker peptides to differentiate not only bovine and porcine, but also species including equine, ovine, piscine and poultry gelatines amongst others [20]. In this work, a library of collagen sequences was prepared using molecular mining of Expressed Sequence Tags (EST) and other databases, coupled with *de novo* sequencing from thirty-two different mammalian species, identified peptides which can be used as species markers in gelatine. Species identity of these peptides was verified by mapping the phylogeny of the peptides [20]. The quality and verification of the database is critical: unlike for other proteins, so aggressive is the gelatine manufacturing process, the modifications caused to the collagen protein during processing cannot necessarily be correctly predicted by conventional proteomics software and database packages in order to build a database. It is critical that the species specificity of marker peptides is independently verified by testing a wide range of same-species collagens so that potentially incorrect sequences are not attributed to species specificity [20]. This HR LC-MS/MS method has been evaluated on a range of foods. Based on the threshold applied by the UK Food Standards Agency to the adulteration of processed meats during the horse meat issues of 2013 which required detection of adulterant at 1 % (w/w), this method was evaluated on a range of

gelatine-rich foods containing an adulterating gelatine at levels of 0.5 % (w/w). Both the accuracy and the precision of the method were 100 % and the maximum specificity was also demonstrated ([19], unpublished data). The tissue origin (bone or skin) of the gelatine can also be determined by this method. Another benefit of untargeted methods is that all the peptide data from a sample can be archived and interrogated at a later date should the need arise in the future to investigate the presence of a new species of interest once the peptide sequence data for that species is available. HR LC-MS/MS methods benefit from confirmatory techniques also, by analysing the fragmentation patterns of the peptides to verify correct marker peptide, and thus species, assignment. This way, matrix interferences can be ruled out meaning the false positive rate of the method is not an issue which is another benefit over techniques such as ELISA or PCR.

Some progress towards developing a quantitative HR LC-MS/MS method has been made by developing internal standards by oxygen-18 labelling of gelatine marker peptides. The incorporation of stable isotopes into peptides results in a fixed mass shift with no effect on the chemical properties of the peptides. Therefore, the relative abundances of the labelled peptides from different samples can be accurately quantified using HR LC-MS/MS [9]. The method was developed on pure gelatines and its future application in the food industry relies on developing an extraction method and accurate measuring method prior to the analysis by HR LC-MS/MS.

Although showing excellent capability to determine the species origin of gelatines, high resolution accurate mass spectrometry methods require very high initial investment, highly trained personnel and elevated instrument upkeep costs. These instruments tend to be used more for research discovery purposes than for the routine analyses which tend to be required to support the food chain in terms of screening for adulteration. In the future, it is likely that more and more targeted methods will be developed from the data generated by these HR LC-MS/MS research instruments to screen for a pre-selected target list of species-specific marker peptides in gelatine food extracts. Such targeted methods, by Selected Reaction Monitoring mass spectrometry (SRM) are relatively low cost and are already used routinely to screen for other contaminants in the food chain including veterinary drugs, pesticide residues, mycotoxins, natural toxins, processing contaminants including acrylamide and materials which migrate into food from containers and packaging materials.

A recent evaluation of a targeted SRM mass spectrometry method tested forty-eight food samples simulating commercial food products and food supplement capsules containing bovine and porcine gelatine mixtures, alongside relevant positive and negative quality control samples. The foods were analysed in two ways: to determine the origin of the adulterating gelatine (a) when present at 1 % of the total mass of the food matrix and (b) when present at 1 % of the total mass of gelatine within the food matrix. The adulterating gelatine was present at as little as 0.07 % of the total food sample mass, depending on the food matrix type. The method showed 100 % accuracy and precision across all samples and the specificity of the method was also of the highest level, screening for fourteen bovine- and eight porcine-specific markers (Project FA0165, Department for Environment, Food and Rural Affairs, 2018[5]). Given that the EU Commissioning Body advised that the threshold for 'deliberate adulteration' of an undeclared meat product is 1 % w/w, this method is well within this tolerance. A further benefit of this form of technology is that there is also evidence that targeted mass spectrometry methods such as this one could offer a greater dynamic range than HR LC-MS/MS methods for quantification of peptides [21]. This is an aspect worthy of future investigation in relation to gelatine peptides in order to inform as to whether deliberate adulteration or accidental low concentration contamination may have taken

[5] Pending publication, https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs

place. SRM methods offer the benefits of screening for a wide range of known species peptide markers, screening for the precursor ion of each peptide and, critically, for each of its four confirmatory product ions, which must all be identified in a product to provide consumers and producers alike the confidence that the results are correct and not due for example to matrix interferences.

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Analytical technique	Indicative data or analyte	Authenticity issue / information
Polymerase Chain Reaction (PCR)	e.g. Mitochondrial cytochrome b gene target	High levels of DNA degradation contribute to the lack of methods to determine the species origin of gelatine in foods.
High Resolution Accurate Mass Spectrometry	Determination of suite of marker peptides for a wide range of species by untargeted analysis.	Relevant to single species or mixtures of gelatines extracted from foods, sensitive to 0.5 % or lower, can screen for a wide range of species in a single analysis. Potential to develop quantitative analysis of food extracts. High financial investment required.
Selected Reaction Monitoring (SRM) Mass Spectrometry	Targeted analysis for a suite of species marker peptides.	Determination of species from a target list of marker peptides. Relevant to single species or mixtures of gelatine extracted from foods, sensitive to 0.07 % depending on matrix. Potential to develop quantitative methods. Suitable for routine, high throughput screening of foods.

5. Conclusion

As markets fluctuate regarding livestock in the food chain, the possibility exists for the carcasses of any animal species, for example horse, to be used to produce gelatine for financial gain.

There is evidence that adulteration of gelatine is occurring in the food chain and indeed the UK Food Standards Agency discovered fraudulent use of hydrolysed collagen in plumping agents added to chicken fillets in 2009. Research work has also discovered mislabelling of gelatines in food products [2]. Methods are required to distinguish gelatines from different species to support authenticity and integrity in the food chain and also to inform consumer choice to support ethical and religious preferences. Gelatine is a highly processed product, manufactured under conditions of high temperature and long-term exposure to acid or alkali. These conditions cause denaturation of DNA and protein structure and therefore conventional animal origin determination methods, such as PCR techniques and ELISA, cannot be applied.

Mass spectrometry methods are emerging for the determination of species origin of gelatine. Full scan (untargeted) technologies, coupled to strictly curated and independently verified databases, offer the capability to screen for a range of species in a single qualitative analysis and therefore the opportunity to uncover unexpected issues in the food chain during routine analysis. The potential now also exists to develop these methods to allow quantitation. The importance of such food

screening capabilities was highlighted by the unexpected discovery of fraudulently-added horse meat in processed foods in 2013. Furthermore, untargeted methods allow data to be archived and re-interrogated should the need arise in the future to investigate the presence of a new species of interest.

While HR LC-MS/MS methods require high initial investment and expert data interpretation, the alternative routine and higher throughput technology of SRM mass spectrometry can be applied to determine species in a targeted method to determine species from a pre-determined list of marker peptides. This technology has been tested both on samples when the adulterating gelatine was present at 1 % of the total mass of the food and when the adulterating gelatine was present at 1 % of the total gelatine content of the food. The method has also been shown to be sensitive to an adulterating gelatine present at less than 0.1 % (less than 0.1 % mass of the total mass of the food sample). The method offers excellent potential for quantitative analysis in the future to further support the food chain in terms of product adulteration. Finally, MS/MS methods offer confirmatory data to ensure correct species identification and to overcome false positive results caused by matrix interferences.

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Additional tools for mitigating the risk of food fraud

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General overview

The primary incentive for carrying out food adulteration and other fraudulent practices is economic and a desire by the dishonest producer or distributor to make money by passing off inferior product as one of a higher value. Unlike food defence where tampering of food is carried out with the aim of harming a company, its employees and even the consumer, the intention of the food fraudster is not directly to cause a public health threat, although in some cases this may be an indirect consequence.

There are a large number of potential types of fraud as described in the introduction to this book. However, they have one aspect in common: their unpredictable nature. This differentiates food fraud from food safety concerns, where contamination is often unintentional and can be linked to a specific source (microbiological contamination in food, excessive use of pesticide residues, mycotoxin production during storage, and so on). Food safety has been the main focus of the food industry over several decades leading to the globally used HACCP (Hazard Analysis and Critical Control Points) approach, a documented food safety system to identify and control biological, physical and chemical hazards in food production. Food fraud on the other hand can occur outside the company's processing and distribution system, and therefore outside the scope of the its food safety management plan.

https://doi.org/10.32741/fihb.22.riskmitigation

There is growing awareness in the food sector for the need for a preventive approach to mitigate the risk of food fraud. Whilst analytical methods such as those described in this handbook play an important role in detecting adulteration, they are not the only solution to preventing food fraud and sometimes provide no solution at all. A more efficient approach is to look at the entire value chain and identify not risks but vulnerabilities in the supply chain and of the product itself. This means taking into account various aspects of the whole chain:

Figure 1: Main aspects to be considered in a comprehensive supply chain analysis

As shown in the above figure, a comprehensive strategy of food fraud mitigation requires placing the food product or ingredient in an all-round context which includes taking into account previous food fraud occurrences, where the product has been sourced from, the complexity of the supply chain involved and the adequacy of traceability within the chain.

Various approaches dealing with all or part of such a strategy have been documented and are available either as guidelines or as specific vulnerability tools. Details of these are given in Section 2 below.

The area of fruit juice fraud has been addressed over the last 30 years and a sophisticated and global approach to controlling this sector has been set in place by the industry itself. Section 3 of this chapter describes the SGF Product Control system, an excellent example of incorporating supply chain monitoring and appropriated analytical testing on an international scale.

Supply chain traceability is an essential part of the overall strategy to mitigate the risk of food fraud. Considerable technological progress has been made in this area. A description of the concept of traceability and the latest tools is given Section 4 of this chapter.

1. Different approaches available to evaluate vulnerability

1.1. General description

Over the past few decades, food safety and quality has greatly improved in the food and food ingredient sectors. Among the main driving forces for this improvement have been the various food safety standards that have provided food operators with a framework for managing product safety throughout their entire manufacturing process. There are currently several food safety management scheme owners, now known as food safety Certification Programme Owners (CPOs), available internationally, all of them recognised by leading retailers and manufacturers worldwide. Examples of CPOs include IFS (International Featured Standards), BRC (British Retail Consortium) Global Standards, SQF (Safe Quality Food), GlobalG.A.P. (Good Agricultural Practices). A food operator can be certified compliant to one or more of these standards through regular audits carried out by a Certification Body (CB), itself authorised to conduct the audit through a formal agreement with the CPO.

Given the number of different schemes in place, many food operators have found themselves having to undergo multiple audits, each one associated with a different standard. Faced with this situation, leading food companies got together to see how they could help manage costs for food businesses by reducing duplication of audits whilst still continuing to provide safe food to consumers across the globe. In the early 2000s the Global Food Safety Initiative (GFSI) was created with the aim of harmonising standards across the global supply chain; its goal “once certified, recognised everywhere” [1]. This was achieved by establishing equivalency between the different CPOs through a set of clear benchmarking requirements that each CPO must include in their standard in order to obtain GFSI recognition.

GFSI’s primary mission is to provide safe food to consumers, and as such its main focus has been on reducing food safety risk. However, with a growing awareness that food fraud was on the increase and could have possible detrimental effects on public health, the GFSI took steps to include this concern in their remit.

In 2012, a ‘Food Fraud Think Tank’ [2] was set up with the support of GFSI, to explore how food fraud could be incorporated into existing CPOs. The work of the Think Tank gained further credence when, in early 2013, the horsemeat scandal hit the headlines. In 2014 GFSI published its position on “Mitigating the Public Health Risk of Food Fraud” where it accepted the Think Tank’s recommendations to include two key elements as part of its Benchmarking Requirements. These are:

1. Companies should perform a Food Fraud Vulnerability Assessment – in which information collected at specified points in the entire supply chain (supply chain mapping) is evaluated on the basis of the potential for food fraud
2. Companies should put in place a Food Fraud Control Plan – consisting of a set of mitigating measures including a monitoring and testing strategy, specifications management, supplier audits and anti-counterfeit technologies.

These recommendations have since cascaded down into the CPOs via the GFSI’s benchmarking process and published in 2017 (GFSI Benchmarking Requirements Version 7.1 [3]).

With these requirements now in place, food companies have been seeking help with implementing the Vulnerability Assessment required by the CPOs. There are now a number of tools available to help companies with this that have been developed either independently from or specifically in reply to the new GFSI requirements for food fraud mitigation. The two main tools that are freely available to food operators are the US Pharmacopeia (USP) Food Fraud Mitigation Guidance Document and SSAFE/PwC Vulnerability Assessment tool. These are described below.

It is worth noting that in all cases the tools that been developed are described as “living” or “dynamic” tools. Food fraud and associated vulnerabilities do not remain static but evolve over time, often influenced by changing environmental conditions, the opening up of new markets, fluctuating economic conditions, the appearance of new adulterants, and so on. It is therefore important that the vulnerability assessment process is carried out on a regular basis.

1.2. USP Food Fraud Mitigation Guidance

The United States Pharmacopeial Convention (USP) published a General Guidance on Food Ingredients as an Appendix to its Food Chemicals Codex. It was developed by the USP Expert Panel on Food Ingredients and Intentional Adulterants to help food companies set up a preventive management system for food fraud [4].

These USP guidelines for Food Fraud Mitigation Guidance (FFMG) are available as a document at www.foodfraud.org and provide a practical framework for companies to follow in order to identify areas throughout the supply chain where their business may be vulnerable to fraud. The document, which has been designed to be generally applicable to any type of food ingredient, describes both a vulnerability and an impact assessment set out in four main steps as shown in Figure 2.

In Step 1, the main factors that may be useful for identifying the susceptibility of a food ingredient to fraud are identified. These contributing factors may be either controllable by the food operator, and include the following:

- Supply chain and its complexity.
- The company’s relationship with its supplier and associated audit strategy. Does the audit specifically address anti-fraud measures?
- The frequency and type of analytical methods used to detect fraud and ensure compliance with specifications. Are the methods used able to detect known adulterants?

Other factors may be outside the user’s control such as:

- The fraud history of the ingredient in question. Has it been implicated in any recent, validated, reports?
- Geopolitical considerations linked to where the product is sourced from.
- Unexpected price fluctuations.

Each factor is then assessed on its contribution to vulnerability (low, medium-low, medium, medium-high, high) in order to build up a “contributing factors assessment matrix”. The USP FFMG document provides guidance on how to categorise each vulnerability factor using illustrative examples from food businesses, and references to where information can be sourced from.

Step 2 then identifies the impact that the food fraud event might have both on the food company and on its wider environment; the premise being that while all foods and food ingredients are possible targets of fraud, not all will impact either public health, consumer confidence or the company's economic situation.

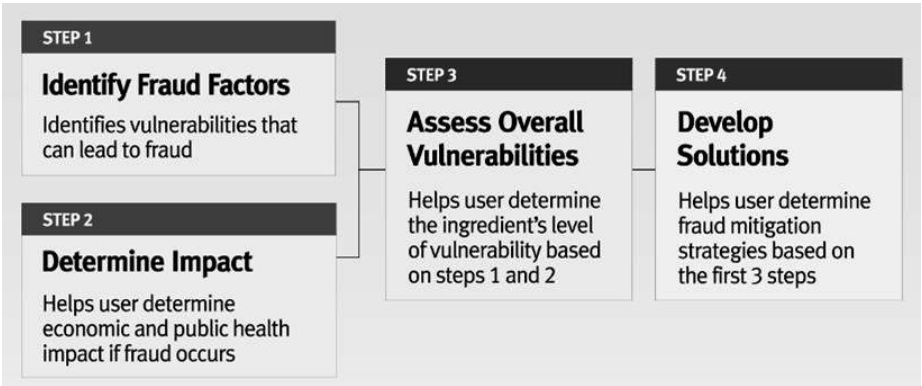

Figure 2: Four steps of the USP Food Fraud Mitigation Guidance Document.
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The results of steps 1 and 2 are then brought together in a "Vulnerability Characterization Matrix" (see Figure 3) to assess overall vulnerabilities and provide an indication of where further fraud mitigation measures are required (Step 4).

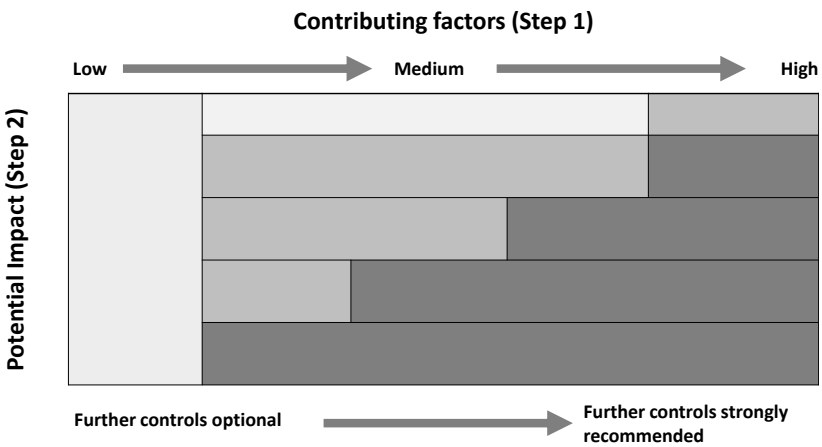

Figure 3: Vulnerability Characterization Matrix
(adapted from USP Food Fraud Mitigation Guidance Document, Food Chemicals Codex Appendix XVII 2016)

1.3. SSAFE / PwC Tool

The SSAFE/PwC vulnerability assessment tool was developed specifically to help companies implement the new GFSI requirements. SSAFE is a non-profit organisation with global food companies as members, and together with PwC (PriceWaterhouseCoopers) and in collaboration

with Wageningen UR and VU University Amsterdam they developed a science-based tool to assess a company's food fraud vulnerabilities. This is available as a free tool, to be used by food operators across the food supply chain, irrespective of size, geographical location or type of food business. It can be downloaded as an Excel file from www.ssafe-food.org or completed online by visiting www.pwc.com/foodfraud.

The SSAFE/PwC Tool has several components starting with a general information sheet in which the user can enter details of the company and the person or team responsible for filling in the questionnaire. It also provides a decision tree that can be used as a pre-filter to help prioritize where the tool should be applied. Its main part is a set of fifty assessment questions structured in two dimensions.

The first dimension explores those elements linked to potential criminal behaviour:

- **Opportunities**: these include the potential for fraud such as the type of product or process and previous fraud history, and the nature of the supply chain.
- **Motivations**: these relate to organizational aspects such as the business culture of the company, its economic situation and that of its customers and suppliers, and any evidence of previous offenses.
- **Control measures**: these include mitigation and contingency control measures, with questions on whether internal or external controls are in place, and whether these are hard or soft controls.

The user provides answers to the different questions by assessing their associated risk levels (low, moderate, and high).

The second dimension brings into play the company and its external environment, such as its suppliers, customers, and supply chain. How these two dimensions the key elements link together is shown in Figure 4.

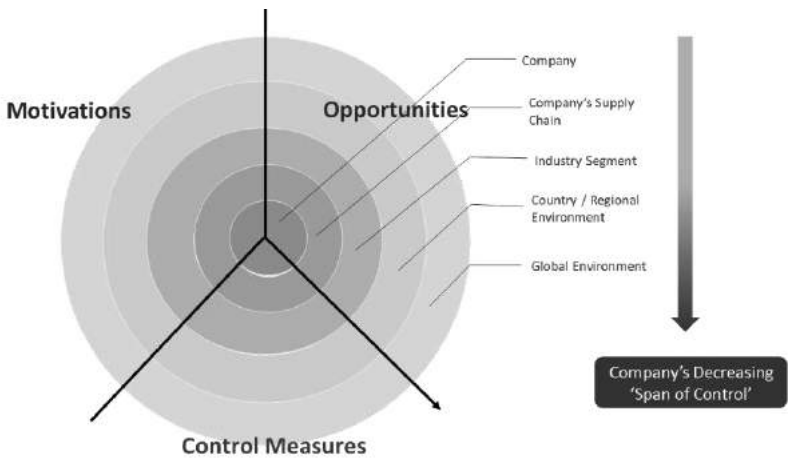

Figure 4: The SSAFE VA tool showing the environment of the company and the three elements of food fraud
Sourced from: Introduction to SSAFE Food Fraud Vulnerability Assessment Tool, December 2015

Once the questionnaire is complete, the tool provides a set of spider webs giving both an overview and a detailed assessment of the findings. Although it does not provide specific recommendations

for mitigation techniques, an overall final report does identify certain areas of vulnerability and this can point the company in the right direction to address the potential risks.

Documentation accompanying the tool vulnerability assessment tool also has a full list for further reading, providing references to other tools and to external sources where more information can be found.

1.4. Other approaches

Other tools or approaches to food fraud vulnerability assessments are described briefly below.

EMAlert™ – Economically Motivated Adulteration Vulnerability Assessment Tool

EMAlert™ is a software tool developed by Battelle in partnership with GMA (association of Food, Beverage and Consumer Products Companies). It can be accessed at www.EMAlert.org. As an interactive tool which is continuously updated, the software provides a company with a quantitative assessment of its vulnerabilities to food fraud in its specific commodity sector. It works on a subscription basis [5].

FDF Food Authenticity Guide – Five Steps to Help Protect Your Business from Food Fraud

This simple guide was developed by the Food and Drink Federation (FDF) in the UK, primarily with the interests of small and medium food business in mind [6]. It can be accessed at www.fdf.org.uk.

The guide describes five key steps to help food operators identify, prioritise and manage upstream supply chain food authenticity risks. These are:

1. Map your supply chain
2. Identify impacts, risks and opportunities
3. Assess and prioritize your findings
4. Create a plan of action
5. Implement, track, review and communicate

For each of these steps, the guide provides a set of questions to consider and guidelines on how to get started. The document is concise and to the point, its main advantage, while still covering the principle aspects a small business needs to address the problem of food fraud.

1.5. Places where information can be found

All the Vulnerability Assessment tools described above rely on obtaining up-to-date information on previous food fraud incidents and possible mitigation measures. Below are some areas where such information is available.

RASFF: Rapid Alert System for Food and Feed

This is the European Commission's online database of food and feed safety notifications. It can be accessed at www.webgate.ec.europa.eu. Information can be searched by date, type of product and, under the Hazard/Category, by selecting adulteration/fraud [6].

USP Food Fraud Database

The USP Food Fraud Database is a continuously updated collection of food fraud records, gathered from around the world. It is available through an annual subscription from www.foodfraud.org [7].

FAIR: Food Adulteration Incidents Registry

FAIR is compilation of historical and current events involving economically motivated and intentional adulteration of foods on a global scale developed by the Food Protection and Defense Institute (FPDI), a department of Homeland Security Centre of Excellence in the United States. Information of events that occurred over 5 years ago are accessible free of charge, more information is available for subscribers only. Information available at https://foodprotection.umn.edu/fair [8].

The FPDI has also initiated a further project, **FIDES (Focused Integration of Data for Early Signals)** which is collating and integrating data specifically to monitor potential food threats around the world. See link at https://foodprotection.umn.edu/innovations/food-systems.fides [9].

Food Integrity Knowledge Base

The Food Integrity Project [10] has built up a comprehensive Knowledge Base linking each food product and its potential fraud or integrity issues to appropriate analytical strategies that can be used for food fraud detection or authenticity testing. The Knowledge Base contains information on the type, frequency and impact of the fraudulent practice, the analytical methods available, including their use and performance criteria. More details on the Knowledge Base are given in a separate chapter of this book.

FARNHub: Food Authenticity Research Network Hub

The “Food Authenticity Research Network Hub» (FARNHub) is an online information hub for resources pertaining to food authenticity. The FARNHub contains an updated overview of scientific publications, past- and ongoing research projects, online resources (databases, web tools, etc.), funding bodies, regulations, and news stories, all concerning food authenticity. The FARNHub was developed in the EU-funded research project Authent-Net, is open access, and can be accessed through http://farnhub.authent.cra.wallonie.be/.

2. Best practice example of sector specific food fraud mitigation by SGF International e.V.

Food fraud is a recognized safety risk for consumers and effective strategies to mitigate this risk are required, including a vulnerability assessment of purchased ingredients and suitable analytical checks. However, food fraud detection calls on particular competencies and means which are not always available at the different links in the supply chain. Criminal energy is often spent on reducing the detectability of fraud and special intelligence is necessary to stay ahead in a constant race between the fraudster and control techniques. Thus there are good arguments to centralise the necessary competencies in a pre-competitive approach to assist raw material purchasing companies as much as possible in this task. Although processing companies are not completely dispensed from carrying out any fraud control, sector specific monitoring systems can reduce significantly the risk of purchasing falsified products and assure fair competition. As a best practice example the control system which is operated by SGF International e.V. (SGF), formerly "Schutzgemeinschaft der Fruchtsaftindustrie e.V." [11] is discussed in this paper.

The Voluntary Control System (VCS) of SGF was established as a company certification system. It started much earlier than other international food certification systems such as the GFSI certified standards or ISO 22000 [12,13] which have gained importance since the scandals such as dioxin and BSE in the nineties. The non-profit organisation SGF [14] was founded in 1974 in Germany by the fruit juice industry. The initial motivation of fruit juice companies to set up the VCS was the wish to combat unfair competition in the marketplace and avoid negative headlines when food fraud incidents came to light. Therefore, control structures were established which have focussed on authenticity and legal compliance right from the very beginning.

It soon became obvious that major food fraud risks were linked to processed semi-finished goods purchased from third parties. For this reason the VCS extended controls along the whole value chain from the first fruit processing step to the distribution to consumers. Farming activities have less potential for food fraud and were not included. Checks of traceability and plant specific technology were intensified successively as support for the interpretation of analytical results. A worldwide unique combination of product and system control thus developed. This includes co-operating independent control systems for consumer goods in a number of European countries.

This paper will focus on food fraud control and not discuss the positive effect of the VCS on other quality aspects, food safety and hygiene.

2.1. Control activities and infrastructure

In the following the operational system of SGF is described. Respective rules are given in the implementing provisions of the VCS which are mandatory for the control body and participating companies which are members of SGF.

All controls are covered by the SGF membership contribution. No additional costs are charged with the exception of reimbursement for investigation costs if fraud incident is proved to have occurred.

The contribution order of SGF considers the companies' turnover. Thus, smaller companies benefit from a lower contribution fee but get full service.

Companies agree to both announced and unannounced audits during normal working days. They also allow SGF auditors to check any production or traceability record.

SGF is in charge of scheduling the control plan and orders audits. Every supplier is audited at least once a year. If considered necessary, for example, if any doubt about the conformity of products from any producer exists or if post controls for already solved issues should be carried out, SGF can increase the frequency of audits or inspections for one specific supplier.

Auditors are trained by SGF and follow an integrity programme.

Every participating company keeps a retained sample from every production unit, every reception of semi-finished goods and every delivery to customers, from which the auditor selects samples for analytical controls. Advice on what to sample is provided by SGF headquarters in function of the specific situation of a company. Specially targeted sampling is carried out when an investigation is underway. An average of about 10 samples per audit are sealed by the auditor and send to SGF headquarters. Analyses are carried out in different independent qualified laboratories to stay flexible in the choice of methods and to benefit from the judgement of independent experts. A legal evaluation is requested from laboratories for analysed samples. If there are reasons to doubt the authenticity of any product, a previously defined procedure for further analytical confirmation is applied.

By covering all links in the supply chain, the identity of retained samples along the whole value chain can be counter checked by comparison with samples taken at both supplier and customer from the same batch. The interpretation of analytical data can be fine-tuned if it appears that the processing conditions have influenced the analytical profile and can be taken into account.

Furthermore, auditors are instructed to take authentic reference material from the running production and to document their history. These samples are used to maintain a worldwide unique analytical reference data base for fruit and vegetable juices. Such samples can also be provided to laboratories to help them develop and test new analytical approaches. The support of analytical development is part of SGF's tasks.

Both analytical results and traceability documentation are evaluated by specialists at SGF's headquarters.

If controls are considered as satisfactory or if required corrective actions have been carried out, the producer is listed as an approved supplier on the SGF-internet member portal which is updated daily.

VCS rules for participating companies also include the purchase of semi-finished goods from SGF approved suppliers with priority or alternatively to apply an extended analytical scope to assure conformity. Such analyses create significant costs and are an additional motivation for suppliers to join the system and to benefit from a list of additional services which are not discussed here.

Products from companies which are not actively participating in the certification scheme are controlled too. Sampling of semi-finished goods from non-participants of the VCS can be carried out during audits at participants who purchase from these sources or who have received commercial samples. Finished products are taken from retail outlets.

In other certification systems food fraud is seen as one safety and quality risk to be controlled by a single company. Thus, only products from one company and their direct suppliers are submitted to controls.

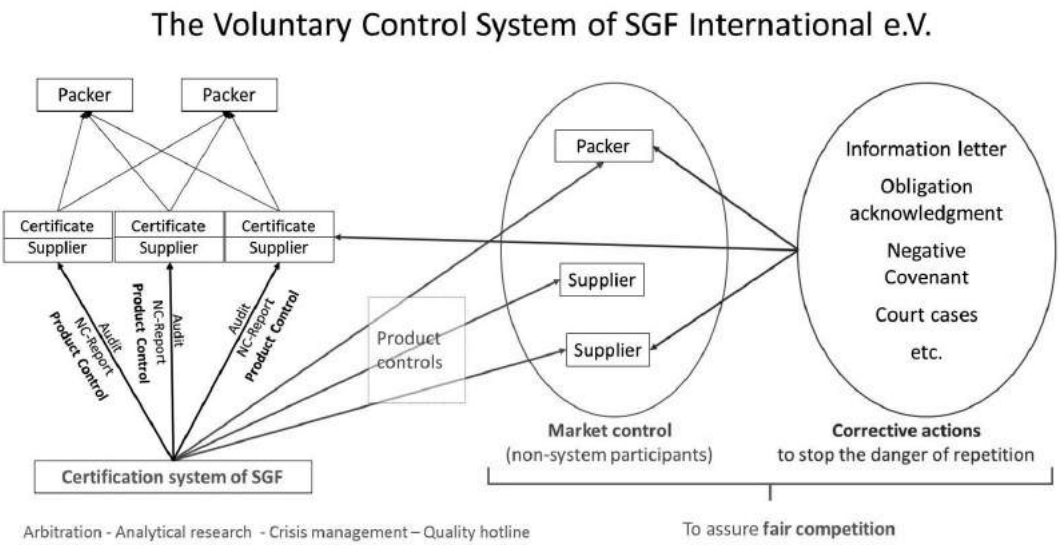

Figure 5: Control activities and corrective actions of the VCS

The major advantage of centralised and independent controls combined with corrective actions is the assurance of fair competition and a clean market. When a purchasing company detects food fraud, the subsequent consequences generally only have an effect on a single supplier-customer-relation. Unscrupulous suppliers remain in the market with adulterated products and harm fair competition and food safety. Therefore general market controls are part of the mandate that SGF had received from VCS participants. The matrix in Table 1 summarises the economic impact of possible frauds.

The VCS is recognized by the industry as control body because the system acts independently. The management and administration of the system must be structured accordingly. Other functions of an industry association such as lobby work in legislation processes and standard setting cannot be carried out by the control system if it is to maintain its neutrality and trust within the industry. The size and economic status of any company should not make any difference when food fraud is detected. For SGF the structure as shown in Figure 6 guarantees this requirement.

Table 1: Impact of food fraud to individual companies and the whole industry

	Fraud not detected	**Fraud detected Source remains active in market**	**Fraud detected Source removed from market**
Company related risks	Liability Food safety risk Official reprimand Recalls Damaged brand image	Less competitive purchasing conditions	No negative impact
Industry branch related risks	Public scandal Damage brand image for product type		No negative impact

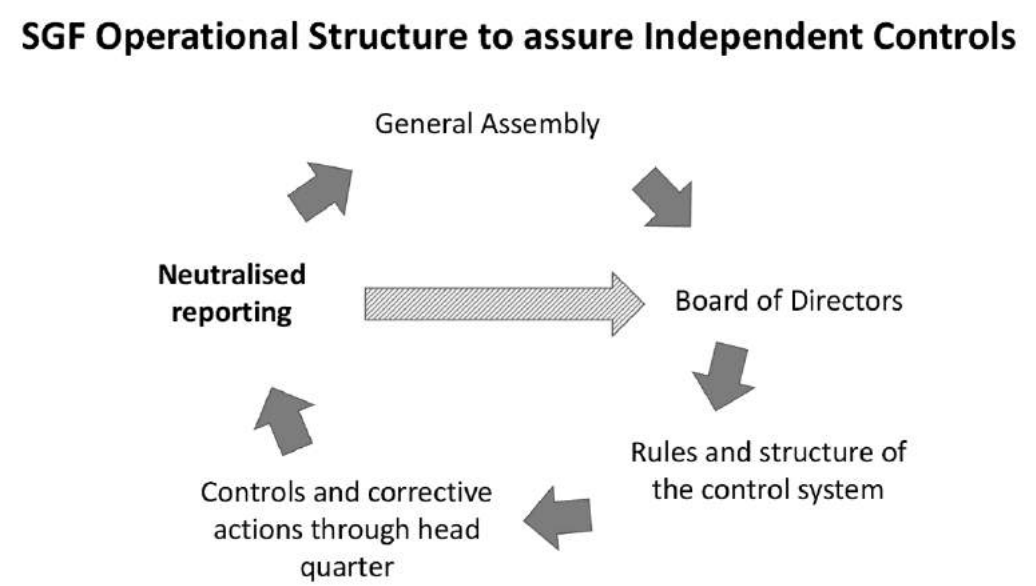

Figure 6: Operational structure of SGF to run an independent control system

As an overriding principle, all company related control results and corrective actions remain confidential between the company concerned and SGF operational headquarters and any problems are discussed solely between these two parties. No names or details are reported or transmitted to third parties. Thus, no direct relationship with the company's customers or with the authorities are affected. This allows constructive solving of any problem to assure that fraud practice is stopped. Furthermore, a tight and targeted follow up through SGF post controls ensures the effectiveness of corrective actions.

Only in a very few cases, for example if in a court case or if official notification for a detected health risk is required, does it become necessary for the operational headquarters to break confidentiality. These exceptional decisions are the responsibility of the board of directors who would be informed about the identity of parties involved.

2.2. Analytical strategy

In well-controlled sectors like the European Fruit Juice Industry, fraudsters need to put in place increasingly elaborate strategies to hide adulteration. This in turn raises the economic threshold for profit from fraud. As a consequence, less opportunistic and more systematically installed fraud can be expected. The higher probability of systematic fraud is considered for the design of control plans. Figure 7 shows a realistic flow of a systematic fraud process including the camouflage of analytical deviation. Experienced and trained auditors are able to identify and report different elements of this type of fraud process, which helps to focus controls.

The VCS adopts its analytical strategy by combining large screenings with selective and specific methods. Beside the widespread monitoring of the market, a risk-based sampling focussed on identified hot spots is necessary to get the best protection for the branch.

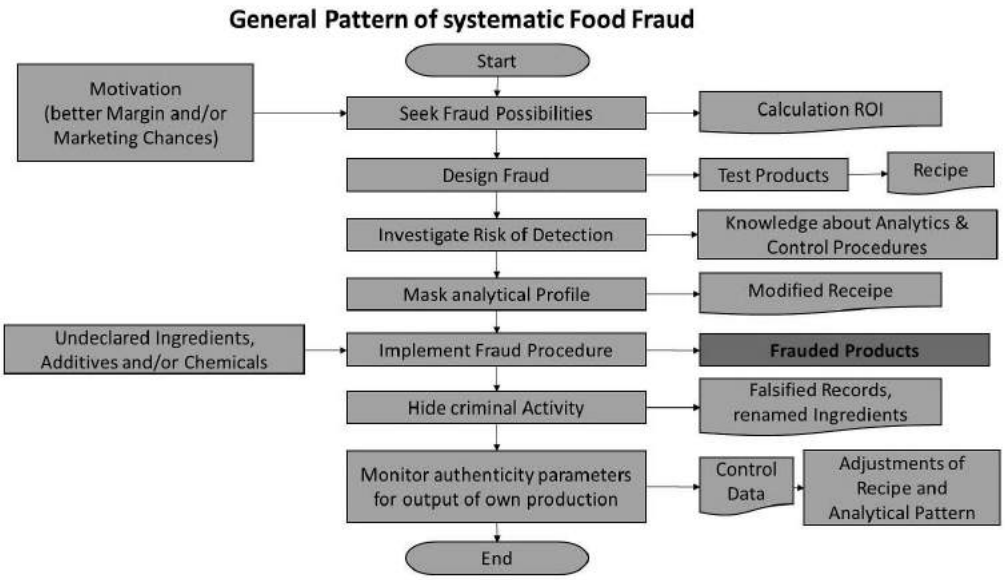

Figure 7: Graphic flow chart for systematic food fraud

Principle analytical objectives are:

- Monitoring of product groups with low fraud risk to maintain a clean market.
- Enforced controls on product groups which are more vulnerable for fraud

One important element to assure the first aspect is the application of proton-NMR-screening (SGF Profiling^{TM}) which covers a number of authenticity aspects for main product types in the fruit juice industry. Every sample taken during plant inspection is submitted to the SGF-Profiling^{TM} first. The method is a non-targeted fingerprint analysis [15,16]. Based on these results SGF decides on the applicable analytical scope.

Since not all authenticity aspects are covered by this screening technique, and for those products for which no reference models exist, other methods must complete the general quality screening. Due to the complexity of possible frauds the approach has a more or less spot check character. Continuously varying the selected checks makes it difficult to predict for the unscrupulous producer which fraud would be checked and which technique would be applied.

The VCS makes it possible to shift resources over company borders to control more intensively where a higher risk of adulteration is expected. This is important in particular for the second analytical objective to set a focus on vulnerable product groups.

Where possible, the analytical methods applied are preferably officially-recognized methods. A number of fruit and vegetable specific methods are recognised as valid by the International Fruit and Vegetable Juice Association (IFU) [17]. Often the best state-of-the-art methods are not referenced as such due to the time required to become an official method. In such cases laboratories must be able to demonstrate their suitability and/or have participated in cross validation checks with authentic and spiked samples organised by SGF.

2.3. Corrective actions

Being an industrial association SGF cannot replace national authorities and does not have the same competencies. However, the control system fulfils similar tasks and keeps the market clean. The system is also more efficient than those carried out by regulatory authorities, since it concentrates specific product know-how and control activities across borders along the entire production chain.

SGF activities follow strict rules that are controlled by external audits. An own management system (ISO 9001:2015) assures continuous improvement, integrity and equal treatment of all market participants.

The key drivers for efficiency are the corrective actions that the system imposes on any participant (see Figure 5) identified as responsible for the marketing of any falsified or adulterated product.

When a case of fraud is detected, confirmation of the analytical results and their evaluation by independent experts are required to avoid unjustified claims. After confirmation, SGF handles the case according to a catalogue of corrective actions which can be divided into internal and external measures. The normal case is the application of internal measures, handled between SGF and the company concerned to ensure confidentiality. This helps to maintain a constructive discussion. Different internal measures are possible:

- Information letter / warning letter
- Acknowledgment of obligation
- Negative covenant with penalty fee agreement for each case of repetition

For exceptional cases and only if internal measures have not had the desired effect, external measures are applied. External measures are all measures where other parties in addition to the co-workers of the SGF secretariat and the concerned company would be informed about the deviation and the identity of the concerned company. As the first step, the board of director is informed and takes the decision for further actions. Possible measures include:

- Formal infringement procedure: Information provided to authorities / Court case
- Information provided to the retailer and/or customers
- Information provided to a consumer organisation or public

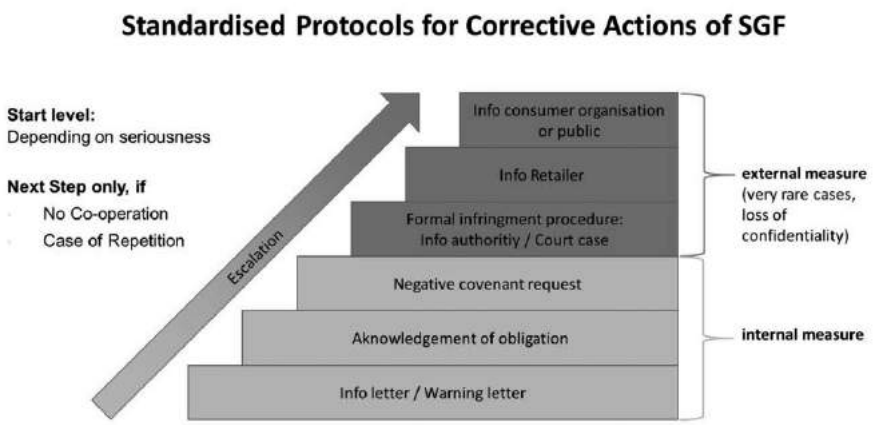

Figure 8: Corrective actions applied by VCS for quality problems

The non-respect of VCS system rules by participants leads also to corrective actions.

Figure 8 shows the different corrective actions. Depending on the seriousness of the case, first one of the internal measures is applied. For food fraud, this is generally the request to sign a negative covenant independently if the company is an SGF member or not. With such a covenant the company confirms that it will refrain from the detected fraud practices and agrees to a dissuasive penalty fee for each case of repetition. If a chosen measure is not successful the next stronger step is initiated. The system is also applied for other quality problems than food fraud, e.g. production errors or increased food spoilage.

2.4. Control results

Even though fruit juice has a high potential for food fraud, the market of consumer goods in VCS covered regions has not been marked by negative headlines due to fruit juice fraud in the last decades. No official statistics exist but in the European Union, it can be estimated that more than 80 % of semi-finished goods come from SGF certified companies. In countries with high fruit juice consumption like France and Germany the figure is even higher. National market coverage above 95 % is not unrealistic.

The control results have remained relatively similar over a number of years. Depending on the market situation some shifts and variations have been observed. About 450 audits per year are carried out by SGF auditors, the number of plants to be controlled is about 400.

As a very rough rule of thumb, from about 4 500 - 5 000 analyses per year, for 10 – 15 % of samples some analytical indicators have led to further investigation. With help of the extended SGF reference data base most cases can be explained by regional, seasonal or process technological particularities. Less than 1 % of samples analysed have shown real detectable authenticity deviation. Because SGF is working with enforced sampling for vulnerable hot spots the real percentage of detectable authenticity problems in the whole industry is likely to be lower than 1 %.

The risk of food fraud is several times higher for products which are marketed from non-VCS participants than those from SGF-approved supplier [18]. On the global level, fruit juices form part of the group of foodstuffs that have a high risk of fraud.

An anonymised overview of control outcomes is published in regular newsletters for SGF members and in annual activity reports, providing companies with information on observed authenticity problems so they can adjust their own food fraud protection measures accordingly.

2.5. Implementing of a centralised control system in other branches

The VCS of the fruit juice industry has been in operation for a long time and a wealth of knowledge encompassing analytical science, market structure, control operations and product specific intelligence has been built up. The experience gained has led to an efficient management of the available budget. Implementation of a similar system in any other branch is principally possible and is definitely recommended for products with a high food fraud risk.

However, the setting-up of a control system would require a certain starting investment and, above all, its acceptance by the industry branch. Only if a major share of market players is in support of its implementation and accept to abide by the rules of conduct, can such a system be rolled out successfully. To limit costs, benefitting from experience of existing control infrastructures is recommended. Limiting it to a defined region for finished goods and/or a reduced product scope could facilitate the start of a new system.

At the end of the day, companies will minimise their own costs incurred in carrying out vulnerability assessments and product control thanks to the advantage of centralisation. Additional market controls would lead to fair competition and fewer risk of scandals. System rules and control mandate must be defined exactly and agreed by all participants. Important points are listed in Table 2 and Table 3.

Table 2: Important characteristics of system rules in a sector specific control system

Rule characteristic	Comment
Rules are defined and agreed by active participants	Rules must be accepted by a major share of the market. Industry reality must be taken into account.
Stimulation to trade and purchase products from participants of the system	Participants must have an interest to purchase semi-finished goods from system approved suppliers preferably.
Enhanced controls when purchasing from outside of the system	No system can be hermetically closed. Therefore the system must include goods from non-controlled suppliers to ensure sufficient protection against food fraud.
Whole chain approach	Authenticity control is more efficient with cross checks along the whole supply chain.
Assure pre-competitiveness	Antitrust rules are prerequisite.

Table 3: Typical points for a mandate of a sector specific control system

Mandate	Comment
Analyses (product checks)	Analyses are necessary to check products and confirm frauds.
Audits (system checks)	Traceability data and knowledge about applied technology and specific circumstances allow refined evaluation of analytical results.
Whole market control	Controls must cover the whole market to assure fair competition.
Positive communication	Blacklisting harms the willingness of defrauding companies to carry out corrective actions. Only publication of achieved certification or approval of companies is recommended.
Maintain confidentiality	Constructive work on corrective actions is possible only if the companies concerned are sure about their anonymity with respect to customers and competitors.
Corrective actions	The system must tend to stop the danger of repetition for any detected source of food fraud.
Development of analytical methods	The system must support the best use and development of applicable analytical science. Access to efficient methods and updated information for market players is important. (e.g. publication of reference databases).
Development of control intelligence	Horizon scanning of fraud possibilities is required for efficient control work. Product specific experience to investigate and to detect fraud must be built up.
Combination with other services (facultative)	Synergies with other branch specific services can be useful. Therefore, pre-competitive character must be maintained.

3. Traceability tools to mitigate food fraud risk

3.1. Traceability

Traceability is the principle of keeping track of and connecting all the recordings that are made, and the existence of some degree of traceability underlies all the supply chain methods for verifying food item property claims.

There are numerous definitions of traceability, most of them recursive in that they define traceability as "the ability to trace" without defining exactly what "trace" means in this context. An attempt to merge the best parts of various existing definitions while avoiding recursion and ambiguity is "The ability to access any or all information relating to that which is under consideration, throughout its entire life cycle, by means of recorded identifications" [19]. This emphasises that any information can be traced, that traceability applies to any sort of object or item in any part of the life cycle, and that recorded identifications need to be involved.

Traceability depends on recording all transformations in the chain, explicitly or implicitly. If all transformations are recorded, one can always trace backwards or forwards from any given food item to any other one that comes from (or may have come from) the same origin or process. In addition, traceability requires relevant information to be recorded and associated with every food item in the supply chain. This makes it possible to find the origin of a given food item (the "parents"), the application of the food item ("the children"), and all properties of every food item (when and where was it created, weight or volume, what form is it in, what species, fat content, salt content, etc.). For the other supply chain methods to work, traceability needs to be present, and the efficacy of the supply chain methods is limited by characteristics of the traceability system. Food items need to be identified in some way (uniquely or as a group), the transformations that the food items go through need to be documented, and the attributes need to be recorded. The specifics of the identification and the documentation of transformations and attributes will decide how much data is present, how well it is connected, and how accurate it is, which in turn will be a limiting factor for the other technologies and methods outlined below.

3.2. Traceability systems

Traceability systems are constructions that enable traceability; they can be paper-based, but more and more commonly they are computer-based. Several detailed descriptions of traceability systems in various food sectors have been published, and there is general agreement on what requirements a traceability system should fulfil:

- It should provide access to all properties of a food product, not only biochemical properties that can be verified analytically.
- It should provide access to the properties of a food product or ingredient in all its forms, in all the links in the supply chain, not only on production batch level.
- It should facilitate traceability both backwards (where did the food product come from?) and forwards (where did it go?).

This means that the following activities must be carried out:

- Ingredients and raw materials must be grouped into units with similar and defined properties, commonly referred to as traceable Resource Units (TRUs)
- Identifiers / keys must be assigned to these units. Ideally these identifiers should be globally unique and never reused, but in practice traceability in the food industry depends on identifiers that are only unique within a given context (typically they are unique for a given day's production of a given product type for a given company).
- Product and process properties must be recorded and either directly or indirectly (for instance through a time stamp) linked to these identifiers.
- A mechanism must be established to facilitate access to the recorded properties.

Practically all food businesses have an internal traceability system; often using software with ample opportunity for browsing data, visualising dependencies (which TRUs are based on which TRUs), and creating reports related to what happens within the company. Implementing a similar functionality for an entire supply chain, examining the whole chain of transformations from raw material source to consumer, is a (and probably "the") major challenge, and requires effort, motivation and cooperation, in addition to the presence of technical solutions that build on well-proven and widely adopted standards. Verification and validation of the data in the traceability system is of course also very important, but these are external processes and not part of the traceability system itself.

3.3. Claims and methods for verification of claims

It is important to keep in mind that a traceability system is made up of statements that are claimed to be true, but it is not known for sure that they actually are true, so that is something that needs checking. Figure 9 illustrates the relationship between food item properties on one hand, and the claims in a traceability system on the other. Claims may be explicitly stated in the traceability system, or they may be implicit in that if the food item had that property (contained nuts, was made from genetically modified material), it should have been declared. The claims, whether implicit or explicit, fall into two categories; those that can be verified by analytical methods, and those that cannot. To verify a claim in the first category ("this product is made from cod"), analytical methods can be used to provide a true/untrue answer, or sometimes a likely/unlikely, answer. To verify a claim that is not related to a biochemical property ("this TRU came from the farm of Jim Jones"), the data recordings in the system have to be investigated, especially the transformations ("Did Jim Jones deliver to the food business that made this TRU?"). Using methods based on analysing data recordings cannot verify the claim, but they can often indicate if the claim might be true or not ("No, according to the records, Jim Jones has never delivered anything to the business that made the food item in question").

This means that analytical methods are very important when we are dealing with traceability, but they do not in themselves provide traceability. What they do provide is a way of verifying most of the claims relating to biochemical attributes of the food item in question. While these claims are only a subset of the total number of claims in a traceability system, they are among the most important ones, because if there is a food safety problem related to a food item, it will be detectable through application of analytical methods, and food safety, as it has been seen, is strongly linked to traceability.

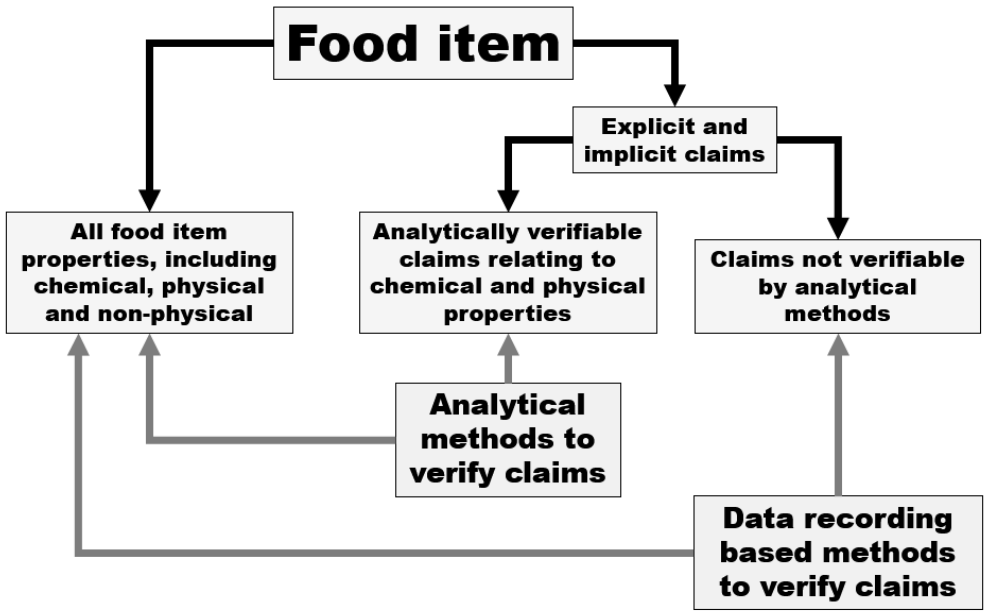

Figure 9: Relationships between claims and methods to verify them

3.4. Blockchain technology

Blockchain technology is not a method for verifying claims in itself, but it is a way of increasing transparency and accessibility of the recordings in the traceability system, and thus for increasing claim veracity, and so indirectly it contributes to verifying claims about food items. Blockchain technology in its current form has been around since 2008; it is what underlies the digital currency known as Bitcoin, and it can be used to document transformations in the supply chain in a secure and transparent manner. Blockchain technology is best described as one that enables records to be shared by all network nodes, updated by miners (system users who, for a fee, keep track of transaction records), monitored by everyone, and owned and controlled by no-one [20]. A significant problem in traceability is that it is difficult to verify that the stated transformations actually took place. Using blockchain technology, the record of all transformations would be in the public domain, openly visible to anyone (although most of the food item attributes would not be visible) [21]. If a buyer received a food item where the transactions were documented using blockchain technology, every single transaction from the food item in question back to the original farming or harvesting would be available for inspection, together with the other food items that came from the same source. This to some degree prevents food businesses from introducing undocumented raw materials or products into the supply chain; if they did, the mass-balance accounting would not add up (a 1200 kg fillet cannot be produced from 1000 kg meat or fish). It also prevents anyone from overwriting the transaction once it has been recorded, which means that if the original data recorded is correct (and it is normally in the interest of high quality producers to record the initial data correctly, to protect their brand and to justify the higher price they get) it becomes very difficult for foods businesses later on in the chain to counterfeit or dilute the product. Blockchain technology will not guarantee accurate recordings, but it will certainly remedy some weaknesses that currently exist.

3.5. Mass balance accounting

Comparing numbers and equalising them has been stated as a way of verifying claims related to large quantities. Material flow analysis is (MFA) is one approach related to mass balancing. This is a methodology developed to assess the flows and stocks of goods and materials within a set time and space [22]. The method is based on the mass balance principle; that matter is conserved in any system, and thus input is equal to output mass. It was developed to describe the metabolic processes of large and complex systems like cities, regions, nations and industrial companies. MFA is based on accounts in physical units (e.g. tons) quantifying the inputs and outputs of those processes [23].

Material flow analysis has often been used as a synonym for material flow accounting; in a strict sense, the accounting represents only one of several steps of the analysis and has a clear linkage to economic accounting. Two basic types of MFA can be distinguished. Type I is concerned with the environmental impacts of certain substances, bulk materials, or products, and therefore the flow of substances and materials linked to these entities are studied. Type II is interested in the performance of firms, sectors, or whole regions or national economies, and thus the throughput of substances and/or materials of these entities is analysed [24]. Whereas the first type is often performed from a natural science or technical engineering perspective, the second type is more directed towards the analysis of socio-economic relationships.

One limitation in using methods such as the MFA is the measuring of the qualitative aspects of material flow [25]. Quantitative changes that are measurable, for instance weight, can be accounted for using a mass balance approach.

3.5.1. Mass balance in fisheries: a study of the Norwegian cod fisheries

The Norwegian seafood industry is regulated by international standards combined with national regulations. The industry is tightly regulated as there are numerous registrations related to catch, landings, production, feeding, slaughtering, storage, transport and export. Despite the wealth of regulatory requirements, periodically there are confirmed incidents of fraud and misreporting [26], as well as accusations and rumours, especially in the cod fishery coastal fleets. Usually the fraud relates to misreporting of the total amount of landed fish. However, there is no agreed assessment suggesting the extent of the fraud, only disputed indications. A 2013-survey among fishermen and buyers conducted by Nofima indicated that the misreporting that year might have been around 5 % of the total catch [27].

In a forthcoming report [28], an analysis of the regulatory framework shows that the whitefish industry in Norway is subject to a complex list of registrations to different authorities that can be used as source data in analysis. When using the data in a material flow analysis, a gap between input and output of cod is found.

The MFA for cod was carried out on a national level for the years 2010-2017. Except for 2012, the output was higher than the input. In 2014, the discrepancy between input and output was as much as 9 %. In total for this period, the output was 5 % higher than the input.

While a certain portion of the gap is likely due to fraud, the discrepancy might also be caused by factors not related to fraud, but rather to the complexity of the production and supply chain. One challenge is that weight is recorded as living weight (round weight) in the landing phase and in product weight upon export. The numbers therefore have to be processed using a national conversion factor to get them in the same format. To obtain more information about the errors in

the conversion factors and other possible sources of discrepancies, an interview was conducted with a company that produces and sells various types of fish products. Among other factors, the interview revealed that the discrepancy in product properties is largely dependent on the product in question. The discrepancy relating to weight, condition and conservation is much higher in the production of highly processed products such as saltfish and clipfish (cod that has been both salted and dried) with a long storage time, than in the production of fresh fish. In general, the more complex the production, the higher the discrepancy. It is also in the production phase of the product that discrepancies are most likely to occur, not in the export and sale of products.

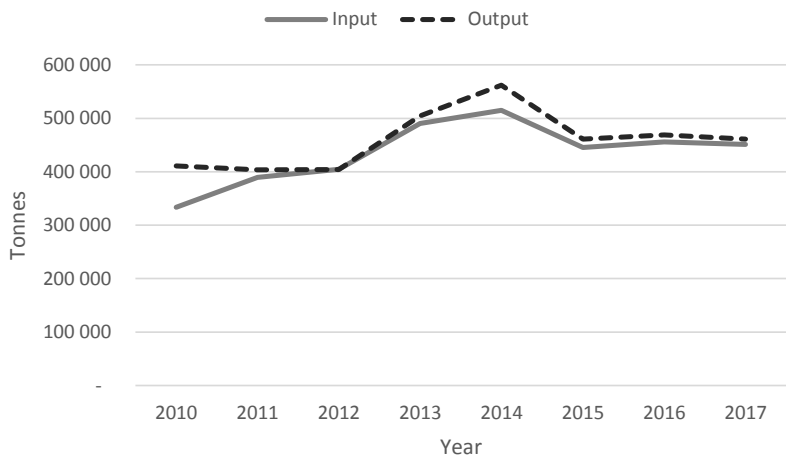

Figure 10: Discrepancy in tonnes between input and output when mass balancing cod in Norway

The conversion factors used to convert product weight into living weight stands out as a significant source of error, but there are also several other factors that can explain why this discrepancy seems to appear annually. Table 4 shows the identified main sources of discrepancies, the different reasons why they occur and the associated responsibility.

Table 4: Sources of discrepancies and associated responsibilities

Source of discrepancy	Reasons	Responsible
1) Errors in source data	Errors in electronic systems	Operator and/or Authority
	Human errors	Operator and/or Authority
	Methodical errors	Authority
	Hidden flows	Actor and/or Authority
	Information gaps	Authority
	Lack of control	Authority
2) Errors in the MFA	Methodical errors	Study
	Human errors	Study
	Time lag/storage	Method limitation
	Conversion factors	Method limitation/authority
	Alternative data sources	Statistics supplier/authority
3) Fraud	Making profit	Operator
	Survival	Operator

The findings from this case study shows that while public record requirements in the Norwegian fishing industry covers a wide range of topics, only a few can be used to trace a product or to identify a discrepancy. The case study shows that tracing claims like origin, time/date and ownership through the production is possible provided there are good systems for recording these properties. Properties like weight, conservation and product condition are more difficult to trace as they may change during the production. As weight often is related to catch volume and illegal, unregulated and unreported (IUU) fishing, this claim is of special interest.

If there are recordings of both input and output in a production, a MFA is of high relevance. However, the case study shows that the reliability is highly dependent on industry structure, the complexity in production, data availability, and data quality. Further, whilst the analysis shows that there is a gap between input and output, it does not identify whether this gap is due to unintentional actions (e.g. production errors, manual error, etc.) or if it is due to criminal activity. As the quantitative approach described above does not identify the source of the discrepancy, it must be supplemented by a qualitative approach, either in-depth interviews with industry actors or more cost-effective methods such as questionnaires or phone interviews, the former used in [27]. These methods can be used to identify weak points in the supply chain, such as those described above, relating to production complexity, conversion factors between product types, etc.

With the MFA-approach being highly dependent on data availability and data quality, it is useful within industries with many control points, but less so in cases where product registrations are few. As the case study shows, the discrepancy can be comparatively higher for highly processed products than products that undergo a much simpler production. For products that undergo a relatively substantial transformation during production, control points throughout the production process itself would be necessary to better account for discrepancies due to inherent product characteristics.

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The FoodIntegrity Knowledge Base

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A whole Work Package of the FoodIntegrity project has been dedicated to the design and creation of a comprehensive **Knowledge Base** linking each food product, its potential integrity issues and appropriate solutions for detection.

For use by industry and regulatory authorities, this tool will make it possible to identify, easily and rapidly, potential food fraud threats to a given food product or ingredient and the existing solutions. The Knowledge Base contains a wealth of information including the type of the fraudulent practice, the analytical methods available, their use and performance criteria, and the availability of reference data with links to literature or standards and open-access databases.

Currently hosted under the umbrella of the FoodIntegrity website [1], a transfer to a European organisation is ongoing in order to ensure its sustainable use in the future.

Gathering information on food fraud detection

Analytical information in the field of food fraud can be found in a wide range of databases or sources. For instance, new techniques are often described in the scientific literature and can be accessed thanks to tools such as Pubmed, ScienceDirect or Scopus.

The FoodIntegrity Knowledge Base offers several specific features to users. First each of the recorded methods is **linked to a food commodity and to a food fraud issue**. It is of utmost importance for users in the food industry who want to have a quick answer to the threats they face in their daily activity and to inspectors monitoring food production to ensure they apply the most appropriate analytical method. The definition of the different types of fraud used in the FoodIntegrity Knowledge base is based on the work done by the GFSI Food Fraud Think Tank [2], a global multi-stakeholder group founded in 2012 to work on recommendations on effective systems to protect consumers from harm due to food fraud.

Entries of the FoodIntegrity Knowledge base include **comprehensive and standardised metadata** for each description of the analytical methods. This provides different kinds of information including for instance the type of food commodity, the analytical target (analyte, molecular marker or physical parameter), or the experimental protocol, giving a wide and summarised outlook of the method. In the case where statistical treatment is applied to the data, its summarised description is included, for instance the name of the multivariate analysis used (PLS-DA, SIMCA, etc.). Standardisation status has been foreseen: users can know if the methods are standardised, if reference material is available or if they are recommended in compendial approaches from non-

standardisation organisations such as the International Organisation of Vine and Wine (OIV) in the wine industry or professional associations such as the International Federation of Fruit Juice Producers (IFU) for juices. Indications if the method has been transferred to several laboratories and is available in routine analysis have been added: this allows users, especially from industry, to assess how easily they will be able to access the method. The Knowledge Base contains a field where the code of the Combined Nomenclature, the systematic list of commodities in use in the EU for classifying goods [3], is stored. This field allows better categorisation for statistics and, acting as a primary key, make future interaction possible with other resources or tools. For easier comparison between methods, information is recorded in a standardised way. When information cannot be stored in the Knowledge Base, links to other sources are provided.

Datasets containing for instance raw data from analytical devices are also attached to some of the entries. Nowadays different analytical approaches are used for food authenticity testing. The majority require a comparison to reliable authentic data to judge the compliance of a food sample. It is of enormous benefit to any organisation carrying out food fraud testing, or embarking on the development of new analytical methodology, to get access to this kind of information. The use of reference data is even more crucial where fingerprinting or profiling approaches are used.

Thanks to public funding from the European Commission and to the work of the FoodIntegrity partners, access to this resource will be **free of charge** for all users. Access to knowledge will be possible for any stakeholder, whether a food manufacturing SME, a researcher in the field of authenticity or a civil servant working in a food safety organisation.

Finally the Knowledge Base will act as a **European focal point** on analytical methods in the field of food fraud standing above the vast array of information that exists in a number of private and public analytical data bases. It will be open to users from any country. The fact that such data sets exist at a single point of reference will benefit most organisations.

Expected use for all food stakeholders

The FoodIntegrity Knowledge Base will be accessed through a reference portal on the web. Different types of users will find an interest in this resource according to their specific needs.

Users from the **food industry** will be able to identify an analytical solution when they are facing an adulteration issue in their premises. All possible solutions will be presented allowing the selection of the most suitable method based on criteria such as compliance with a standard, transfer to laboratories in routine, complexity of the method, etc. A second possible use of this Knowledge Base is during the preparation of food fraud mitigation plans. Since the recommendation via the GFSI Benchmarking Requirements Version 7.1 published in 2017 [4], cascaded to Global Food Safety Certification Programmes such as IFS (International Featured Standards) or BRC (British Retail Consortium), food companies are required to carry out a Food Fraud Vulnerability Assessment, then put in place a Food Fraud Control Plan consisting of a set of mitigating measures including a monitoring and testing strategy. The Knowledge base will help these companies in identifying the right analytical methods to mitigate risks identified in their vulnerability assessment.

For **food safety authorities**, the expected use of this Knowledge Base is first to enrich early warning tools such as the RASSF portal [5] or incident databases. Along with the case, the analytical solution to detect the fraudulent practice will be provided.

For **standardisation organisations**, the Knowledge Base will help to determine gaps in the coverage of food fraud detection by standards. Furthermore future candidates for standardisation or methods which need extended validation will also be identified.

The Knowledge Base will also be useful for **food testing laboratories**, whether academic or private. Thanks to the description and to the links to scientific papers containing the full description of the protocol, analytical methods can be easily implemented in routine practice. The service portfolio of these companies will expand, allowing a better monitoring of fraud risk in industry. Furthermore it will enable proficiency tests to be organised among more and more laboratories, facilitating dissemination and recognition of these methods throughout the European Union. For research laboratories, access to authentic and standardised datasets of analytical methods which can be reused will promote the development of new and improved analytical methods.

Ultimately the Knowledge Base will be used by all stakeholders as a knowledge reference in the field of food fraud. It will help to disseminate the idea that food fraud is not inevitable and that tools exist to tackle it.

Food Integrity Knowledge base, Result #300, 04 May 17

RESULTS #300

FOOD INTEGRITY ISSUE

Food Category	Other Seafood (crustaceans / bivalve molluscs / cephalopods)
Commodity Detail	King and Queen scallops
Description	Mislabelling of fresh and frozen King ("Pecten maximus") and Queen ("Aequipecten opercularis") scallops. The introduction of the Fish Labelling Regulations in 2003 (and as amended thereafter) stipulated that certain fish and aquaculture products must be labelled with the commercial designation of the fish species it contains.
Type of Fraud	Substitution Mislabelling
Issue	Product composition Botanical/Cultivar/Varietal/Species
Importance	Often
Location in Supply Chain	Storage/transport Food manufacturer Trader Distributor

ANALYTICAL STRATEGY

Type	Fingerprinting Screening Method	
Target	Production of PCR-RFLP fingerprints for the identification of King and Queen scallops. Species-specific profiles are produced using DNA extracted from scallop samples.	
Analytical Technique	Electrophoresis	Other type of Electrophoresis - Lab-on-a-chip capillary electrophoresis (CE)
	PCR (DNA- and RNA-based methods)	Other type of PCR - Restriction fragment length polymorphism (RFLP) followed by Lab-on-a-chip capillary electrophoresis (CE)
Compendial Approach links	SOP extraction - http://tna.europarchive.org/20141103165934/http://www.foodbase.org.uk/results.php?f_report_id=2	
Sample Preparation	Species-specific profiles are produced using DNA extracted from scallop samples. DNA should be extracted using either a CTAB DNA extraction or other suitable commercial kit method (Tepnel, Promega, Genescan, R-Biopharm, Qiagen etc.). Perform DNA extraction from individual scallops in duplicate for the first sample and for every 10 samples thereafter.	
Further Information on the Method	see page 8 for further details: final project report: Appendix F, page 133ff	

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Figure 1: Example of a method recorded in the FoodIntegrity Knowledge Base

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The FoodIntegrity knowledge Base is the collaborative result of the work of 19 European organisations from the FoodIntegrity project:

FERA, EUROFINS, JRC IRMM, BFR, SITEIA.UNIPR , CRA-W, FiBL, , UCPH, DLO, VSCHT Praha, FEM, UCLM, BARILLA, TEAGASC, Isolab GmbH, CSIC, FAO, SOLTUB, SWRI.

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Further reading

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The FoodIntegrity Network, a group that had members representing the key stakeholders impacted by food fraud (regulators, industry, academics, research providers and customers) was used to identify key areas where scientific guidance was required. The output from this was the production of several opinion papers. The aim of the scientific opinions produced as part of the FoodIntegrity project was to provide independent scientific advice and communicate to all interested parties on topical issues concerning food integrity. The scientific opinions provide objective, science-based advice, and clear and coherent communication, grounded in the most up-to-date scientific knowledge and data. These opinions are recommended as further reading. The titles, an abridged abstract and the corresponding author are contained below. At the time of writing, several of these papers are under peer review and it is expected that these will be available in open access journals at the time of reading.

Stable isotope techniques for verifying the declared geographical origin of food in legal cases

Corresponding Author: Federica Camin (Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM))

Consumers are increasingly interested in the provenance of the foods and European laws require protection against the mislabelling of premium foods. Methods for testing authenticity require robust analytical techniques that can be utilised by the various regulatory authorities. Of the many techniques, the most widely-used method is stable isotope ratio analysis. Scope and approach: Focus is on the use of stable isotope ratios of H, C, N, O, S and Sr for verifying the geographical origin of food, cross-referencing it with examples of legal cases. State of the art including rules for building an authentic sample reference database (commonly called databank) and for interpreting the results obtained in actual cases is described. The overall objective is to provide stakeholders and competent authorities dealing with fraud, with a best-practice guide for its use. Key findings and conclusions: Stable isotope ratios can differentiate foods on the basis of their geographical origin and, especially for light elements, can be measured reliably in routine work in different matrices and compared successfully between different laboratories. Examples of legal applications are grape products, orange juices, olive oil, cheese, butter, caviar. Sometimes, the cases are not brought directly to the court, but before further verifications (e.g. paper traceability, forensic accounting) are conducted. The system can satisfy the court when a robust databank of authentic samples exists, the methods used are officially recognized, validated and accredited, and the expert demonstrates that the conclusions are sufficiently robust and reliable to stand up to the required level of proof.

Role of analytical testing for food fraud risk mitigation – principles of cost-benefit determination for analytical fraud testing

Corresponding Author: Francis Butler (University College Dublin)

Food fraud is of high concern to the food industry. A multitude of analytical technologies exist to detect fraud. However, this testing is often expensive. Available databases detailing fraud occurrences were systematically examined to determine how frequently analytical testing triggered fraud detection. A framework was developed for deciding when to implement analytical testing programmes for fraud and a framework to consider the economic costs of fraud and the benefits of its early detection. Current regulatory issues relating to food fraud detection are explored as well as some of the main factors associated with statistical sampling for fraud detection.

What are the scientific challenges in moving from targeted to non-targeted methods for food fraud testing and how can they be addressed? – Spectroscopy case study

Corresponding Author: Terry F. McGrath (Institute for Global Food Security, ASSET Technology Centre, School of Biological Sciences, Queen's University Belfast, Northern Ireland, United Kingdom)

Background: The authenticity of foodstuffs and associated fraud has become an important area. It is estimated that global food fraud costs approximately $US49b annually. In relation to testing for this malpractice, analytical technologies exist to detect fraud but are usually expensive and lab based. However, recently there has been a move towards non-targeted methods as means for detecting food fraud but the question arises if these techniques will ever be accepted as routine.

Scope and approach: In this opinion paper, many aspects relating to the role of non-targeted spectroscopy based methods for food fraud detection are considered: (i) a review of the current non-targeted spectroscopic methods to include the general differences with targeted techniques; (ii) overview of in-house validation procedures including samples, data processing and chemometric techniques with a view to recommending a harmonized procedure; (iii) quality assessments including QC samples, ring trials and reference materials; (iv) use of “big data” including recording, validation, sharing and joint usage of databases.

Key findings and conclusions: In order to keep pace with those who perpetrate food fraud there is clearly a need for robust and reliable non-targeted methods that are available to many stakeholders. Key challenges faced by the research and routine testing communities include: a lack of guidelines and legislation governing both the development and validation of non-targeted methodologies, no common definition of terms, difficulty in obtaining authentic samples with full traceability for model building; the lack of a single chemometric modelling software that offers all the algorithms required by developers.

The scientific challenges in moving from targeted to non-targeted mass spectrometric methods for food fraud analysis: A proposed validation workflow to bring about a harmonized approach

Corresponding Author: Michele Suman (Barilla Advanced Laboratory ResearchParma, Italy)

Background: Detecting and measuring food fraud is a challenging analytical task since a very wide range of food ingredients and types may be adulterated by numerous potential adulterants, many of which are yet unknown. To date most of the methods applied for the control of food fraud are targeted methods, which are focused on the detection of one or a few classes of known compounds.

Scope and approach: There is an increasing availability of solutions and applications based on high resolution mass spectrometry (HRMS), allowing parallel non-targeted approaches, novel compound identification and retrospective data analysis. For these types of methods sample-handling must be minimal to allow the inclusion of as many as possible chemical categories. However data-handling of such methods is much more demanding, together with the potential requirement to integrate multiplatform data as well as conducting data fusion. To allow the processing of massive amounts of information based on the separation techniques and mass spectrometry approaches employed, effective software tools capable of rapid data mining procedures must be employed and metabolomics based approaches does appear to be the correct way forward. To verify the relevance of modelling results, appropriate model validation is essential for non-targeted approaches, confirming the significance of the chemical markers identified.

Key findings and conclusions: The present paper is devoted to review and assess the current state of the art with regards non-targeted mass spectrometry in food fraud detection within many food matrices and to propose a harmonized workflow for all such applications.

Sampling guidelines for building and curating food authenticity databases

Corresponding Author: James Donarski (Fera Science Ltd, York, UK)

Background: Food fraud is a global issue and one that can often be detected through the use of analytical testing. Analysis of suspect foodstuffs and comparison of their results to those contained within a food authenticity database is a typical approach. This scientific opinion was commissioned as part of the FoodIntegrity EU project to provide guidance for the creation of these food authenticity databases.

Scope and Approach: This opinion paper provides what the authors believe are the most important considerations which must be addressed, when creating a food authenticity database. It covers three broad sections, relating to aspects that need to be considered before, during and after the analytical data has been collected. Specifically, the areas of database scope, analytical methodology, sampling, collection and storage of data, validation and curation are discussed.

Key Findings and Conclusions: The globalisation of foodstuffs brings new and novel commodities to consumers throughout the world. When foodstuffs are new to a specific population, it can be the case that consumers or even inspection laboratories cannot easily recognise when a fraud has taken place. The provision of available, reliable and robust food authenticity databases is a tool in preventing such fraud. This opinion was produced to facilitate the sharing of these databases.

Use of NMR applications to tackle future food fraud issues

Corresponding Author: Luisa Mannina (Institution Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma)

Background. NMR targeted and untargeted methodologies are widely recognized as important tools for food authentication and the detection of counterfeit products. Targeted approaches allow the identification of specific markers of identity/adulteration for a given foodstuff. In the untargeted approach, the chemical profile of the whole foodstuff is used to create a unique fingerprint as a reference for suspect samples. The untargeted analysis methodology typically follows the metabolomics approach.

Scope and Approach. In this manuscript we discuss how both targeted and untargeted NMR methodologies are applied in routine use for food fraud monitoring. The cost effective approaches for routine application are discussed using examples of Food Screener™ and benchtop low-field instruments. Key Findings and Conclusions. Several examples of routine consolidated NMR targeted and untargeted applications are reported and the food matrices that are problematic for the NMR application are discussed. The future NMR implementation into routine practice will rely on the further exploration of FoodScreener™ like platforms for simultaneous targeted and untargeted applications and the continued development of applications for low-field benchtop instrumentation.

The future of NGS (next generation sequencing) analysis in testing food authenticity

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Food authenticity is a big concern for consumers, food authorities and food producers and processors, since incorrect food labelling and other types of fraudulent practices have been demonstrated to negatively affect the confidence and even the safety of the final consumer. European Union regulation (EU) No. 1169/2011 requires that consumers should be appropriately informed regarding the food they consume. This is vital in order to achieve a high level of health protection and to guarantee their right to information, as well as to protect the businesses of scrupulous producers from unfair competition. Consumers' choices can be influenced by health, economic, environmental, social and ethical considerations. In fact, the general dictionary definition of "*authenticity*" is "*the quality of being authentic, trustworthy, or genuine*", and the relevant dictionary definitions of "*authentic*" include "*not false or copied; genuine; real*" and "*having an origin supported by unquestionable evidence; authenticated; verified*". More specifically regarding food authenticity, a recently produced CEN standard defines authenticity in a food and feed context as the match between the *food product characteristics and the corresponding food product claims* (CEN WS86). These labelling requirements, which are legally specified and differ depending on the product, may include the scientific name or breed, and production method (e.g. organic, free-range, wild-caught etc.). However, other features of the product can also be included by producers to inform the consumer, including (i) ethical issues (halal, vegetarian, etc.), (ii) nutritional composition (vitamins, omega 3, etc.), (iii) the area where the product was caught or farmed (for sustainability reasons, or with particular regard to EU legislation regarding protected designation of origin (PDO), protected geographical indication (PGI), traditional specialities guaranteed (TSG) etc.), (iv) status of the product (such as whether the product has been previously frozen and defrosted) and (v) the presence of undeclared ingredients that can also represent a health risk for the consumer (allergens such as gluten, nuts, etc.).

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The authors of the Food Integrity Handbook gratefully acknowledge the previous work compiled in the book "FAIM Food Authenticity – Issues and Methodologies" (EU-funded Concerted Action AIR3-CT94-2452, 1994 – 1997). Published in 1998, this early work inspired both the format and content of this new Handbook on Food Authenticity. The contributors to the FAIM document are listed below together with their organisations at the time the book was published.

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FoodIntegrity Handbook

Food Fraud has been around a long time but following several highly mediatised incidents such as the milk and infant formula contaminated with melamine in 2008 and the horsemeat scandal in 2013, all authenticity issues have become big news. Regulators and customers now require food operators to keep abreast of any potential risks and to regularly assess their raw material and ingredient supply chains for vulnerability to food fraud. It is hoped that this FoodIntegrity Handbook will be a useful companion to help the food industry achieve this aim.

The Handbook has been written for food business operators and is primarily aimed at quality control managers working in food production and to those actors involved in the food supply chain. It may also be useful to young scientists, students and researchers with little prior knowledge of the area.
Written by nearly 50 experts in food authenticity, it is the result of European collaboration through the EU-funded FoodIntegrity project.

The book's editors, Jean François Morin and Michèle Lees, are respectively the current and former Directors of Collaborative Research at Eurofins Analytics France, an analytical laboratory renowned for its pioneering research into Food Authenticity.

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