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Analytical approaches for food authentication*

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1 Introduction

The driving force behind any adulteration is to maximise revenues by either using a cheap ingredient to (partially) substitute a more expensive one, or to (partially) remove the valued component in the hope that the altered product passes undetected by the final user or consumer. Adulteration of foods has a history reaching back to ancient times. One of the earliest accounts of adulteration of foodstuffs was given by *Cato* in his treatise *De Agricultura*, who reported on the adulteration of wine. Likewise, Roman civil law regarding the sale of food was as complex and detailed as Community legislation nowadays is. Although various commodities have been met within an adulterated state (pepper has been adulterated with gravel, leaves, twigs, etc., tea with spent and re-dried tea leaves, milk and wine have been watered, to coffee roasted grains or chicory have been added, saw dust to bread, butter yellow to butter), adulteration of high commercial value was not much of a problem up to the beginning of the 19th century. However, some of the adulterants used were toxic, e.g. butter yellow (a hepatocarcinogen) to give butter a more attractive appearance or toxic lead acetate for sweetening wine. Then food was produced from small businesses or individuals, and purchasing involved a high degree of personal accountability. Intentional adulteration increased greatly as a result of modern practices in food production, i.e. the interruption of the direct link between a food producer and his customers. Major advances in agronomy, large-scale transport, processing capabilities, and the introduction of efficient distribution systems have resulted in increased technological complexity, a higher degree of globalisation and lower product prices. The economics of farming are very complex and mostly governed by intricate guidelines and laws to balance production and demand, to stabilise prices and protect local interests. Although prices for staple food are nowadays comparatively low in most Western countries, consumers have to rely on product labelling and advertising to find out details regarding the nature of the foodstuffs they buy. Consumer protection represents therefore one of the earliest forms of governmental supervision and regulation of commercial enterprises. It has become the responsibility of public law enforcement agencies to police the claims made

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by producers and to bring to justice fraudulent operators. The establishment of Food Chemistry as a scientific discipline enabled society to adopt appropriate countermeasures to pursue fraud. Unfortunately, unscrupulous purveyors of fraud also benefited from this development, since the availability of compositional data enabled them to fine-tune their illicit manipulations in order to evade legal action. In general, fraudsters tend to be quite innovative and well informed about weak points in food inspection systems. Therefore, food control authorities need to develop new, and constantly adapt existing methodology to detect fraud to protect fundamental rights of consumers.

2 Food adulteration

Being aware of the adulteration/misbranding problematic the European Commission initiated a project to canvass current trends and methodologies in food authentication (1). According to this the major issues are:

- Non-compliance with legal requirements/product standards, e.g. max/min content of water, fat, protein, etc.
- Economic adulteration of food, i.e. blending of a cheaper product with commodities of higher economic value
- Food produced according to special production systems (organic food, minimally processed food, conventional food, genetically modified food)
- Characterisation and denomination of geographical origin

In response to a number of "food scandals" and "food scares" the European Commission issued the White Paper on Food Safety (2), which was later-on followed by an EC Regulation laying down general principles of food law (3). Regulation EC 178/2002 establishes in EU law that the three inter-related components of risk analysis (risk assessment, risk management and risk communication) shall provide the basis for food law in any Member State. Besides that the regulation also stipulates that the interests of consumers have to be protected, with a particular view to food adulteration (Table 1). In the context of authenticity testing two main cases have to be differentiated: economic adulteration and misbranding of products (production system, denomination of geographical origin, genetically modified food/food ingredients). Current issues for food authentication are exemplarily listed in Table 2.

Table 1

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

Regulation EC 178/2002 Article 8

Protection of consumers' interests

1. Food law shall aim at the protection of the interests of consumers and shall provide a basis for consumers to make informed choices in relation to the foods they consume. It 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.

Table 2

Major food authenticity issues

Milk/dairy products	Watering/skimming Differentiation of milk/dairy products from animal species (bovine, ovine, caprine milk) Addition of foreign proteins (e.g. soya) and foreign fat (e.g. tallow, lard, etc) Altered casein/whey protein ratio Heat load Geographical origin
Meat/meat products	Differentiation of animal species Differentiation of sex, breed, specific cuts Use of offal, mechanically-recovered meat Addition of foreign protein Differentiation fresh – frozen meat Addition of water Geographical origin
Fish	Differentiation of animal species Differentiation wild – farmed fish Differentiation fresh – frozen fish Geographical origin
Wheat/pasta	Differentiation between common wheat and durum wheat
Rice	Differentiation of rice varieties (long-grain Indica and paddy Japonica rice, Basmati rice) Geographical origin (Basmati, North American, European)
Coffee	Differentiation between Arabica and Robusta varieties Geographical origin Adulteration of instant coffee by addition of coffee husks or non-coffee materials (chicory, malt, caramel, starch, maltodextrins, etc)
Fruit juices	Watering/reconstitution of concentrate below statutory limit Undeclared sugaring (addition of sugar or sugar syrups) Addition of other fruit types/varieties Addition of organic acids Peel extract/pulp wash Flavouring Geographical origin
Wine	Watering Chaptalisation Addition of glycerol Geographic origin Vine variety
Honey	Differentiation blossom – honeydew honey Botanical origin Geographical origin
Oils and fats	Watering/sugaring Differentiation of botanical origin Undeclared addition of foreign fats/oils to monovarietal products Geographical origin Processing technology (cold pressed oils)
Spices/herbs/flavourings	Addition of inferior herbal parts Differentiation between natural – nature-identical – artificial flavourings

2.1 Economic adulteration

It is common practice to blend e.g. edible oils to prepare suitable products, but if the resulting blend deviates from the mixture proportions given on the label, or if the blend is traded as genuine, the vendor eventually commits fraud. One of the most cited cases was the co-mingling of cottonseed oil imported into Egypt with palm olein produced in Malaysia. Quite recently, European police have made raids and arrested 30 people involved in the production and sale of 16 000 tonnes of fake butter. It was reported that for its production "chemicals, oils, and laboratory-produced substances" were used and the product was sold for manufacture of cakes, biscuits and ice cream.

2.2 Production systems

Organically grown and/or minimally processed food commodities have gained wide attention in developed countries, since wide-held belief attributes superior nutritional qualities to such products, mostly because their "naturalness" should be preserved to a higher degree by deliberately avoiding conventional agronomical and food processing techniques (application of agro-chemicals, heating, refining, additives, etc.). Likewise, marketing of agricultural products has recently focused on promotion of premium goods in Western countries. Such products command a premium due to the fact that they were e.g. produced by applying only traditional methods of (artisanal) production, and/or using only certain ingredients. To capitalise on market demands and improve on profits, goods of less economic value may therefore be co-mingled into higher priced commodities, thereby affecting honest producers who suffer from unfair competition and consumers, whose right for correct product information are violated by fraud.

2.3 Characterisation and denomination of geographical origin

Products manufactured in a particular way in a specific geographical region have always found a following, although they usually command a higher price. Denomination of geographical origin ("appellation d'origine") is meanwhile relevant for a larger number of food products (cheese varieties, meat and meat products, oils and fats, olives and fruits, beer, etc). In 1992, the European Union created systems known as PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Speciality Guaranteed) to promote and protect locally produced food which has a high reputation for certain quality characteristics and to deter unfair competition which not only discourages producers but also misleads consumers (4) (Table 2). To be eligible to use a PDO or a PGI label, an agricultural product or foodstuff must comply with strict specifications. On-site inspections by a control authority are currently the only accepted way to safeguard the PDO/PGI label, as reliable and validated analytical testing methods do not exist yet. The most promising approach seems to be to characterise the products by determining stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{16}\text{O}/^{18}\text{O}$) and subsequent appli-

cation of mathematical pattern recognition techniques. It has been applied to the characterisation of a number of food products such as wine, fruit juices, honey, cereals, edible oils, etc (4–10). It was found that the feeding regimen, in particular maize silage, can influence the $^{13}\text{C}/^{12}\text{C}$ ratio, use of industrial fertilisers the $^{15}\text{N}/^{14}\text{N}$ ratio and the water supply the $^{16}\text{O}/^{18}\text{O}$ ratio of milk and milk products. Although some promising results have been obtained, the analytical approach is in its infancy and much needs to be done to give a reliable indication that verifies the origin of a product.

Table 3
Number of Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) registered products (status November 2004)

Belgium	4	Luxemburg	4
Czech Republic	3	Hungary	–
Denmark	3	Malta	–
Germany	67	The Netherlands	6
Estonia	–	Austria	12
Greece	83	Poland	–
Spain	76	Portugal	91
France	138	Slovenia	–
Ireland	3	Slovakia	–
Italy	137	Finland	1
Cyprus	–	Sweden	2
Latvia	–	United Kingdom	28
Lithuania	–		

3 Strategies for authentication

Strategies for solving an authenticity problem may belong to one of the following categories:

- on-spot audit
- permanent supervision by an independent authority (certification body)
- paper trail (book checks)
- analysis

A common analytical strategy is to compare information from specimens to be authenticated with that obtained from collections of genuine specimens (Figure 1). The ideal situation would be the following: the adulterant contains a marker substance which is not present in the genuine product. The adulteration of orange juice with industrial preparations of malic acid can easily be detected by determining D-malate, which is virtually not present in the genuine product. The situation becomes much more complicated when such a fundamental difference between the adulterant and the genuine product does not exist. In many cases, the marker is present in both products but in different concentrations. As a consequence, concentration ranges of potential marker substances have to be established for the genuine product, taking into account possible sources of variation due to variety, climate,

production system, processing, etc. Such data bases require continual updating to ensure that state-of-the-art agricultural and processing technologies are properly reflected, otherwise they will become corrupted. It goes without saying that impartial and disinterested persons or organisations have to be entrusted with data collection and maintenance of the data base. Furthermore, all stakeholders in the food chain should have access to the data bases, otherwise customers of authenticity tests have no means to evaluate how good the analytical services they receive from the testing laboratory are. This creates a paradoxical situation: it is quite understandable that organisations, e.g. law enforcement agencies, but also commercial labs offering authenticity tests, guard their data base, because they do not want that sensitive information comes to the attention of unscrupulous operators and/or that other laboratories offer a competing service. Some data bases are publicly available, e.g. the fatty acid composition of a number of edible fats and oils have been published in the *Codex Alimentarius*.

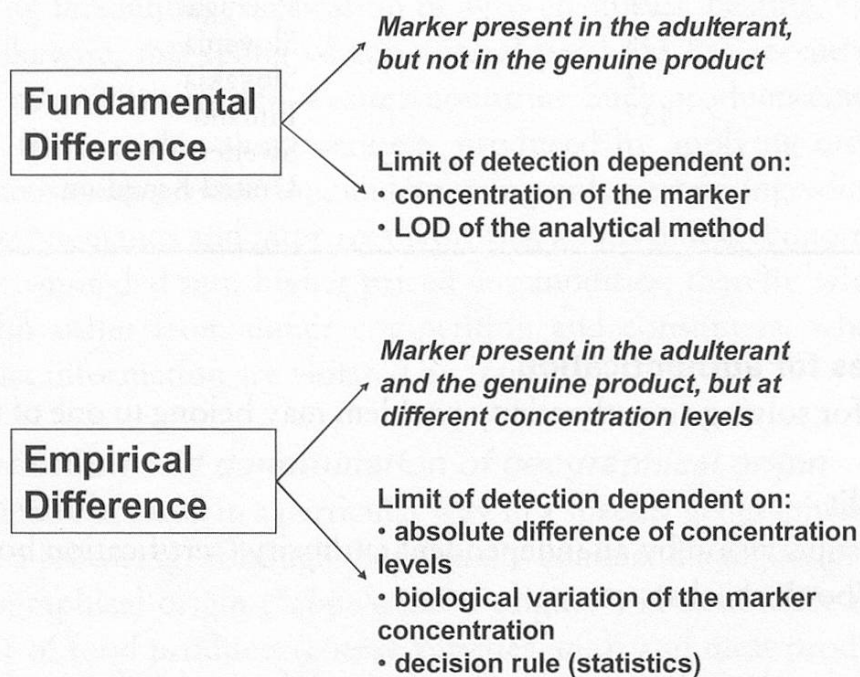


Figure 1 Principal strategies for the detection of food adulteration

4 Tools for authentication

4.1 Analytical methods

A whole arsenal of methods, including every facet of instrumental analytical chemistry, is nowadays available for the determination of marker substances. Very often hyphenated methods, mostly an on-line combination of chromatographic and spectroscopic methods are deployed to solve authentication problems. Further-

more, methods primarily developed for molecular biology have found wide-spread application, in particular for the discrimination of different species and the detection and quantification of genetically modified food. An overview of analytical methods and a number of useful marker substances for food authentication is given in Table 4. Pattern recognition methods based on the application of spectroscopic techniques such as (FT)-IR, Raman, and NMR spectroscopy and subsequent data evaluation by multivariate statistical methods have gained attraction and are likely to become ever more important as sample preparation for such methods is minimal and the necessary statistical software packages for classification are becoming more powerful (11–19).

Table 4
Analytical methods and targeted markers used for food authentication

Chromatographic methods: HPLC GLC (Chirale separations)	Fatty acids, triacylglycerols, sterols, steradienes Terpenes (chiral flavour compounds)
Spectroscopic methods: (FT)-IR, Raman ICP-OES, ICP-MS NMR IRMS	Phenolic compounds (hydroxycinnamic acid derivatives, flavanones, flavanoles, anthocyanidines) Organic acids, amino acids, saccharides
Electrophoretic methods: PAGE IEF CE	Fingerprints Stable isotopes ($^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$, $^{34}\text{S}/^{32}\text{S}$, $^{87}\text{Sr}/^{86}\text{Sr}$)
Biochemical methods: ELISA PCR	Elements Proteins, peptides Nucleic acids

The whole subject has been covered in much more detail by a number of books and dedicated review articles (20–33).

4.2 Statistical methods

Given the complexity of some problems, univariate statistics (measures of location and dispersion) have to be substituted by intricate statistical algorithms to aid in pattern recognition and classification of genuine and adulterated products. Chemometrics, the discipline concerned with application of statistical and mathematical methods to chemistry, is usually applied to compositional data in order to solve authenticity and classification problems. The merits of such advanced procedures (principal components analysis, linear discriminant analysis, various cluster-

ing techniques, artificial neural networks, genetic algorithms) although scientifically sound, are difficult to comprehend for those not familiar with advanced statistical data interpretation techniques and might, therefore, find little acceptance in a court of law. Furthermore, one danger of chemometric methods is that results are sometimes interpreted in a manner which is too optimistic.

5 Conclusion

Detection of fraud, though powerful analytical tools are at hand, is complicated by the fact that the quantity of certain indicators varies due to biological, climatic, agronomical and temporal factors. Moreover, processing can dramatically change the composition of minor constituents. Therefore, too stringent specifications can not be set by food inspection as this will eventually increase the number of false-positive results. Since unscrupulous manufacturers or vendors have developed an excellent understanding of the underlying principles to detect fraud, they have managed in many cases to tailor blends in a way that they comply with product specifications.

In many cases no fundamental differences, ideally the lack or presence of a product specific component, between the genuine and the adulterated product exist. Consequently, purity criteria have to be empirically determined by analysing a wide array of genuine products and creating and regularly updating a database holding information about the concentration ranges of certain indicative components of the commodity concerned. In order to solve difficult cases more than one analyte has to be considered for detecting fraud. Likewise, a combination of different analytical techniques to determine dissimilar characteristics of a commodity (e.g. by a combination of spectroscopic and chromatographic methods) could be more useful than relying on one single methodology.

The challenge for food law enforcement agencies is to be a step ahead and to develop constantly new methods to get a better insight into the complex chemical mixture representing food, in order to identify a set of possible marker components for authentication purposes.

Whatever type of fraud is perpetrated, authenticity testing relies either on a fundamental difference between the original and the adulterant, or on an intimate knowledge of their composition and possible ranges of compositional variation. The former case is much more tractable than the latter. Detection of foreign proteins added to milk of a certain species by exploiting differences in their electrophoretic mobilities is an example where a fundamental dissimilarity is used to check the purity of the product. If no tangible differences exist, compositional data of authentic samples have to be gathered, taking into account all possible natural variations, e.g. due to variety/breed, production systems, geographical origin, etc. The authenticity of a product is confirmed when its compositional data fit into the data space represented by authentic samples.

Summary

The proof of authenticity of foods is a key issue in the area of food chemistry since ancient times. One of the earliest accounts of adulteration of foodstuffs was given by *Cato*. Extensive sections of the legislation of the European community are dedicated to adulteration and imitation of food. Formerly the protection of health (e.g. addition of toxic lead acetate to wine) and the protection against fraud (e.g. addition of spent and re-dried tea leaves to tea) were at the centre of attention. Nowadays the emphasis is put on the verification of the geographical origin and the type of production of foods. Especially with high quality products the question of authenticity is a main topic. Basically the proof of authenticity can be based either on fundamental or on empirical differences between the original and the adulterated product. A fundamental difference is the presence or absence of a specific component either in the original or in the adulterated product whereas for an empirical difference a specific marker is present in both products but in different concentrations. In the latter case an extensive database describing the natural variation of concentration of the marker is required for assessing authenticity. For the analytical proof of authenticity a wide spectrum of chromatographic and spectroscopic methods is available. Methods based on the determination of the stable isotope ratio of different elements ($^1\text{H}/^2\text{H}$, $^{12}\text{C}/^{13}\text{C}$, $^{16}\text{O}/^{18}\text{O}$, etc.) are becoming increasingly important. Especially with the empirical methods the quality of the databases used is of eminent importance. In the literature multivariate statistical analyses of distinguishing features are increasingly described. If these methods will also be recognized officially, e.g. in legislation or in a court of law needs to be awaited as the uncertainty of the prediction of these methods are scientifically not yet fully evaluated.

Résumé

La démonstration de l'authenticité d'un produit est depuis toujours (Caton l'ancien citait déjà au début de notre millénaire un cas de contrefaçon d'aliments) un problème crucial de la chimie alimentaire et de nombreux chapitres du droit européen en matière d'alimentation sont dédiés à l'adultération ou la contrefaçon d'aliment. Si à l'origine la protection de la santé (adjonction d'acétate de fer dans le vin) respectivement la protection contre les contrefaçons (coupage du thé noir par adjonction de feuilles séchées) étaient recherchés en priorité aujourd'hui c'est plutôt l'origine géographique d'un aliment, la façon de le produire ou de le préparer et l'utilisation, respectivement la non utilisation d'ingrédients ou d'additifs qui prennent une place privilégiée dans leur analytique. Pour les produits « Premium » qui ont pu voir ces dernières années une grande progression de leur vente, la question de leur authenticité (grec: crédibilité) se pose avec une acuité encore plus particulière. En principe, la démonstration de l'authenticité peut être basée soit sur des différences fondamentales, soit sur des différences empiriques entre le produit original et celui falsifié. Une différence fondamentale est mise en évidence par la présence ou l'absence exclusive d'un composant spécifique soit dans l'original soit dans la copie,

tandis qu'une différence empirique se démontre par une différence de concentration d'un ou de plusieurs marqueurs également présents dans le produit original et dans la contrefaçon. Dans ce dernier cas, la démonstration de l'authenticité doit se baser sur des vastes données incorporant les variations naturelles de la teneur des marqueurs spécifiques. Pour apporter la preuve de l'authenticité, une large palette de méthodes chromatographiques et/ou spectroscopiques est à disposition. A cet égard, les procédés basés sur la détermination des rapports isotopiques des éléments légers ($^1\text{H}/^2\text{H}$, $^{12}\text{C}/^{13}\text{C}$, $^{16}\text{O}/^{18}\text{O}$, etc.), prennent de plus en plus d'importance. Tout particulièrement avec les méthodes empiriques, la représentativité et la qualité de la base de données nécessaire à la prise de décision joue un rôle déterminant. Dans la littérature, des procédés d'évaluation statistiques multivariées sont de plus en plus largement utilisés pour mettre en évidence une différenciation entre les échantillons. Cependant, il faut encore attendre un peu avant de savoir dans quelle mesure de telles méthodes doivent obtenir une reconnaissance officielle, p. ex. dans la législation ou devant un tribunal car l'incertitude des interprétations obtenues par ce procédé n'est pas encore connue avec précision.

Zusammenfassung «Analytische Möglichkeiten des Authentizitätsnachweises von Lebensmitteln»

Der Authentizitätsnachweis stellt schon seit altersher eine zentrale Fragestellung der Lebensmittelchemie dar. Einer der ältesten Hinweise auf die Fälschung von Lebensmitteln findet sich schon bei *Cato*. Umfangreiche Kapitel der europäischen Lebensmittelgesetzgebung sind der Verfälschung oder Nachmachung von Lebensmitteln gewidmet. Stand ursprünglich der Gesundheits- (z.B. Zugabe von Bleizucker zu Wein) bzw. der Täuschungsschutz (z.B. Streckung von schwarzem Tee mit gebrauchten und getrockneten Teeblättern) im Vordergrund, so nimmt in jüngerer Zeit der Nachweis der geographischen Herkunft eines Lebensmittels, die Art der Gewinnung bzw. Bearbeitung und die Verwendung bzw. der Nichtgebrauch bestimmter Zusätze und Ingredienzien einen besonderen Stellenwert in der Lebensmittelanalytik ein. Speziell bei Premiumprodukten, die ja in den letzten Jahren hohe Zuwachsraten verbuchen konnten, ist die Frage nach deren Authentizität (griech.: Glaubwürdigkeit) besonders aktuell. Grundsätzlich kann der Nachweis der Authentizität entweder auf fundamentalen oder auf eher empirischen Unterschieden zwischen dem Original und der Fälschung beruhen. Ein fundamentaler Unterschied bezieht sich auf die ausschliessliche Anwesenheit/Abwesenheit einer spezifischen Komponente entweder im Original oder in der Fälschung, während bei empirischen Unterschieden der Marker sowohl im Original als auch in der Fälschung vorhanden ist, jedoch in unterschiedlichen Konzentrationen. Im letzteren Fall ist zur Beurteilung der Authentizität umfangreiches Datenmaterial zur Beschreibung der natürlichen Variation des Markergehaltes eine Grundvoraussetzung. Zum analytischen Nachweis der Authentizität steht eine breite Palette von chromatographischen bzw. spektroskopischen Methoden zur Verfügung. Verfahren, die auf der Bestimmung

von Stabilisotopenverhältnissen der Leichtelemente ($^1\text{H}/^2\text{H}$, $^{12}\text{C}/^{13}\text{C}$, $^{16}\text{O}/^{18}\text{O}$, etc.) beruhen, gewinnen in diesem Zusammenhang immer mehr an Bedeutung. Speziell bei empirischen Methoden spielt die Repräsentativität der zur Entscheidungsfindung notwendigen Datenbank und die Datenqualität eine herausragende Rolle. In der Literatur werden in zunehmendem Ausmass multivariate statistische Auswerteverfahren zur Merkmalsunterscheidung beschrieben. Inwieweit solche Methoden auch offizielle Anerkennung, z.B. in der Gesetzgebung oder vor Gericht, finden bleibt abzuwarten, da die Unsicherheit mit der die Aussagen dieser Verfahren behaftet sind, wissenschaftlich noch nicht genügend durchleuchtet wurde.

Key words

Authentication, EU-Regulation

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