thermo scientific

Authenticity and Adulteration

of food and beverages

Overview of Regulation, Analysis and Future Trends

Table of Contents

ThermoFisher
SCIENTIFIC

Executive summary

Table of Contents

Food adulteration has a long history, but recent scares such as melamine adulteration of milk powder and the horsemeat scandal have focused media attention on this area. The deliberate adulteration of food and misrepresentation to deceive the consumer is illegal world-wide. Consumer protection is enshrined in general food law, compositional standards for agricultural commodities are protected, and general labeling regulations require detailed information about food and beverages to be provided to consumers. Additionally, specific labeling regulations provide information to consumers concerning organic foods, genetically modified organisms (GMOs) and foods of designated origins as well as ensuring traceability throughout the food chain.

To support regulations, there are "targeted" and "untargeted" methods of analysis. Targeted methods are used to detect and quantify a known substance used for adulteration. Untargeted methods can be used to initially screen for adulteration, leading to identification and subsequent target analysis. Untargeted methods can also be used to "fingerprint" foods, by measuring a number of different variables and looking for characteristic patterns employing statistical techniques (chemometrics).

A wide range of instrumental techniques such as chromatography, mass spectrometry, elemental and isotopic analysis, molecular spectroscopy and molecular biology are used for monitoring adulteration and authenticity. In the future more "hand-held" devices will become available for use in the field for rapid testing for adulteration. And increasingly sophisticated untargeted methods will improve the ability to detect subtle differences between genuine and fraudulent food and beverages.

Table of Contents

Historical background

The deliberate adulteration of food has a long history and dates back to times when trading first began with people producing and selling food to their customers. Adulteration was frequently very crude and involved commodities such as bread flour, which was commonly adulterated with, for example, sand, ash, sawdust or mustard flour. White flour, as opposed to whole meal flour, at that time was considered a luxury and demanded a premium price, and a whiter appearance could be achieved by adding ground animal bones to flour. Tea was adulterated using dried beech leaves, milk with the simple addition of water and coffee could be bulked up with maize and other cereal grains. In the UK, the first food laws were not concerned with food safety, but were introduced to combat adulteration. The Adulteration of Food and Drugs Act 1860 (later revised in 1872) and the Sale of Food and Drugs Act 1875 established food adulteration as a criminal offense and also made provision for the appointment of public analysts for the official testing of foods.

Despite the passing of 150 years since adulteration was recognized as being a major concern for the food chain, it still remains a significant problem. As the food supply chain is now global, is exceedingly complex and there are many players involved between production and consumption, it is in fact much easier to conduct fraud without being easily detected. Nowadays, milk adulteration is a major issue in the dairy industry and has been causing concerns among consumers and food manufacturers. Milk is one of the most common targets for adulteration, usually accomplished by the addition of water, whey, sodium hydroxide (caustic soda), urea or melamine, among other substances. Adulteration is carried out to increase volume, mask inferior quality, and replace the authentic substances for the seller's economic gain. Food adulteration has become increasingly sophisticated, often being specifically designed to avoid detection through routine analysis.

> In the UK, the first food laws were not concerned with food safety, but were introduced to combat adulteration.

Historical background

There are a number of different terms used in this area such as adulteration, economic adulteration, authenticity, food fraud, deliberate mislabeling, substitution, misbranding and counterfeiting. Adulteration is the deliberate addition to food of a substance or substances with the aim of deceiving the consumer in terms of the quality of the food being traded. This addition could be of a single chemical such as Sudan I dye aimed at improving the color of spices to give the appearance of a higher quality. Alternatively, it could be simply blending (dilution) of a premium product such as virgin olive oil with a low-value vegetable oil at levels which cannot be readily detected by taste by the consumer. Although adulteration is a deliberate attempt to mislead in terms of the authenticity of a foodstuff, the same result can also be achieved by deliberate mislabeling without any adulteration taking place. Examples are deliberate mislabeling of wine or honey in terms of geographical or botanical origin. Again the deception is about misleading the consumer into believing a product is of a higher quality than it actually is and therefore misleading the consumer into paying a higher price. The process of adulteration and mislabeling in terms of food authenticity is sometimes described as "food fraud" or is referred to as "economic adulteration" indicating it is part of a deliberate attempt to defraud.

Counterfeiting refers to the deliberate manufacture of a food or beverage mimicking a well-known product and using imitation packaging and labeling to pass-off as a recognized premium brand. This has most frequently occurred in the area of alcoholic beverages where illegally distilled alcohol is sold as a branded product such as vodka.

The term "traceability" is frequently used in the area of authenticity. It describes the process whereby all raw materials and all ingredients in a food or beverage can be "traced" back through the food chain, so there is documented evidence supporting their source and provenance. In EU and USA law there is a statutory requirement on food suppliers to maintain a traceability system for all food products.

Product	Adulteration	Years	Financial & Health Effects
Olive oil	Industrial oil denatured with aniline	1981	600 deaths reported in Spain
Orange juice	Beet sugar syrup, water and malic acid	1980's	Prosecutions of juice suppliers
Wine - Austria	Ethylene glycol	1985	Market recall – huge damage to Austrian wine industry
Chili spices - Asia	Sudan and other illegal dyes in spices, palm oil and processed foods	2005	Largest supermarket recall ever in UK costing £millions
Milk powder - China	Melamine & cyanuric acid	2008-2009	300,000 victims, 6 infant deaths & 54,000 babies hospitalized
Animal feed - Ireland	Adulteration of pig feed with waste oil	2008	PCBs and dioxins in pork – Estimated €200 million financial losses
Animal feed - Germany	Adulteration of feed with contaminated waste cooking oil	2010	PCBs and dioxins in meat – restrictions on 5000 farms
Sports & tea drinks - Taiwan	Phthalates (DEHP) 2-20 ppm added as clouding agent to replace palm oil	2011	Health effects unknown but exposure above TDI for up to 15 years
Meat & meat products - Europe	Horse meat	2013	Large scale food recalls, RASFF alerts and prosecutions of processors
Cumin spice – India, Turkey	Ground peanut & almond shells	2015	Dangerous to allergen suffers but no individuals identified

Table showing high profile food and beverage scandals

Regulations to prevent adulteration and ensure food authenticity

Table of Contents

General food law - The framework for food safety in the EU is covered in **REGULATION (EC) No 178/2002** which lays down the general principles and requirements of food law. This regulation, which also established the European Food Safety Authority (EFSA) as the independent EU risk assessment body, lays down all procedures in matters of food safety. Specifically with respect to adulteration and authenticity Article 8 – covering protection of consumers' interests states that food law shall aim at the prevention of (a) fraudulent or deceptive practices; (b) the adulteration of food; and (c) any other practices which may mislead the consumer. While these general principles make it very clear that it is illegal to deliberately adulterate food or to fraudulently deceive the consumer, it is more difficult to translate this principle into more detailed provisions until specific cases of adulteration are detected.

In the USA the **Food Safety Modernization Act** was introduced in 2011 recognizing the global nature of the food supply chain and the need for a radical update in food safety law. Much of this act is similar to the general provisions of EU food safety law concerning, for example, the need for HACCP, GMP and accreditation requirements for food testing laboratories. Section 106 deals with protection against intentional adulteration and is aimed at preventing acts intended to cause wide-scale harm to public health, including acts of terrorism targeting the food supply. This section says that "such acts, while not likely to occur, could cause illness, death and economic disruption of the food supply." Rather than targeting specific foods or hazards, this rule requires mitigation (risk-reducing) strategies for processes larger registered food facilities. These provisions relate primarily to acts of terrorism in the food supply chain rather than economic adulteration.

Generally, the safety of food is regulated through approval of what can and cannot be added to foods, and risk-based limits are applied, for example, to additives, residues and adventitious contaminants. Thus, there are positive lists of approved substances that can be added to food (food additives), such as colors with associated maximum permitted levels and provisions as to which foods they can be added. Pesticide and veterinary drug residues in foods are similarly controlled by licensing approved agrochemicals and veterinary drugs and then stipulating maximum residue limits (MRLs) based on risk assessment. Maximum limits (MLs) of adventitious contaminants such as metals, mycotoxins, PCBs and dioxins in specific foods are stipulated in a number of EU contaminant regulations. There may be differences in detail between the EU and the USA but the principles are the same, and in many cases there has been some harmonization through the setting and agreeing of food standards through bodies like Codex Alimentarius.

> "General food law prohibits fraudulent or deceptive practices, adulteration of food and practices which mislead the consumer"

Regulations to prevent adulteration and ensure food authenticity

Thus, for food additives the regulations clearly make it illegal to add any unauthorized color such as Sudan dye to any foodstuff. So adulteration using an illegal additive is covered by existing food safety law. However, what constitutes the cut-off point in terms of enforcement action for the presence of illegal dyes in processed foods is not given in regulations, and has proved controversial in view of the low level of detection achievable with modern analytical instruments. In some areas of deliberate adulteration, it has proved necessary, after the event to provide specific limits for the substance in question e.g. EU Regulation 594/2002 sets a maximum level of 2.5 mg/kg for melamine in foods and specifically a limit of 1.0 mg/kg for melamine in powdered infant formula and follow-on formula. Similarly, China set a maximum limit of melamine in baby formula milk of 1 mg/kg, in liquid milk including raw milk, milk powder and other formula milk powder of 2.5 mg/kg and in other foods containing 15% and above milk to 2.5 mg/kg. A national standard method (including a rapid high performance liquid chromatography method) for determination of melamine in raw milk and dairy products was issued by the PRC Standardization Administration. For adulteration of meat and meat products by horsemeat a cut-off point of 1% was generally accepted as being the action limit, although methods of analysis are capable of detecting far lower levels of adulteration.

Area regulated	Scope	Regulations
Adulteration - general	Prevention of adulteration	Regulation 178/2002
Authenticity - general	Fraudulent & deceptive practices	
Intentional adulteration	Prevent terrorists acts of food adulteration & requires mitigation	FSMA 2011 (US)
Melamine adulteration	Limits of 2.5 ppm in food & 1.0 ppm in infant formula	Regulation 594/2002
Authorized food colors	Prevention of use of illegal dyes	Directive 94/36/EC
Emergency measures for illegal dyes in imported foods	Provision for import controls of chilli, chilli products, curcuma & palm oil	Decision 2005/402/EC
Food Labeling	Prevention of misleading information on food labels	Regulation 1169/2011
Olive oil – vertical regulation	Compositional and labeling standards for olive oils	Regulation 1019/2002
Wine – vertical regulation	Organization of agricultural markets including wine	Regulation 1308/2013
Wine vinegar – vertical regulation	Compositional and labeling standards for wine vinegar	Regulation 479/2008
Agricultural products	Protected designations of origin Establishes PDO Establishes TSG	Regulation 607/2009, Regulation 510/2006, Regulation 509/2006
GM foods	Labelled as GM if more than 0.9%	Regulation 1829/2003
Organic foods	Labeling of organic foods	Regulation 834/2007
Traceability	Traceability must be established at all stages of food production	Regulation 178/2002

Examples of regulations related to authenticity and adulteration

Regulations to prevent adulteration and ensure food authenticity

Food labeling regulations

Protection against fraudulent and deceptive practices intended to mislead consumers is also provided through very detailed food labeling regulations. **REGULATION (EU) No 1169/2011 of 25 October 2011** covers the provision of food information to consumers and ensures that food descriptions are tightly defined to prevent misleading branding. In the EU the indication of geographical origin has been mandatory for a number of years for beef and beef products following the BSE (mad cow) crisis, and it has created consumer expectations for origin labeling, with the origin of meat appearing to be consumers' prime concern.

For some commodities other than beef, mandatory origin provisions have been developed on the basis of vertical approaches, for instance honey, fruit and vegetables, fish and olive oil. For the future it has been recognized that there is a need to explore the possibility to extend mandatory origin labeling to other foods. Types of meat such as swine, sheep, goat and poultry, meat used as an ingredient, unprocessed foods, milk and milk used as an ingredient in dairy products, single-ingredient products, and ingredients that represent more than 50% of a food will probably, in the future, require mandatory labeling of origin. Milk is one of the products for which an indication of origin is considered of particular importance. An example of vertical controls in place in the EU is provided by **Regulation (EC) No 1019/2002** which sets out marketing standards for olive oil. The labeling of olive oil must show in clear and indelible lettering the following information on the category of oil:

a) Extra virgin olive oil: "superior category olive oil obtained directly from olives and solely by mechanical means"

b) Virgin olive oil: "olive oil obtained directly from olives and solely by mechanical means"

c) Olive oil composed of refined olive oils and virgin olive oils: "oil comprising exclusively olive oils that have undergone refining and oils obtained directly from olives"

d) Olive-pomace oil: "oil comprising exclusively oils obtained by treating the product obtained after the extraction of olive oil and oils obtained directly from olives," or "oil comprising exclusively oils obtained by processing olive pomace oil and oils obtained directly from olives"

These categories are very clearly defined and the various regulations on olive oil provide a substantial amount of compositional data related to quality parameters. There are no definitive methods to distinguish these categories or detect whether a lower grade oil such as deodorized oil has been added to "extra virgin olive oil" as economic adulteration.

Table of Contents

Regulations to prevent adulteration and ensure food authenticity

EU regulation No 1308/2013 establishes a common organization of the markets in agricultural products and outlines the system for the control of 24 specified agricultural sector products including wine. **EU regulation (EC) No 607/2009** lays down rules as regards to protected designations of origin and geographical indications, traditional terms, labeling and presentation of certain wine sector products. This regulation provides detailed labeling rules and annual verification requirements for specified categories of wines. Chaptalization is the process of adding sugar to unfermented grape must in order to increase the alcohol content after fermentation. In the EU, chaptalization is not permitted except in designated wine growing zones in more northerly areas, where grapes might not ripen sufficiently to produce sufficient glucose and fructose. Control of chaptalization is strictly controlled in EU countries, although permitted in the USA apart from California. Detection of illegal addition of sucrose during fermentation is generally detected by SNIF-NMR or isotopic measurements using IRMS.

Another example of a vertical regulation concerns wine vinegar which is defined by **EC Regulations 479/2008** (Annex IV, sections 1 and 17) as a product obtained from the acetous fermentation of wine, which is in turn defined as a product obtained exclusively from the alcoholic fermentation of fresh grapes, whether crushed or not, or from grape must.

In the EU there is no legal requirement to label food as being Halal or non-Halal. If a description "Halal" is made, then it must be clear which product the description refers to, if the business is not to run the risk of committing offenses of misrepresenting the foods on sale. At present there are few recognized systems of certifying that a particular food is Halal. However, certain Muslim organizations in the UK and elsewhere are collaborating to develop an umbrella certification board for Halal foods.

Regulations to prevent adulteration and ensure food authenticity

Table of Contents

Labeling of genetically modified (GM) foods

There are specific requirements laid down in **Regulation 1829/2003** on genetically modified food and feed. In the EU, if a food contains or consists of GMOs or contains ingredients produced from GMOs, this must be indicated on the label. The rules cover all GM food and animal feed, regardless of the presence of any GM material in the final product. This means products such as flour, oils and glucose syrups have to be labelled as GM if they are from a GM source, whereas products produced with GM technology (cheese produced with GM enzymes, for example) do not have to be labelled. Products such as meat, milk and eggs from animals fed on GM animal feed also do not need to be labelled. Any intentional use of GM ingredients at any level must be labelled. There is a threshold for the adventitious, or accidental, presence of GM material in non-GM food or feed sources, which is set at 0.9% and only applies to GMOs that have an EU authorization. In the USA there is a requirement for GMOs to be authorized by the FDA, but there are no specific requirements concerning labeling of GM foods.

Table of Contents

Regulations to prevent adulteration and ensure food authenticity

Labeling of organic foods

Aims and overarching rules of organic food production are set out in **EU Regulation No 834/2007** which also stipulates how organic products must be labelled. Foods may be labelled "organic" only if at least 95% of their agricultural ingredients meet the necessary standards. In non-organic foods, any ingredients which meet organic standards can be listed as organic. The code number of the certifying organization must be provided. In the UK there are nine approved organic control bodies such as the "Organic Food Federation" and the "Soil Association." Organic production outlaws the use of GMOs and derived products. The regulation on GM food and feed lays down a threshold (0.9%) under which a product's GMO content does not have to be indicated, and therefore products with GM content below this threshold can be labelled organic.

Producers of packaged organic food are required under EU law to use the EU organic logo. However, this is not a binding requirement for organic foods from non-EU countries. Where the EU organic logo is used, the place where any farmed ingredients were produced must be indicated.

Organic products from non-EU countries can be distributed in the EU market only if produced and inspected under conditions that are identical or equivalent to those applying to EU organic producers. Control bodies (certifying organizations) operating in non-EU countries must be directly authorized and monitored by the European Commission and EU countries. The European Commission therefore supervises and monitors the import of organic products and the checks carried out on organic guarantees.

thermoscientific

Table of Contents

Regulations to prevent adulteration and ensure food authenticity

Schemes to protect specific traditions of foods and beverages

There are three different schemes aimed at protecting specific traditions and qualities of food, agricultural products and wines, and aromatized wines and spirit drinks produced in the EU or in other countries. Each scheme has a well-recognized logo which is only permitted to be displayed on foods or beverages which fulfil the specific requirements. Protected Designation of Origin (PDO) and the Protected Geographical Indication (PGI) have a specific link to the region where the product comes from, while Traditional Speciality Guaranteed (TSG) highlights a traditional production process. Food products are eligible for all three logos: PDO, PGI and TSG, while wine is eligible for PDO and PGI status, and spirit drinks and aromatized wines only qualify for PGI recognition.

Council Regulation (EC) No 510/2006 makes provision for the designation PDO covering agricultural products and foodstuffs which are produced, processed and prepared in a given geographical area using recognized know-how.

Council Regulation (EC) No 509/2006 makes provision for TSG which highlights traditional character of specified foods.

For **Protected Geographical Indication**, rather than specific regulations, the EU has concluded 17 bilateral agreements with trade partner countries such as USA, Canada, Australia and South American countries that provide for geographical indication protection. PGI covers agricultural products and foodstuffs closely linked to a geographical area.

For PGI, at least one of the stages of production, processing or preparation must take place in the protected geographical area. High-quality, renowned products are frequently the target of counterfeiting, in particular the copying and passing off of well-known geographical indications such as "Chablis" or "Queso Manchego" or "Prosciutto di Parma," The level of protection granted and the list of the protected geographical indications is defined in each bilateral agreement.

In the EU there is Database of Origin & Registration (DOOR) which supports the agricultural product quality policy by providing a modern information technology system for the dissemination of public data with regard to registered PDOs, PGIs and TSGs.

Regulations to prevent adulteration and ensure food authenticity

Dietary supplements and herbal medicines (botanicals)

The use of the words dietary supplement, food supplement, botanical, nutraceutical, functional food, Traditional Chinese medicine, dietetic food, energy or slimming food is confusing as there is significant overlap in definitions. Different terms are also being used for the same products, and the same terms used in different countries to mean different products. Botanicals are herbal/plant derived products, which are used as ingredients in some dietary supplements, in some nutraceuticals, and in some functional foods. Chinese herbal medicines are wholly composed of botanicals or derived from botanicals. Dietary supplements sit somewhere between pharmaceuticals and foods, being available in dose form (tablets, capsules, etc.) but not regulated as medicines. The global market for vitamins, minerals, and nutritional and herbal supplements (VMHS) has been estimated to be around $82 billion, with roughly 28% of that in the US, where sales increased by approximately $6 billion between 2007 and 2012. Growth is expected to be between 5–6% a year both globally and in the US. With such a high value market, it is not difficult to see that there is a significant incentive for adulteration.

In the USA and EU there is less regulatory control for dietary supplements than foods, and stricter controls on vitamins and minerals than on botanicals. In the USA no pre-market approval of dietary supplements is required, but manufacturers must produce dietary supplements to minimum quality standards and ensure that they do not contain contaminants or impurities, and are accurately labelled. In the EU food supplements (vitamins and minerals) require EFSA risk assessment to ensure safety for which supporting analytical data is required. EFSA has not to date specifically evaluated botanicals, but EFSA has a compendium of 900 botanicals which lists those approved and those currently not permitted for use in either dietary supplements or foods.

Due diligence rules apply to dietary supplement manufacturers in a similar way to the food sector, and routine testing of dietary supplements should therefore be at a similar level or higher. In the EU and US there are no indications of any forthcoming regulatory changes, and existing regulations already provide good consumer protection in the area of vitamins and minerals. There is no obvious need to strengthen existing regulations, but there is a need to develop better tools (analytical methods). Investment is also needed both by dietary supplement manufacturers to improve quality assurance and by enforcement authorities to increase the extent of monitoring these products.

Table of Contents

Traceability

Traceability is important as a food safety measure in the event of the need for a product recall due to microbiological or chemical contamination, for example, in instances when eggs were contaminated with dioxins and a large number of egg-containing processed food products needed to be identified for recall. Article 18 of **EU REGULATION (EC) No 178/2002** stipulates that the traceability of food, feed, food-producing animals, and any other substance intended to be, or expected to be, incorporated into a food or feed shall be established at all stages of production, processing and distribution. With respect to authenticity and adulteration, traceability provides in many instances the only method to reliably check, for example, geographical origin, where other indicators such as isotopic ratios or trace elemental analysis may not be sufficiently well developed. Section 204 of the USA **2011 Food Safety Modernization Act** is very similar to the EU as it also requires "tracking and tracing" by food suppliers. The FDA established some pilot projects to develop methods and technologies to enhance "tracking and tracing" though the food supply chain including imported foods.

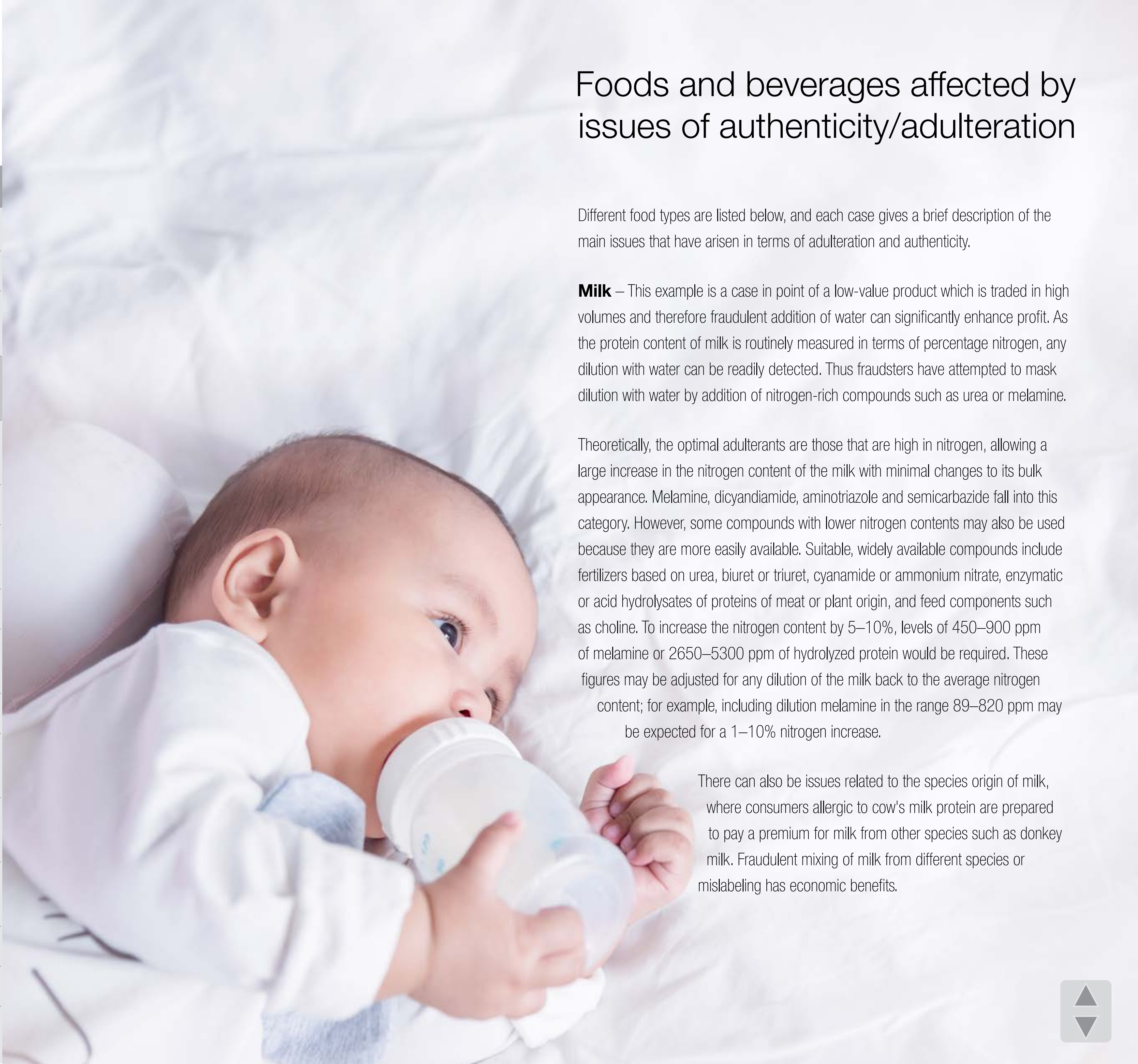
Foods and beverages affected by issues of authenticity/adulteration

Table of Contents

The list of foods that have been affected either by deliberate adulteration or by issues surrounding their authenticity are discussed below with brief explanations. The accepted wisdom has been that economic adulteration and false claims regarding authenticity tend to be limited to high-value food products where there is most to be gained financially by this fraud. It is also the case that commodities traded in high volumes, despite relatively low values, are targets for fraud as small dilutions when scaled up can lead to high profits.

In a published study from China covering a period from 2004 to 2014, media reports of economically motivated food fraud and adulteration were studied. From a total of 1553 reports, it was established that 38% involved animal products, 23% grain-based foods and 13% beverages. The main issues of adulteration were the use of illegal additives (23%) and counterfeit foods (11%) with recycled or discarded foods counting for 10% of the media reports. These media reports all appear to concern crude adulteration as opposed to rather more subtle cases of misrepresentation in Europe, for example, concerning geographical origin.

Table of Contents



Foods and beverages affected by issues of authenticity/adulteration

Different food types are listed below, and each case gives a brief description of the main issues that have arisen in terms of adulteration and authenticity.

Milk – This example is a case in point of a low-value product which is traded in high volumes and therefore fraudulent addition of water can significantly enhance profit. As the protein content of milk is routinely measured in terms of percentage nitrogen, any dilution with water can be readily detected. Thus fraudsters have attempted to mask dilution with water by addition of nitrogen-rich compounds such as urea or melamine.

Theoretically, the optimal adulterants are those that are high in nitrogen, allowing a large increase in the nitrogen content of the milk with minimal changes to its bulk appearance. Melamine, dicyandiamide, aminotriazole and semicarbazide fall into this category. However, some compounds with lower nitrogen contents may also be used because they are more easily available. Suitable, widely available compounds include fertilizers based on urea, biuret or triuret, cyanamide or ammonium nitrate, enzymatic or acid hydrolysates of proteins of meat or plant origin, and feed components such as choline. To increase the nitrogen content by 5–10%, levels of 450–900 ppm of melamine or 2650–5300 ppm of hydrolyzed protein would be required. These figures may be adjusted for any dilution of the milk back to the average nitrogen content; for example, including dilution melamine in the range 89–820 ppm may be expected for a 1–10% nitrogen increase.

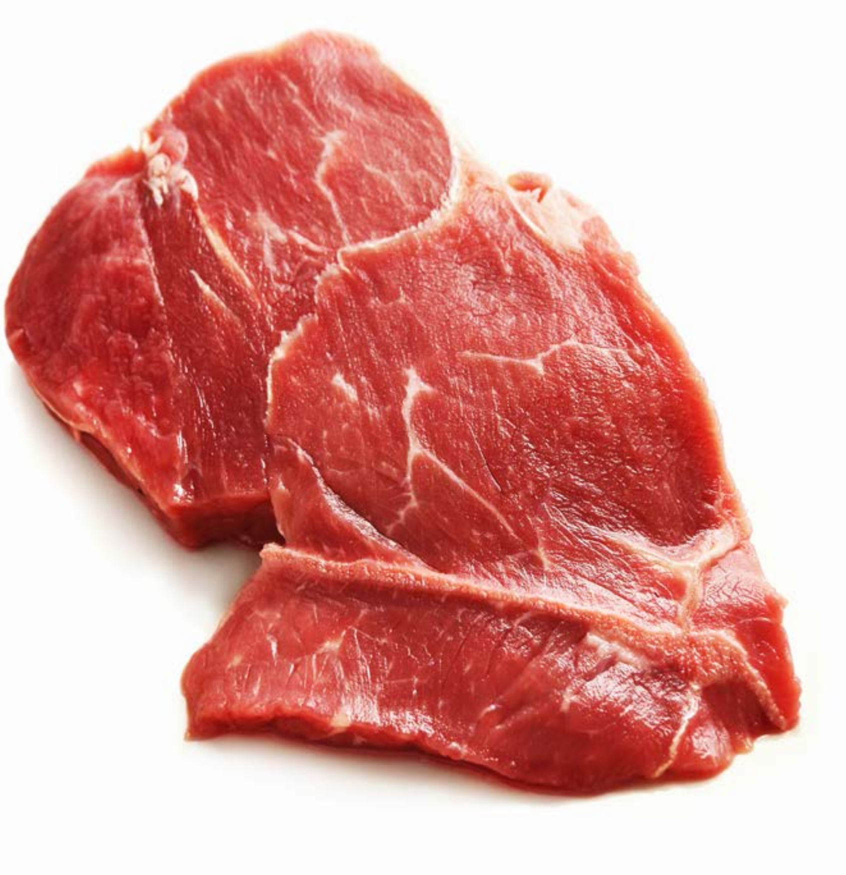
There can also be issues related to the species origin of milk, where consumers allergic to cow's milk protein are prepared to pay a premium for milk from other species such as donkey milk. Fraudulent mixing of milk from different species or mislabeling has economic benefits.

Foods and beverages affected by issues of authenticity/adulteration

Olive oil – The biggest food scandal in recent times was the "toxic oil syndrome" in Spain in 1981 where around 600 deaths were reported. An industrial oil denatured with aniline was deliberately sold as olive oil by street traders. Olive oil is one of the commodities where substantial fraud is believed to occur largely because of large price differences between premium grade "extra virgin olive oil" and other grades. Geographical origin is also important in determining price. Blending of olive oils with other oils such as hazelnut oil and adding "deodorized olive oil" to virgin oil is an adulteration by dilution which is difficult to detect.

Oils – There have been cases of waste cooking oils being "cleaned" and resold as edible oils. Adulteration of sesame oil, walnut oil, pumpkin seed oil, peanut oil and soybean oil with cheaper lower grade oils has also been detected.

Vinegar – Wine vinegar cannot contain acetic acids obtained from either petroleum derivatives or pyrolysis of wood (synthetic acetic acid) or from the fermentation of non-grape sugars. Neither can wine vinegar be produced from dried grapes diluted with water. Therefore “raisin vinegar”, produced by fermenting dried grapes and rehydrating with water, cannot be considered wine vinegar. Balsamic vinegar “aceto balsamico di Modena IGP” is obtained from cooked and/or concentrated grape must and aged in oak for different periods of time. There is plenty of scope for misrepresentation in this area where there are large price differentials between low grade and genuine IGP balsamic vinegar.



Foods and beverages affected by issues of authenticity/adulteration

Meat and meat products – The adulteration of beef products with horsemeat in 2013 was a major food scandal in Europe, which not only involved economic fraud, but raised concerns about social and religious taboos. The adulteration of comminuted meat products, e.g., with offal and the detection of undeclared soy protein, can also be of concern. The addition of pork to other higher value meat products also infringes on religious practices. This extends to labeling of food products containing gelatin as being Halal, which has driven the need to demonstrate the origin of the gelatin in a variety of processed food products including confectionary. Another issue with meat, particularly beef, can be whether it is organic or whether from a specific geographical region such as "Prime Aberdeen Angus", which are particularly difficult claims to challenge. There have also been cases of adulteration intended to change the appearance of fresh meat to enhance the red color by illegal use of carbon monoxide.

Fish and other seafood – Partial or complete substitution of one fish species by a lower value one can be easily carried out in comminuted processed fish products, e.g., partial substitution of prawn or sampi by cheaper white fish meat. There are also price differences between "farmed" and "wild" fish of various species such as salmon, which makes mislabeling farmed fish as wild financially attractive. For commercial fishing operations, it is illegal to dip fish in formaldehyde to prevent parasitic or fungal diseases or to use it as a preservative to increase the shelf life of the fish. Recent media coverage has indicated possible illegal use of formaldehyde on cod and other commercially sold fish, and that certain fish products available in the US were contaminated with formaldehyde.

Table of Contents

Foods and beverages affected by issues of authenticity/adulteration

Honey and bee products – Adulteration of honey can involve dilution with inexpensive sugar syrups such as high-fructose corn, glucose or saccharose syrups, which are produced from beet or canes. Excessive feeding of bees with sucrose or other syrups is also not permitted. In addition to adulteration of honey, mislabeling occurs to a significant extent with mislabeling of either botanical origin (pollen type) or geographical origin of honey as there are large price differences between different honey types. Single floral types of honey command a premium as does, for example, Manuka honey from New Zealand which is particularly prized for its claimed bioactive properties. Similar issues surround Royal jelly production where artificial sugar should not be used. The adulteration of high commercial value beeswax, which is important to bee-keepers for the production of high quality honey, has also been reported where paraffin waxes have been deliberately added to the beeswax.

Cereals including rice – Melamine and other nitrogen-rich compounds have been used to adulterate wheat flour to enhance apparent protein content. For some cereals such as rice, there are varieties like basmati which command a significantly higher price, and mislabeling is believed to occur to a significant extent. There are also issues concerning labeling of organic and conventional wheat, and a need to verify whether cereals are genetically modified and whether appropriately labelled.

Foods and beverages affected by issues of authenticity/adulteration

Table of Contents

Nut products – Hazelnuts and their derivatives (oils and pastes) are widely used as ingredients in many desserts, ice creams and chocolates. The price of hazelnuts depends on geographical origin, abundance of harvest, etc. When there are unfavorable economic conditions, the supply can be maintained by adding other ingredients such as ground almond, because of its physical similarity. Or other more unexpected products might be added, for example chickpea.

Fruit juices – Orange juice has been adulterated by dilution with water and masked by the addition of sugar and malic acid. With high value juices like pomegranate and lemon juices there can also be authenticity issues, and with some juices like apple the variety and origin can affect the price, offering scope for mislabeling.

Fruit – Lemons grown in a typical lemon-growing area of southern Italy (Siracusa), have been awarded the PGI recognition as "Limone di Siracusa". This PGI status commands a higher price and therefore provides scope for mislabeling. Essential oils from lemons are also of high value, and their authenticity is therefore important in determining the price. The popularity and high value of cranberries with claimed health benefits also has potential for adulteration and mislabeling.

Table of Contents

Foods and beverages affected by issues of authenticity/adulteration

Cocoa, coffee & tea – Arabica coffee beans provide a high-quality brew with intense aroma and a finer taste than Robusta beans. The market price for Arabica beans is about 2–3 times higher than Robusta, providing the temptation to dilute by blending to increase profit. Geographical origin is also important as again the market price can be significantly different depending on the where the beans have been grown. The deliberate addition of other materials such as coffee husks to the ground beans during the roasting process helps to reduce costs. Roasted fine ground coffee husk has a similar physical appearance to ground coffee beans making it difficult to detect coffee adulterated with husk by a simple visual inspection.

For cocoa after harvesting of the pods, beans are fermented and dried to improve the final quality of the product. Some farmers may willfully mix unfermented cocoa beans with fermented ones, especially when they are under time pressure to meet demand. Unfermented cocoa beans have an astringent taste and unpleasant flavor, and affect the desired characteristics of the derived chocolate.

Similar to coffee, the geographical origin of tea can determine the price, particularly for some favored green teas, driving the temptation to mislabel. Some herbal teas particularly when used for medicinal purposes, can be adulterated either deliberately or inadvertently with ingredients which are not the intended botanical species.

Table of Contents

Foods and beverages affected by issues of authenticity/adulteration

Vegetables – There have been some publications concerned with the geographical origin of Chinese cabbage and whether tomatoes are correctly labelled as organic or conventional. In general, vegetables are a commodity of only minor concern for authenticity and adulteration.

Cheeses – This is a high-value product and the control of geographical origin is an important factor for PDO cheese as well as for cheese types produced in different countries and sold under the same name. There can also be issues of adulteration of cheese, where milk casein produced for industrial (non-food) use has been deliberately added back to cheeses like mozzarella.

Eggs – Whether eggs are "free range" or produced from caged birds affects the price, and eggs can easily be deliberately mislabeled as "free range".

Foods and beverages affected by issues of authenticity/adulteration

Wine – As there are significant price differences depending on varieties and geographical origin of wine, there is considerable scope for misrepresentation. It is also not permitted to add sugar to grape juice prior to fermentation except for certain approved geographical areas, and this can be a source of adulteration. The only major scandal with wine occurred with Austrian wine in 1985, when diethylene glycol was deliberately added to make the wine appear smoother to the palate and increase its apparent quality and market value.

Beer – The geographical origin of beer can be an issue when specialist beers are exported, but generally this is not a major area of fraud. Several cases of beer counterfeiting have been recently reported, wherein the counterfeiters have switched the labels and bottle caps of cheap brands for the labels and caps of the more expensive market leader brands.

Spirits and liquors – There are some very special liquors such as "Limoncello" which must be made using special lemons and following a defined process. Cheaper products are fraudulently sold, and are made from lower cost fruit and not following the required process. There is also a market in counterfeit spirits either misrepresenting high-value brands such as whisky or counterfeiting white spirits illegally distilled and sometimes containing with dangerously high levels of methanol.

Table of Contents

Foods and beverages affected by issues of authenticity/adulteration

Herbs and spices – As the value of some spices such as chili powder is determined by the color, the illegal addition of dyes to spices can significantly enhance the price. Sudan I dye, which is not approved for food use, was first detected as present in chili, and as the authorities began monitoring, the fraudsters switched to using other dyes with a wide range of unauthorized dyes eventually being found. A shortage of cumin in 2015 led to cases of adulteration of this dried, powdered herb with ground peanut shells or almond shells, presenting a risk to allergen sufferers. Expensive spices like saffron are susceptible to substitution by other materials.

Flavorings – It is important in terms of price to know whether flavorings are naturally derived or chemically synthesized. For example, vanillin flavoring from vanilla beans is far more expensive than the chemically identical, but chemically synthesized "vanillin", making substitution financially attractive. Consumers are also prepared to pay a premium for products containing "natural flavorings" as opposed what are seen as "chemical" additives.

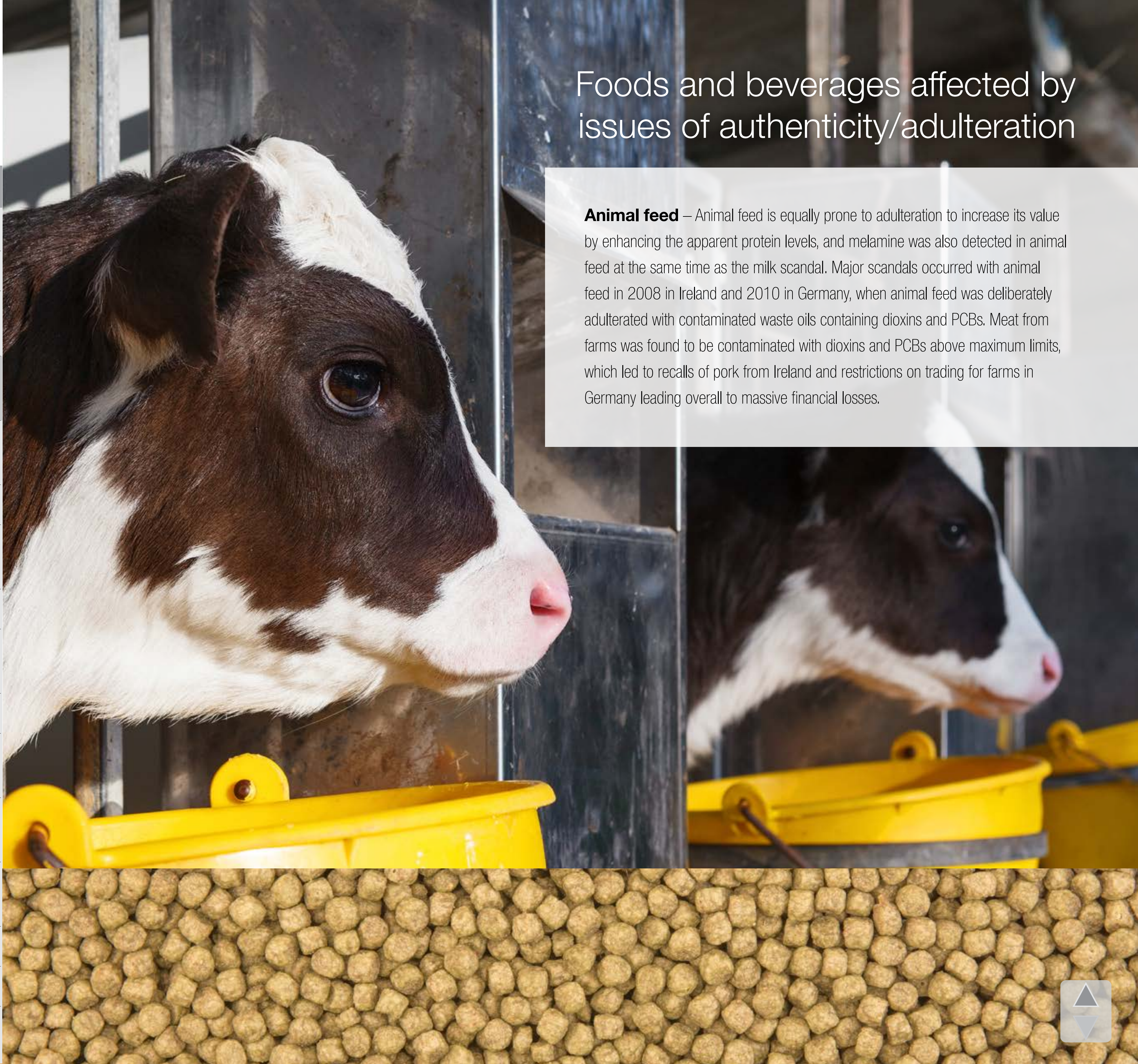
Table of Contents

Foods and beverages affected by issues of authenticity/adulteration

Dietary supplements – There is a considerable market for "natural products", mostly herbal medicines, with claimed health benefits such as herbal products sold as dietary aids or for erectile dysfunction. Consumers are attracted to such products as they deliberately wish to avoid taking licensed pharmaceuticals. As these natural products are mostly ineffective, they are increasingly being found to be deliberately adulterated with synthetic drugs. Unscrupulous producers may add licensed drugs and unlicensed analogues of various classes, such as phosphodiesterase type 5 (PDE-5) inhibitors, weight loss, hypoglycemic, antihypertensive and anti-inflammatory agents, or anabolic steroids, to develop or intensify biological effects of natural products. Some licensed drugs such as sildenafil and tadalafil have been detected, but more frequently untested analogues have been found raising questions of safety. There have been 852 rapid alerts (RASFF) for dietary supplements in the 5 years between December 2011 and December 2016, and 186 notifications in just the last 12 months.

Ginkgo biloba (ginseng) – Ginkgo biloba is very expensive, and one of the top-selling botanicals in the world, its high value leading to frequent adulteration. The most common form of adulteration is addition of a less expensive flavonol or flavonol glycoside to ginseng extract to achieve the required regulated level of flavonol glycoside.

Table of Contents



Foods and beverages affected by issues of authenticity/adulteration

Animal feed – Animal feed is equally prone to adulteration to increase its value by enhancing the apparent protein levels, and melamine was also detected in animal feed at the same time as the milk scandal. Major scandals occurred with animal feed in 2008 in Ireland and 2010 in Germany, when animal feed was deliberately adulterated with contaminated waste oils containing dioxins and PCBs. Meat from farms was found to be contaminated with dioxins and PCBs above maximum limits, which led to recalls of pork from Ireland and restrictions on trading for farms in Germany leading overall to massive financial losses.

Rapid Alert System for Food & Feed (RASFF)

A search of the RASFF portal for a 5-year period from 2011-2016 shows 665 notifications that were categorized as being adulteration or fraud. Many of these alerts relate to the absence of proper documentation such as health certificates or detection of fraudulent health certificates, suggesting attempts to export products that have not undergone proper checks or may not be fit for human consumption. This period encompasses the horsemeat scandal as evidenced by 83 alerts related to detection of horsemeat DNA in products as varied as canned & frozen beef, diced steak, beef burgers, meatballs, corn beef, sausages, salami, kebabs, cottage pie and pasta products like lasagna, ravioli, tortellini and Bolognese. These reports concern levels of horse DNA from 1% in composite products to some being almost entirely horsemeat, rather than the beef which was indicated on the label. Detection of horsemeat in samples seized by authorities has proved to be relatively easy, due to widespread availability and widespread expertise in applying PCR to DNA detection.

Other interesting examples of adulteration from RASFF notifications concern illegal use of red dyes with pork tenderloins which were labelled as being beef, mislabeling fish species such as puffer fish as monkfish, adulteration of milk powder with vegetable fats, adulteration of honey with sugar and mislabeling geographical origin of honey. There were also cases of adulteration of animal feed with a protein supplement reported as containing 27% ammonium sulfate to artificially enhance the nitrogen value. Apart from the period when there was a focus on horsemeat detection, in general there are far fewer reports of issues of authenticity than one might expect, probably reflecting the difficulties in detection and monitoring of these more subtle cases of misrepresentation which are undoubtedly occurring in international trade.

To support health and consumer protection, the European Commission Joint Research Centre (JRC) has launched a monthly summary of articles on food fraud and adulteration. The objective is to inform stakeholders of potential fraud cases in the global feed/food chain, giving them the opportunity for taking actions to counter fraud. The monthly summary is derived from articles from media globally, retrieved from the Medical Information System (MedISys), JRC-developed internet monitoring and analysis system, on the Europe Media Monitor and RASFF. The types of foods being searched are olive oil, fish, organic products, grains, honey, coffee, tea, spices, wine, certain fruit juices, milk and meat. The first report was published in September 2016.

Targeted and untargeted analysis

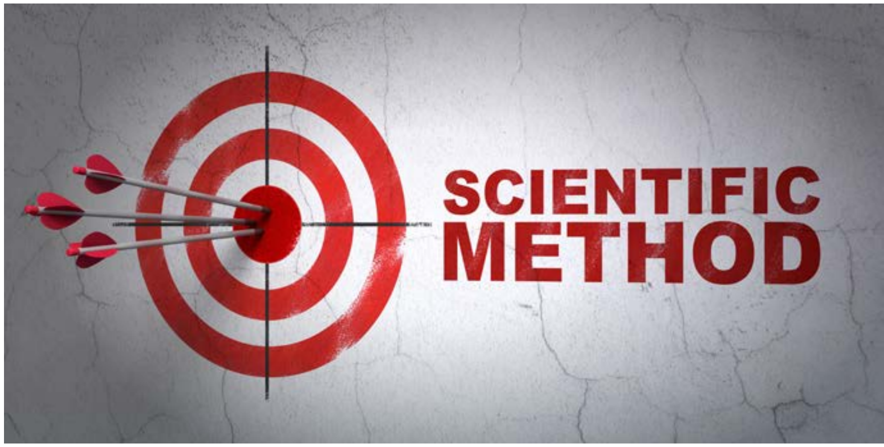
To enforce regulations on authenticity and adulteration there are "targeted" and "untargeted" methods of analysis. Targeted methods are used to detect and quantify a known substance used for adulteration. Untargeted methods can be used initially to screen for possible adulteration, leading to identification of the substance responsible and then subsequent target analysis. For authenticity, untargeted methods are primarily used to "fingerprint" foods, by measuring a number of different variables and looking for characteristic patterns employing statistical techniques (chemometrics).

For target analysis, the approach is the same as that used for the analysis of residues or contaminants in foods. Thus, for the analysis of target compounds chromatographic and mass spectrometric techniques are generally used typically, for example for the detection of melamine or Sudan dyes. However, frequently what starts out as target analysis for a single chemical progressively expands to cover a wider range of possible adulterants, as it becomes evident that other substances might be implicated. Thus, target analysis in spices was initially only for Sudan I, but screening subsequently needed to be expanded to include Sudan II, III and IV as well as Para Red and Basic Red 4G.

In some cases it may be that the adulterant itself cannot be readily detected, but the adulterant may contain "marker compounds" not found naturally in the commodity being adulterated, and these marker compounds can be targeted. 2-Acetylfuran-3-glucopyranoside has been used as a marker for honey adulteration by rice syrup in China, and biuret and triuret as markers for detection of urea-based fertilizer adulteration of milk.

Once there is a known adulteration problem then target analysis using molecular spectroscopy can be developed with a view to rapid screening, preferably without sample extraction and cleanup. This is where FTIR and Raman are being employed, particularly where it is possible to develop hand-held devices which can be used in the field. The lead-time required to develop and validate these techniques for rapid analysis can often mean that by the time the technology is available, the adulteration is no longer being carried out.

By its very nature, adulteration is inevitably difficult to detect, so increasingly "profiling" or "fingerprinting" of foods is being used as a means of highlighting any unusual features of the matrix, e.g., unexpected peaks in LC-MS. Once the unusual peak has been pin-pointed, it then requires classical identification techniques to be applied to identify and establish whether adulteration has occurred. This is a very challenging approach, as it relies on establishing a "normal profile" for a foodstuff in the form of a database and getting a good understanding of the natural variations that can occur. The drawback of this approach is that analytical techniques inevitably are selective as the whole food cannot be analyzed directly. Gas chromatography (GC) can only give a profile of volatile compounds in a foodstuff, while HPLC gives only a picture of non-volatiles after some selective pre-treatment like extraction. Comprehensive profiling probably requires a combination of fingerprinting to be conducted to cover both volatiles and non-volatiles, and possibly also to profile polar and non-polar non-volatiles in separate analyses. A technique like direct analysis in real time (DART) mass spectrometry overcomes some of these issues of discrimination as the whole food can be analyzed without pre-treatment. The downside is that NOT all chemicals are ionized using DART, and possibly the substance used for adulteration might not be seen.



Chromatography and mass spectrometry

Chromatography and mass spectrometry (GC, HPLC, GC-MS, LC-MS/MS and LC-HRMS) are the most widely used instrumental techniques for monitoring adulteration and verifying authenticity. Although GC and HPLC lack specificity of MS hyphenated techniques, once methods have been developed, they are frequently transferred to GC or HPLC particularly for authenticity when there is a need to generate results to populate databases.

After conducting a search using 'Web-of-Science' 389 peer-reviewed publications on food authenticity and adulteration were found, where the authors have used Thermo Scientific™ instruments. Of these publications 29 papers involved use of GC or GC-MS, mainly for volatiles in wine, honey and oils. The same search reveals 135 publications using HPLC and various forms of LC-MS being dominated by use of accurate mass spectrometry (HRMS) using Thermo Scientific™ LC-Orbitrap™ MS or variations such as Thermo Scientific™ LC-Q-Exactive™ MS (42 papers).

LC-Orbitrap MS has proved to be the definitive technique for unequivocal identification of chemicals in adulterated foods, such as Basic Red 4G detected for the first time in sumac spice, copper-chlorophyll color (E-141) in edible oils and numerous reports of the identification for the first time of synthetic drug analogues used to adulterate herbal products (medicines) and dietary supplements.

In addition to using LC-Orbitrap MS for unequivocal identification of previously unknown adulterants, it has also been used to initially identify individual substances within a class of compounds that might be employed for subsequent fingerprinting. For example, LC-Orbitrap MS has been used to identify individual polyphenols in apple juices for development of a means to classify juices based on apple variety and geographical origin as some juices have PDO status. In another study into the profile of unifloral sage honey using a non-targeted metabolomic approach, a significant number of flavonoid glycosides and phenolics were identified using LC-Orbitrap MS. After this initial identification work, a method was developed with cleanup combined with UHPLC with diode array detection (DAD) and MS/MS for target analysis. The selection of chemical markers from the group of phenolic compounds was based on their being sage nectar-pollen derived, propolis characteristic flavonoids and abscisic acid. These examples of apple juice and honey show how LC-Orbitrap MS has been used initially for untargeted identification of potential compounds that can be subsequently used for fingerprinting.

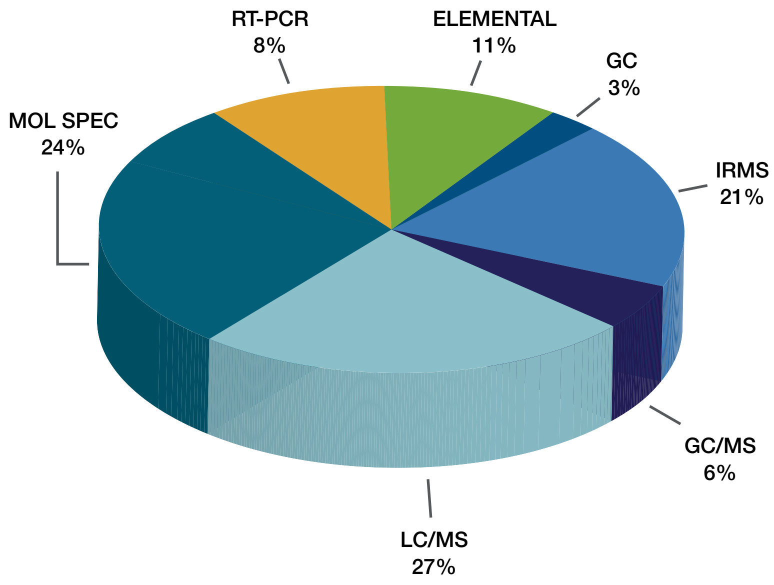


Table of Contents

Chromatography and mass spectrometry

Ion chromatography has been successfully employed where sugar profiles give unique indications of origin. In a study comparing high-value maple syrup with other natural sweeteners, anion exchange chromatography with pulsed amperometric detection (Thermo Scientific Dionex Ion Chromatography system) showed that maple syrup exhibits unique carbohydrate profiles dominated by high sucrose content, followed by glucose and fructose traces. Additionally, three oligosaccharides not previously reported in maple syrup, were detected with potential to be used as authenticity markers. Ion chromatography has also been used for the identification of adulterants in food products like honey and fruit juices; as well as for the characterization of the carbohydrate profile of agave syrups, coffee and natural sweeteners.

GC and GC-MS have proved to be very effective when the character of an authentic product is dictated by its volatiles and if unique marker compounds can be identified. In one study of wine authenticity, headspace solid phase microextraction (SPME) was employed for quantification of δ-ionone and δ-damascenone. The data obtained from a wide set of authentic wine samples led to the conclusion that the determination of both the enantiomeric distribution and concentration of δ-ionone can serve as a potential marker to detect a fraudulent addition of flavor essences to wine. GC-Orbitrap™ MS has been shown to be a promising approach to fingerprinting volatiles in either untargeted analysis in combination with chemometric techniques (PCA), or for identification of unique marker compounds. In a preliminary study of whisky samples using a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole Orbitrap™ GC-MS/MS system operating at a resolving power (FWHM) of 60,000, Scotch whisky samples from different distilleries could be distinguished from one another as well as from other whiskies such as Bourbon. The GC-MS profiles were different between Scotch and Bourbon, and some five volatile compounds were tentatively identified by accurate mass measurement as being at elevated levels in the Bourbon.

Table of Contents

Elemental analysis

Most trace elements naturally occur in agricultural products, but importantly for the same commodity, there are differences in the levels and patterns of multiple elements depending on different factors. These differences can enable the successful discrimination of products from different regions and countries, as trace elements are derived primarily from the soil and local environment unique to a geographical location. There is an assumption that the concentrations of the majority of elements will not be significantly altered during food processing, transportation or storage. Multi-element analysis involving as many as 50–60 elements has been successfully employed to determine the geographical origin of wine, cereals including rice, and coffee. As it is rarely possible to discriminate geographical origin based only on concentration differences of a small number of elements, the complex multi-element datasets need statistical methods (chemometrics) to be used. If sufficient discrimination cannot be achieved only with multi-elemental analysis, other variables like stable isotopes can additionally be employed by chemometric techniques.

In a study from Australia, 56 elements were measured in 1397 samples of wines from different regions. From this dataset, twelve elements (Ba, Sr, Mg, Si, Cs, Mn, Ca, Ni, Tl, Cr, Fe, and Rb) were found to be sufficient to discriminant red wines from different regions in Australia.

In animal production the type of feed, the use of nutritional supplements, exposure to soils and exercise regimes can influence the trace element composition of meat and meat products. Multi-element analysis has therefore been used to distinguish organic from conventionally produced meat. Concentrations of trace elements such as manganese, chromium and copper among others were shown to be different between different rearing systems. An example from China illustrated the separation of organic and conventional pork using partial least squares to discriminate based on multi-element analysis.

The analytical techniques most commonly reported in the literature for multi-element analysis are based on **inductively coupled plasma mass spectrometry (ICP-MS)** and **ICP-atomic emission spectroscopy (ICP-AES**). Samples need pre-treatment prior to instrumental analysis and, while wines can be simply diluted, other matrice such as rice and meat need to undergo acid or microwave digestion. There is a comprehensive range of instruments for elemental analysis ranging from ICP-OES to ICP-HRMS, for which the choice of instrument will be dictated by required sensitivity, specificity and sample throughout. Trace Elemental Analysis thermoscientific

Table of Contents

Isotope Ratio Mass Spectrometry

Isotope ratio mass spectrometry (IRMS) can differentiate between samples containing identical chemical components, but which have very small differences, for example determining place of origin of coffee beans and wine. The isotope ratios of elements most commonly used for verification of food authenticity are $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$. IRMS is being increasingly used for authenticity measurements when more traditional methods cannot give the desired discrimination. The geographical origin of beverages such as wine and food products such as milk, coffee and organic grown vegetables can be identified using isotope fingerprints.

In nature there is an inter-relationship between soil, fertilizer, water, plants and animals. Uptake from soil and fertilizers, evaporation of water from the sea and precipitation, photosynthesis by plants, consumption of plants by animals and metabolism all lead to different isotope fingerprints. Evaporation of water from the sea and re-precipitation as rain leads to differences in $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios in rainwater depending on the distance from the sea and latitude. Different plants photosynthesize CO_2 through C_3, C_4 or crassulacean acid metabolism (CAM) pathways leading to characteristic differences in $\delta^{13}C$ values. The detection of commercial C_4 cane and corn derived sugar syrups in C_3 agricultural products (fruit juice, honey, maple syrup) can thus be detected by differences in $\delta^{13}C$ values.

$\delta_{2}H$ and $\delta^{18}O$ fingerprints are valuable isotopic probes for discrimination of geographical and botanical origin, for example determining place of origin of coffee beans and wine.

In addition, the $\delta^{2}H$ value of non-exchangeable hydrogen atoms in sugars are used to detect the addition of C_3 beet sugars to other C_3 products such as fruit juice. $\delta^{15}N$ fingerprints provide an insight into the use of artificial fertilizers and has been used to distinguish organic from conventionally grown vegetables.

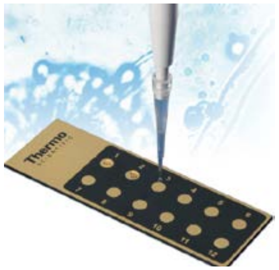
There are two primary approaches to determining stable isotope ratios of foods and beverages. The whole sample can be subjected to total combustion using Elemental Analysis Isotope Ratio Mass Spectrometry. Alternatively, individual compounds can be separated by GC or HPLC prior to combustion and isotope analysis. Bulk stable isotope analysis (BSIA) involves passing the sample through a reactor, separating the resultant gases by GC and then measuring ($\delta^{2}H$ and $\delta^{18}O$) or ($\delta^{13}C$ and $\delta^{15}N$ and $\delta^{34}S$) using for example a Thermo Scientific™ EA IsoLink IRMS System.

Compound specific isotope ratio analysis (CSIA) involves initial GC or HPLC separation and individual compounds are passed into a capillary reactor prior to IRMS determination. The later approach is particularly suitable, for example, for flavorings like vanillin where only one target compound is of interest for authentication for example with the GC IsoLink IRMS System.

Molecular spectroscopy

Table of Contents

Methods of vibrational spectroscopy, such as **near infra-red (NIR)** and **fourier transform infra-red (FTIR)**, can be used as fast and easy-to-use fingerprinting techniques. Both techniques can be used with no sample preparation or with minimal sample preparation. They are also non-destructive, and coupled with chemometric data processing have shown to be very effective for food authentication. **Raman spectroscopy** is another vibrational spectroscopic tool which has potential for addressing authenticity issues. It shares the practical advantages of infrared methods (i.e., speed, minimal sample preparation, low cost per analysis) but has specific spectroscopic characteristics that facilitate collection of information which is complementary to infrared techniques. These include its relative insensitivity to water and ability to reveal significant information about composition and structure of macromolecules, especially protein, in foods. Fluorescence can be a problem with Raman, especially with biological materials, and Raman signals are weak. However, fluorescence can be minimized by both selection of appropriate laser wavelengths and mathematical processing of collected spectra. With modern instruments, weak Raman signals are no longer a serious disadvantage due to improved detector sensitivity and the feasibility of using longer collection times. **Surface enhanced raman spectroscopy (SERS)** measurements are made by depositing the sample onto metallic colloidal or metallic solid substrates of nanoscale roughness. These metallic nanostructures enhance the Raman signal intensity by several orders of magnitude. This occurs because of chemical enhancement in which charge transfers between adsorbed molecules and the metallic substrate are involved and electromagnetic enhancement.



SERS slide - nanoscale rough metallic substrate cups for sample

SERS has the advantage that it makes it possible to overcome fluorescence quenching which a common interference found in Raman spectra. Various SERS substrates have been proposed, but the use of solid metallic substrates such as electro-polished aluminium foils provides a simple and practical approach.

FTIR and NIR have been applied to widely used authentication of virgin olive oils, floral origin of honeys, milk and cheese authentication, vinegar, herbs, cocoa and wine authentication as well as detection of adulteration of meat, milk and animal feed.

Thermo Scientific™ Nicolet™ iS™ 5 FTIR spectrometer

There has been commercialization of portable spectroscopy devices which provide ideal performance for product assurance and authenticity testing of a wide variety of food types. A choice of either a Mid-IR or a Near-IR version enables you to bring your spectrometer to where you need the answers most.

Molecular biology provides the technology for highly specific and ultra-sensitive detection of DNA in complex matrices. The **polymer chain reaction (PCR)** is the most commonly used format for analysis of target DNA sequences, selected as being unique to an animal or plant species. PCR can be conducted on very small sample sizes (mg or less), and because the technique is based on amplification, it can detect very small quantities of DNA. When PCR is used in forensic work, small sample size and sensitivity are big advantages, but in food adulteration there is usually plentiful sample and levels of economic adulteration are such that ultra-sensitivity is not needed. In fact, only employing small sample sizes can be a drawback when it is necessary to take representative food samples from a heterogeneous matrix, and high sensitivity can be a problem in terms of potential cross-contamination in the laboratory. Unlike other areas where the limit of detection of the method is set as the action threshold, because of the high sensitivity of PCR, a more pragmatic approach has been adopted. Usually 1% has been taken as a reporting limit for meat adulteration.

PCR initially involves extraction and purification of DNA from the sample. Then after addition of "primers", which are essentially probes for the target DNA sequence, the extract is subjected to annealing, and then the amplification occurs in a light cycler. In real-time PCR the amplification is not measured retrospectively, but as the name implies it is conducted in "real-time". Quantification is possible based on the Ct (threshold cycle) which is the intersection between an amplification curve and a threshold line.

Real-Time PCR

TaqMan™ instruments from Applied Biosystems™ have become synonymous with real-time PCR. A range of instruments are available meeting different needs of sample throughput together with all supporting consumables in the form of test kits. Real-time PCR has been extensively used for detection of horsemeat in a wide range of raw and cooked meat products, as well as more generally for meat and fish speciation. PCR is a target method of analysis which necessitates the use of specific DNA probes which are commercially available for a range of targets. Probes for pork are available which are used in testing for Halal authentication. Even in highly heat-processed products like gelatin, it has proved to be possible to extract high quality DNA for sensitive detection of pork. Although generally PCR tends to be used to look for a single target such as horsemeat or porcine DNA with multiple probes, it can also be used in multiplex format for simultaneous screening of a number of targets. In this format PCR is mostly used for screening for GMOs where there can be a number of different modifications known to be authorized for GM maize or soy bean. Unauthorized and therefore previously unknown GMOs are not easily detectable by PCR because the modification needs to be known in order to develop a suitable probe.

Chemometric pattern recognition techniques

When foods are being "fingerprinted", typically large numbers of variables are generated, whether these be δ values derived from IRMS, multi-elemental compositions, mass spectra, retention times or combinations of these parameters. To handle these very large datasets, statistical techniques employing multivariate analysis must be applied, and this can be done using what are known as "supervised" or "unsupervised" methods. Unsupervised methods involve classification of samples without any knowledge about their origin, while supervised methods look for a similarity of unknown to authentic materials. Choosing the most appropriate method is really the key to successful analysis.

Supervised methods employ discriminant techniques such as:

- Linear discriminant analysis (LDA) used to characterize or separate two or more classes of variable
- Partial least squares which looks for fundamental relationships between two different matrices
- Artificial neural networks (ANN) which involves modelling of complex relationships

Unsupervised methods employ techniques such as:

- Analysis of variance (ANOVA) which is a collection of statistical models
- Multivariate analysis of variance (MANOVA) which is a procedure for comparing multivariate sample means
- Principal component analysis (PCA) which is a method which reduces dimensionality
- Cluster analysis (CA) which is a method of grouping of samples into "clusters" based on their similarity

There are various sophisticated variations on the above methods such as orthogonal partial least discriminant analysis (OPLS-DA). The quality of unsupervised models is frequently evaluated by several parameters such as the goodness-of-fit parameter, the proportion of the variance of the response variable that is explained by the model, predictive ability and recognition ability parameters.

Multi-isotopic and elemental analyses were combined by chemometric techniques to differentiate rape honey, acacia honey, vitex honey, and jujube honey from China. Samples have been analyzed using both IRMS and ICP-MS. Hydrogen and oxygen isotopes were found to be more suitable than the carbon isotope for discriminating the floral origins of these honeys, and there were also significant differences in the contents of most elements between or among different floral origins. The combination of IRMS and ICP-MS methods provided the most effective and accurate approach for classifying Chinese honeys according to their floral origins.

Food authenticity research projects

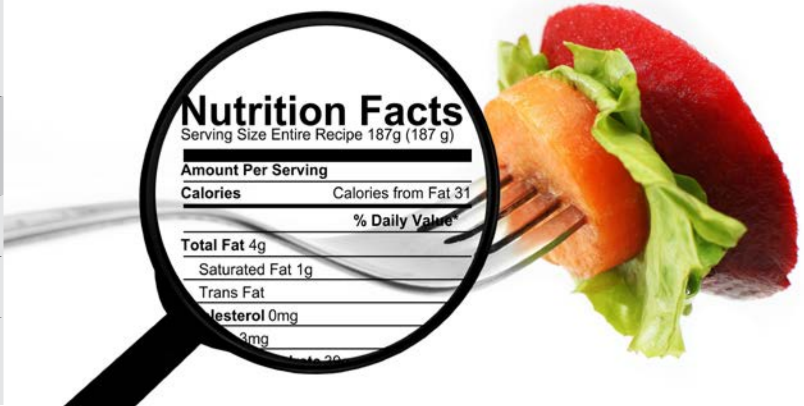
The EU has had a long history of funding large projects concerned with authenticity. The FP6 project TRACE started in January 2005 and was completed at the end of December 2009 with 53 partners including 9 SMEs. It was worth €18.8M.

The focus of this project was the origin of food and the application of suitable traceability systems. A follow-up project known as **FOODINTEGRITY** worth €12M involving 38 participants from 18 European countries and one from China started in 2014 and will be completed in 2018.

The key focus of FOODINTEGRITY is to consolidate, harmonize and mobilize European capability on food authentication. The project will reduce the current barriers to data-sharing and utilization that is crucial to combating food fraud. It will do this by supplying methods and tools that will address both enforcement and industry needs. The project seeks to enhance early warning capabilities, and working with industry is developing methods, systems and processes that will assure the quality, authenticity and safety of the food chain. Olive oil, spirits drinks and seafood are core areas being used as model foods in this project.

Under the Horizon 2020 framework, the **OLEUM** project (advanced solutions for assuring the overall authenticity and quality of olive oi) started in September 2016 with a 4-year duration and funding of €5M. There are 20 partners from 15 countries in this project. The overall objective of OLEUM is to better guarantee olive oil quality and authenticity by empowering detection and fostering prevention of olive oil fraud. This overall objective is supported by three strategic objectives:

1. To develop new and/or improved analytical methods for assuring the quality and authenticity of olive oil
2. To develop the OLEUM databank - an online integrated quality assurance database of olive oil analytical methods and data related to chemical and organoleptic characteristics (e.g., related to the sensory experience such as taste, odor and texture)
3. To develop and support a worldwide community of proficient analytical laboratories involved in the analysis of olive oil, thus establishing a wide OLEUM network



The project is too new to have a website as yet. It does not seem to be very radical in the analytical area, and it is said that OLEUM will revise existing analytical methods for verifying olive oil quality and detecting fraud by identifying drawbacks and improving performance and efficiency (e.g., improved, sensitivity and usability, decreased time and cost of analysis). The project will aim to enhance methodology for organoleptic assessment by improved reproducibility and developing a quantitative support procedure. OLEUM will also aim to identify novel analytical markers for detecting illegal blends, measuring olive oil freshness and best-before quality, and for monitoring compliance with labelled geographical origin. The project is coordinated by Prof. Tullia Gallina Toschi of the Department of Agricultural and Food Sciences of the University of Bologna, Italy.

AUTHENT-NET - Food Authenticity Research Network is a small (0.5 M) project running from April 2016 to March 2018 and coordinated by FERA Science Ltd (UK.) AUTHENT-NET will mobilize and coordinate relevant research budget holders in the area of food authenticity in order to facilitate the eventual development of a transnational European funding vehicle that will allow members states (MS) to jointly fund anti-fraud research.

PhasmaFOOD is a new project which will start in January 2017, last for 2 years and will involve development of portable photonic miniaturized smart system for on-the-spot food quality sensing. The project is worth €3M, has 9 partners and is coordinated by the Belgium company "Intrasoft International SA". Of three areas indicated where detection devices will be developed, it is planned to work on detection of food fraud, and specifically adulteration of alcoholic beverages, oil, milk and meat.

Table of Contents

Future trends

The practice of adulterating food and misrepresenting its authenticity both have strong economic drivers, making it unlikely there will be any decline in the future. As scandals are exposed, the perpetrators switch to new fraudulent practices, which are difficult to identify, especially as food fraud grows ever more sophisticated. Regulations controlling adulteration and protecting authenticity are already strong and it seems unlikely that increased consumer protection will result from more regulation.

Chromatography, mass spectrometry, elemental analysis, molecular spectroscopy including NMR and stable isotopic ratio measurement are all powerful tools which are being widely employed to combat food fraud. Untargeted food analysis has developed fast in recent years, particularly with improved ability to generate very specific "fingerprints" of foods, using, for example, high resolution mass spectrometry.

The analytical skills implicit in those working in a food testing laboratory can be readily applied to measurements of a range of different parameters to detect adulteration and test authenticity of a food or beverage. Although there are many powerful statistical tools that are employed for multivariate analysis of complex datasets, applying these chemometric methods requires considerable experience. This is not always necessarily available in a typical food control laboratory. For the future, this gap needs to be filled with "user-friendly" chemometric packages becoming available and being supplied, perhaps together with the instruments being used. Additionally large databases of varied parameters for authentic foods need to be generated and shared by users. However, this can only happen when some standardization is agreed upon both in terms of measurement and data storage.

GC and GC-MS Methods

Title	Authors	Publication
Fingerprinting of red wine by headspace solid-phase dynamic extraction of volatile constituents	Laaks J, Letzel T, Schmidt TC, Jochmann MA. (2012)	Anal Bioanal Chem 403:2429–2436
Sesquiterpene fingerprinting by headspace SPME–GC–MS: Preliminary study for a simple and powerful analytical tool for traceability of olive oils	Damascelli A, Palmisano F. (2013)	Food Anal. Methods 6:900–905
Atmospheric pressure chemical ionisation mass spectrometry analysis linked with chemometrics for food classification – A case study: Geographical provenance and cultivar classification of monovarietal clarified apple juices	Gan H-H, Soukoulis C, Fisk I. (2014)	Food Chem. 146:149–156
Characterization of the authenticity of Pasta di Gragnano protected geographical indication through flavor component analysis by gas chromatography–mass spectrometry and chemometric tools	Giannetti V, Mariani MB, Mannino P. (2016)	JAOAC Int 99:1279–1286
Quantitative determination of α-ionone, β-ionone, and β-damascenone and enantio differentiation of α-ionone in wine for authenticity control using multi-dimensional gas chromatography with tandem mass spectrometric detection	Langen J, Wegmann-Herr P, Schmarr H-G, (2016)	Anal Bioanal Chem. 408:6483–6496

LC and LC-MS Methods

Title	Authors	Publication
HPLC–PDA–MS fingerprinting to assess the authenticity of pomegranate beverages	Borges G, Crozier A. (2012)	Food Chem. 135:1863–1867
Discovery and structural elucidation of the illegal azo dye Basic Red 46 in sumac spice	Ruf J, Walter P, Kandler H, Kaufmann A. (2012)	Food Additives & Contaminants (2012) 29:897–907
The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry	Keckeš S, Gašic U, Velickovic TC, Milojkovic-Opsenica D, Natic M, Tešic Z. (2013)	Food Chemistry 138:32–40
Authentication of closely related fish and derived fish products using tandem mass spectrometry and spectral library matching	Nessen MA, van der Zwaan DJ, Grevers S, Dalebout H, Staats M, Kok E, Magnus Palmblad M. (2016)	J. Agric. Food Chem. 64:3669–3677
High-throughput analysis by SP-LDI-MS for fast identification of adulterations in commercial balsamic vinegars	Guerreiro TM, Noin de Oliveira D, Ferreira MS, Catharino RR. (2014)	Anal Chim Acta 838:86–92

ICP-MS Methods

Title	Authors	Publication
Effect of organic and conventional rearing system on the mineral content of pork	Zhao Y, Wang D, Yang S. (2016)	Meat Science 118:103–107
Geographic determination of coffee beans using multi-element analysis and isotope ratios of boron and strontium	Hou-Chun Liu, Chen-Feng You, Chiou-Yun Chen, Yu-Ching Liu, Ming-Tsung Chung (2014)	Food Chem. 142:439–445
Discrimination of geographical origin of rice based on multi-element fingerprinting by high resolution inductively coupled plasma mass spectrometry	Cheajesadagul P, Arnaudguilhem C, Shiowatana J, Siripinyanond A, Szpunar J. (2013)	Fish Sci., 80:1089–1096
Identifying the origin of Corbicula clams using trace element analysis	Iguchi J, Isshiki M, Takashima Y, Yamashitan Y, Yamashita M. (2014)	Food Control 59:714–720
Determining the geographical origin of Chinese green tea by linear discriminant analysis of trace metals and rare earth elements: Taking Dongting Biluochun as an example	Ma G, Zhang Y, Zhang J, Wang G, Chen L, Zhang M, Liu T, Liu X, Lu C. (2016)	Food Control 59:714–720

Table of Contents

IC Methods

Title	Authors	Publication
Identification, classification, and discrimination of agave syrups from natural sweeteners by infrared spectroscopy and HPAEC-PAD	Mellado-Mojica E, López MG (2015)	Food Chem., (2015) 167:349–357
Comparative analysis of maple syrups and natural sweeteners: Carbohydrates composition and classification (differentiation) by HPAEC-PAD and FTIR spectroscopy-chemometrics	Mellado-Mojica E, Seeram NP, Mercedes G. López MG. (2016)	J Food Composition & Analysis 52:1–8
Multiple Applications of Ion Chromatography, Oligosaccharide Fingerprint Profiles To Solve a Variety of Sugar and Sugar–Biofuel Industry Problems	Gillian Eggleston and Eduardo Borges, (2015)	J. Agric. Food Chem. 63 2841–2851
Chemical markers for the authentication of unifloral Salvia officinalis L. honey	Gasic UM, Natic MM, Misic DM, Lusic DV, Milojkovic-Opsenica DM, Tesic ZL, Lusic D. (2015)	J. Food Composition & Analysis 44: 128–138
Potential of ion chromatography coupled to isotope ratio mass spectrometry via a liquid interface for beverages authentication.	Guyona F, Gaillard L, Brault A, Gaultier N, Salagoïty M-H, Médina B. (2013)	J. Chromatography A, 1322:62–68

Table of Contents

IRMS Methods

Title	Authors	Publication
The use of IRMS, ^{1}H NMR and chemical analysis to characterise Italian and imported Tunisian olive oils	Federica Camin, Anita Pavone, Luana Bontempo, Ron Wehrens, Mauro Paolini, Angelo Faberi, Rosa Maria Marianella, Donatella Capitani, Silvia Vista, Luisa Mannina, (2016)	Food Chemistry 196:98–105
Detection of counterfeit scotch whisky by ^{2}H and ^{18}O stable isotope analysis	Meier-Augenstein W, Kemp HF, Hardie SML. (2012)	Food Chem., 133:1070–1074
Stable isotope analysis of cattle tail hair: A potential tool for verifying the geographical origin of beef	Xiaoling Liu, Boli Guo, Yimin Wei, Junling Shi, Shumin Sun, (2013)	Food Chem., 140:135–140
Stable, isotope and chemical compositions of European and Australasian ciders as a guide to authenticity	Carter JF, Yates HAS, Tinggi U, (2015)	J. Agric. Food Chem. 63:975–982
Multi-element (C, H, O) stable isotope analysis for the authentication of balsamic vinegars	Werner RA, Roßmann A. (2015)	Isotopes in Environmental and Health Studies 51:58–67

Molecular Spectroscopy Methods

Title	Authors	Publication
Feasibility study on the use of Fourier transform near-infrared spectroscopy together with chemometrics to discriminate and quantify adulteration in cocoa beans	Teye E, Huang X, Lei W, Da H. (2014)	Food Research Int., 55:288–293
FTIR spectroscopy combined with chemometric for analysis of sesame oil adulterated with corn oil	Fadzlillah NA, Che Mann YB, Rohman A. (2014)	J. Food Properties 17:1275–1282
Determination of the adulterants in adulterant–brandy blends using fluorescence spectroscopy and multivariate methods	Markechova D, Majek P, Kleinova A, Sadecka J. (2014)	Anal. Methods 6:379–386
FT-Raman and NIR spectroscopy data fusion strategy for multivariate qualitative analysis of food fraud.	Márquez C, López MI, Ruisánchezn I, Callao MP. (2016)	Talanta 161:80–86
Methods for detection of pork adulteration in veal product based on FT-NIR spectroscopy for laboratory, industrial and on-site analysis	Schmutzler M, Beganovic A, Beohler G, Huck CW. (2015)	Food Control 57:258–267

Find out more at **thermofisher.com/FoodIntegrity**

ThermoFisher
SCIENTIFIC