Recent developments in food authentication†

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1 Introduction

Food authentication is the process by which a food is verified as complying with its label description. Labelling and compositional regulations, which may differ from country to country, have a fundamental place in determining which scientific tests are appropriate for a particular issue. As an example of this, the European Community does not permit the use of pulpwash (an aqueous extract of the albido) in orange juice whereas the use of “in-line pulpwash” (i.e., made as part of the juice process) is permitted in the USA. Thus different tests are appropriate to establish the authenticity of “pure orange juice” in the different countries. The question of whether in-line pulpwash can be differentiated chemically from off-line pulpwash is an issue which, as yet, has not been considered.

Labelling legislation is there to ensure that food is properly described. It seeks to protect the consumer from being sold an inferior product with a false description in addition to protecting honest traders from unfair competition. Enforcement of this legislation ensures that correctly described products remain available to the consumer and that consumer confidence is maintained, which in turn ensures a market place for these foods. Thus the availability of sound analytical methods which can ensure the authenticity of foods plays a fundamental role in the operation of modern society.

The desire to make a fraudulent profit from the misrepresentation of food has been a feature of society from historical times. One of the earliest scientific surveys of the authenticity of food was undertaken by Arthur Hill Hassall in 1861.1 He employed microscopy, which until this time had been reserved for medical studies, to investigate the authenticity of coffee, an extremely expensive commodity at that time. His survey found that 31 out of 34 samples contained adulterants such as chicory, roasted wheat and burnt sugar. In 1995 the UK Ministry of Agriculture Fisheries and Food (MAFF) undertook a survey of instant coffees and evaluated their authenticity on the basis of their sugar composition; 15% of the coffees examined were considered as not authentic.2 This demonstrates two important points. First, adulteration issues do not go away. If there is the potential for an illegal profit to be made, only continued vigilance provides reassurance against this type of fraud. Second, the development of new technologies will often discover food authenticity issues which current techniques can not. Hassall’s idea of using the microscope for food studies was a good example of this point which subsequent scientific developments in food authentication have regularly confirmed. This review will therefore record some of the recent advances in food authentication.

2 Classification of authenticity issues

As the example of pulpwash demonstrates, there can be many different and indeed subtle issues concerning labelling which it may be desirable to check by performing chemical tests. However, it is possible to classify the issues into a number of similar topics.

2.1 Species of origin

A common authenticity problem is for the species from which a food was made to be misdescribed. This may take the form of
substitution of one species for another. Thus in a survey of battered fried fish sold to the consumer, the species of fish was found to be misdescribed in 5% of cases. In this instance there was no evidence of a fraud being carried out for economic benefit since as often as not, an equally expensive fish was substituted.

Of course, claims concerning the species of origin are effectively claims concerning the genetic make up of the organism and the definition of a species may make this a rather arbitrary classification. Some claims may go beyond the species barrier to the variety of the organism. Thus claims that beef has come from, for example, Aberdeen Angus may potentially require testing. Such claims confer a commercial advantage because some consumers consider that pure bred beef herds are less likely to have contracted bovine spongiform encephalopathy (BSE). To my knowledge, tests for variety have not yet been established; however, the application of recent developments in DNA technology (described further below) suggest that variety specific authenticity testing could be developed.

The introduction of genetically modified organisms (GMOs) into food commerce may constitute a special case for variety specific authenticity testing if labelling makes claims about the presence or absence of these materials.

Another authenticity issue which may commonly arise is the need to determine whether food products from one species have been mixed with similar material from a cheaper species. The question of whether durum wheat pasta contains common wheat represents a typical example. In a MAFF survey, only one sample out of 249 was found to exceed the limit of 8% common wheat in durum wheat pasta, which was the limit which the methods employed could be confident constituted misdescription. A further 14 samples showed evidence of containing between 3 and 8% common wheat by at least one method. The chosen methods involved the determination of protein composition using either HPLC or electrophoresis.

### 2.2 Geographical region of origin

It is common for certain foodstuffs to be described as coming from a particular country or region. Very often this description is used as a cipher for product quality. The price of good quality wines is often largely based on the region they come from but the same can also be said of cheeses, sausages, olive oil and so on. European legislation (EEC No. 2081/92) has been developed for the protection of geographical indications and designations of origin for agricultural produce and foodstuffs. Chemical tests to determine region of origin remain in the early stages of development and it is likely that, to be practicable, databases of authentic produce parameters would necessarily have to be large. Thus food producers have tended towards the development of quality schemes certified by approved inspection bodies in order to control the quality (and hence the value of the labelling) of specified foodstuffs from a region.

### 2.3 Commercial treatment

There are a number of commercial treatments to which foods may be subject. Some are considered desirable by the consumer, e.g., pasteurisation (although this does not add value to the product). Cold pressed (i.e., virgin) olive oil represents another example of a quality process (although the test for this procedure seeks to demonstrate the absence of refined oil). Other procedures, such as food irradiation or the use of GMOs, seek to benefit the consumer, in these cases through the retardation of spoilage or through the production of cheaper food. However, in much of Europe (and in contrast with the USA) these developments have been treated with considerable suspicion by consumers.

### 2.4 Water

Water was one of the earliest adulterants of milk and beer. It still remains a common extender of foodstuffs and has been detected both in liquid products such as wine and also in meat products.

### 2.5 Brands

Protecting the authenticity of brands is an important issue for most businesses whether it be Rolex watches or Levi jeans and foods are no exception. The Scotch whisky industry goes to considerable lengths to protect its brands from being counterfeited, particularly in some third world countries where Scotch can command premium prices and counterfeiting of brand is rife.

### 3 Recent developments in meat authentication

Authenticity issues in meat and meat products have recently been reviewed by Hargin. Geographic origin, meat species (particularly in admixture) and treatment (whether meat described as fresh had in fact previously been frozen) were seen as key issues. Differences in the legislation of meat composition (e.g., use of blood plasma) between EC member states was seen as a potential problem. MAFF has undertaken two surveys in order to establish the prevalence of potential meat frauds in the UK market place. It was discovered that 16 out of 164 samples of cured meat failed to properly declare the amount of added water. It was also found that 44 out of 534 fresh meat samples had been previously frozen but were not labelled to this effect. This survey employed a comparative measurement of the activity of the enzyme β-hydroxacyl-CoA dehydrogenase (HADH) before and after freezing the sample. This enzyme is released when mitochondria are disrupted by freezing; thus there is little change in enzyme activity when a previously frozen piece of meat is refrozen and then thawed whereas a considerable difference is found when fresh meat is analysed initially and is then reanalysed after being frozen and thawed. A survey of species substitution of raw and cooked meats carried out by the Florida Department of Agriculture and Consumer Services found 22.9% of cooked products and 15.9% of raw products contained meats of species other than that described, at levels in excess of 1%. Enzyme linked immunosorbent assay (ELISA) and agar-gel immunodiffusion were the techniques employed for the analysis.

Research continues to develop better methods for determining the authenticity of meat. Myers and Yamazaki have investigated a new immunological technique in which antibodies to meat immunoglobulin (IgG) are bound to a polyester cloth. This format was considered superior to traditional microwell formats for ground meat samples containing fine meat particles because, in the latter, the meat particles may retard the diffusion of the sample IgG molecules.

The development of DNA methods continues to have a major place in meat authentication. Hunt et al. used oligonucleotide probes to identify the species of origin of raw and cooked meat. The benefits of this procedure were that the polymerase chain reaction (PCR) approach was not required. This was advantageous because this equipment is not available in all laboratories and it may also give rise to undesirable assay variability. It was also considered superior to immunological techniques because the latter detect soluble plasma proteins. It has been argued that these are not meat and may arise from adventitious contamination with meat juices or blood. The oligonucleotide probe method works on intracellular DNA. A sample wash procedure can therefore be used to eliminate any cross-contamination from blood, making the procedure particularly effective for enforce-
ment purposes. Because the probes recognise relatively short segments of DNA, the method is applicable to processed and canned meat products.

Consumers have a right to expect properly labelled meat products. However, when the mislabelling issue contravenes ethnic or religious mores, the issue becomes particularly sensitive. Al-Rashood et al.\(^\text{11}\) describe a method for detecting the presence of pork fat in processed foods. The method employs HPLC of the triacylglycerols and can detect as little as 5% pork fat (on a fat basis) in admixture with other meats.

Meat products are potential adulterants in a much wider variety of foods than just other meat products. Agullo and Gelos\(^\text{12}\) described how the determination of free and bound cholesterol using gas chromatography (GC) can be applied to the detection of bovine blood plasma in egg pastas.

### 4 Recent developments in fish authentication

The main issue in fish authentication is one of species. This is usually in the filleted product since before filleting the morphological features provide a good means of determining the species. The traditional technique for determining the species of raw fish is polyacrylamide gel electrophoresis (PAGE) of the sarcoplasmic proteins. After fish is cooked, the use of a denaturant, sodium dodecyl sulfate (SDS), is necessary to give good electrophoretic profiles. Craig et al.\(^\text{13}\) described the use of SDS-PAGE for the detection of other fish species in raw, reformed breaded scampi (*Nephrops norvegicus*). Heavily processed (e.g., autoclaved) products may require cyanogen bromide (CNBr) cleavage of proteins and for different species of closely related families such as tuna or salmon DNA procedures are preferred.\(^\text{14}\)

Rehbein et al.\(^\text{15}\) reported a method of DNA analysis which could be applied to canned products from closely related species. They applied the PCR to amplify sections of the mitochondrial cytochrome *b* gene. These were then analysed using single strand conformation polymorphism (SSCP). In this procedure, single stranded DNA is folded such that complementary sections become bound together. Differences in the three dimensional structure caused by alterations in base pair sequences of the single strand lead to different electrophoretic mobilities which are visualised by silver staining. Differentiation of four eel species and three types of caviar was achieved.

Ram et al.\(^\text{16}\) employed a different DNA technique for authentication of canned tuna and bonito. The mitochondria cytochrome *b* gene was again the focus of attention and the PCR was used to amplify the amount of DNA. However, in this study, sequencing followed by restriction site analysis was performed. This technique uses restriction enzymes which cleave double stranded DNA at defined base pairs to generate species specific DNA fragments. One DNA base pair change between species can therefore be detected by choosing a restriction enzyme which cuts the DNA at the site of this change. Different sized DNA fragments are then produced by the action of the restriction enzyme in the two species.

Thus restriction analysis, SSCP and the use of oligonucleotide probes (as described for meat) represent the three major strands of research on the application of DNA methods to food authentication. Each of them has advantages for different authenticity issues and a more detailed description of these important methods has recently been published by Davidson.\(^\text{17}\)

### 5 Recent developments in milk and cheese authentication

The methods which have been applied to milk and cheese authentication are similar to those for meat products. However, since milk from healthy animals does not contain cellular material, the DNA methods are not applicable. The preferred methods are largely based on protein analysis and either involve typical protein chromatographic techniques (such as electrophoresis) or the use of antibody technology.

Recio et al.\(^\text{18}\) have contributed a very useful review of capillary electrophoretic methods. CZE has been used to detect the adulteration of fresh milk with milk powders and for the determination of the fraudulent addition of rennet whey solids to dairy products (through detection of caseinomacropeptide), and is potentially useful for detecting the adulteration of milk with milks from other species. It is also useful in monitoring proteolysis in cheese production and in providing a measure of thermal treatment of milk.

An electrophoretic ripening index for the evaluation of proteolysis using PAGE provided a means for assessing the quality of Parmesan cheese.\(^\text{19}\) Retail samples purchased in Italy were generally of good quality but those purchased in Austria suggested that adulteration with products with low proteolysis (e.g., cheese rind or very young cheese) had occurred. PAGE has also found application in determining bovine milk in Halloumi (ovine) cheese using analysis of the αs1-casein to provide a detection limit of 2.5%.\(^\text{20}\) The issue of detecting the presence of milk from different species has also been addressed using immunological techniques.\(^\text{20,21}\) These methods can permit detection of as little as 0.1% milk from a foreign species.

Geographic origin is an authenticity issue which is of particular concern to purchasers of cheese. The measurement of the stable isotopes \(^1^3\)C and \(^1^5\)N has been shown to be influenced by the region of origin of the milk. This is because the isotope values in milk are related to those of the fodder on which the cows are fed. Milk from regions dominated by grassland typically shows relatively negative \(\delta^{13}\)C values, but in regions dominated by crop cultivation the \(\delta^{12}\)C values are more positive. The \(\delta^{15}\)N values are influenced by factors such as soil conditions, the intensity of agricultural use and the climate.\(^\text{22}\) As yet, there are insufficient data to determine whether this approach is practicable for certifying the origin of milk and its products; however, the approach shows promise and may form an important component of a suite of tests for geographic origin.

Another important area in the authenticity of milk products is the detection of non-milk fat. Ulberth\(^\text{23}\) applied multivariate regression analysis of fatty acid composition to the detection of tallow in admixture with milk fat. The method was able to determine as little as 1.2% tallow in milk fat using partial least squares (PLS) regression, which is superior to the traditional method of using the butyric acid (C4:0) content alone. The use of advanced statistical techniques is becoming increasingly important to food authentication since they represent the most convenient methods of interpreting data from a number of discrete analytes or methods. A number of different approaches are available and these have been cogently reviewed by Adams.\(^\text{24}\)

### 6 Recent developments in vegetable oil authentication

In common with milk, vegetable oil does not contain sufficient DNA to enable the newer biotechnological techniques to be used to determine the plant origin of the oil. Techniques for authenticating oils have therefore centred on compositional analysis, but there is always the danger that an adulterant can be found which will not be detected by these techniques.

Stigmastadiene is a dehydration product of stigmastasterol formed during the refining process. It is therefore a valuable
the virgin and refined olive oil at levels as low as 5% by measuring the isotope ratio of individual components of oils further. They linked数学 techniques (such as multivariate statistics and fuzzy logic) applied to other instrumental methods. Linking sensory characteristics with the concentration of chemicals provides a potentially powerful means of authenticating oils which is directly linked to features which the consumer demands.

Nuclear magnetic resonance (NMR) is the third spectroscopic technique which is being increasingly applied to food authentication. It can be used in a number of different ways. The entire spectrum can be used to generate a database which is subsequently interpreted by statistical techniques. In this it is analogous to using FT-IR or FT-Raman spectroscopy. Alternatively, it can be used to measure small amounts of specific compounds in the sample which are then used as markers of authenticity. Finally, the isotopic specificity of NMR can be exploited to provide a measurement of species specific isotopic ratios.

Shaw et al.37 adopted the multivariate approach using 13CNMR spectra and found that they were able to differentiate the cultivar of a number of extra virgin olive oils in over 90% of cases and were also able to give some indication of the region of origin.

Sacchi et al.38 were able to identify a number of minor components using high field 2H NMR which were markers of adulteration or were related to oil quality and freshness; however the technique is not yet sufficiently developed to represent an authentication technique.

7 Recent developments in essential oil authentication

Essential oils are so called because they are “essences” rather than because they are considered “necessary”. The essential oils tend to have a very high unit cost, making their extension potentially very profitable. The use of NMR to provide isotopic measurements at defined chemical positions of the test molecule plays a key role in ensuring the authenticity of many of these materials. The 2H nucleus represents one of the best studied nuclei and this site-specific approach has been widely adopted by the Eurofins company under its SNIF-NMR trademark. This approach has been used for determining the authenticity of vanillin and p-hydroxybenzaldehyde from vanilla essence.39 As in so many of these cases, inclusion of δ-13C data provides an extra analytical dimension and enhances the scope of the authentication. The 2H NMR approach has been adopted for benzaldehyde from bitter almond oil and cinnamon oil40 and for phenylethanol and phenylethyl acetate,41 and the combined 2H and δ-13C approach for mustard oils.42 These isotopic methods provide one of the best means of determining the source of food components but the high value of the natural product can make it cost effective to go to considerable lengths to overcome them. Thus Remaud et al.39 demonstrated the presence of a deuterated methoxyl group in a sample of vanillin which could only have occurred through the synthesis of an isotopically labelled adulterant prepared with the deliberate intention of subverting the test.

Another method of authenticating essences is to use the chemical composition of minor components of the essence. This approach has been applied to vanillin.43 Often it is the case that many of the biologically important components of essences are chiral. Thus chiral GC methods have proved valuable and their value can be extended by including on-line δ-13C measurements.44 The use of two isotopic measurements by GC-IRMS (13C and 15N) was found valuable for the authentication of methyl N-methanthranilate.45

The most notable recent development in authentication of flavours comes from 3H (tritium) analysis.46 This has been applied to the analysis of benzaldehyde where the short half-life of 3H (12 years) means that it is never found in petroleum derived materials. As yet, only the potential of the method has been demonstrated since the methodology used requires a large amount of sample. However, this difficulty might be overcome.
by the use of accelerator mass spectrometry, which is designed to measure radioactivity from small samples.

8 Recent developments in fruit products authentication

As with fish, fruits are relatively easy to authenticate when they are whole. It is the act of processing them into other products such as fruit juice or wine which gives rise to the possibility of extension with cheaper materials. Fruits are largely composed of simple sugars and the ready availability of commercial sweeteners means that the potential for adulteration is great. H NMR and δ-13C are the core methods for detecting the addition of beet sugar and cane or corn syrups to wine and fruit juices, respectively, from C3 plants (e.g., orange). These methods have also been extended to maple syrup and citrus honey.

These methods are not easy to apply to pineapple juice since the δ-13C value of pineapple is similar to that found for cane and corn products. Jamin et al. addressed this difficulty by comparing the carbon isotope ratio of the juice with those of the organic acids in the juice, since it was considered likely that these compounds would need to be added in order for the juice to retain an acceptable sugar/acid ratio. This approach of using an internal reference is common for isotopic methods and has been collaboratively tested for δ-13C measurements from fruit pulps and sugars. This principle has recently been applied to detecting the addition of mixtures of beet and cane or corn sugar to fruit juices by analysis of the δ-13C content of individual sugars. At this time, an area of great interest is the development of rapid, automatable sample treatment processes. Interest has been sparked by the measurement of δ-18O in sugars from fruit juice which provides complementary information to other isotopic techniques. This research will certainly lead to further developments in food authentication.

Isotopic methods seek to detect a signal from the major adulterant of a product. An alternative approach is to look for a minor component which might be present in the potential adulterant but which is present in much smaller amounts in the foodstuff tested. Oligosaccharide analysis has proved particularly useful for detecting the presence of commercial sweeteners in fruit juices. Initial work in this area used HPLC with pulsed amperometric detection, although it is now more common to use capillary GC of the silyl ethers. These approaches have been reviewed by Low and have also been applied to honey and maple syrup.

Anthocyanins represent another key marker for detecting the addition of cheap fruits to more expensive fruit purées, particularly when maintaining colour is important. The method has been applied to detecting elderberry in red wine and jams. Species which do not contain colours, phenolic components such as dihydrochalcones can prove useful markers.

Spectroscopic approaches which consider the entire sample composition have also been applied to fruit products. The approach taken is similar to that used for vegetable oils. Again, the main issue still to be resolved is the reliability of interpretation for unknown samples. If the basis on which samples are being classified is not well understood, then the effects of changes in, e.g., growing environment cannot be taken into account in the future. Nevertheless the approach has demonstrated promise for orange juice (NIR), and for fruit purées and jam (MIR), for distinguishing the arabica and robusta coffee varieties and for detecting the adulteration of instant coffees with carbohydrates. It is noteworthy that a chemical basis was proposed for the ability to distinguish the coffee varieties, namely the chlorogenic acid and caffeine contents. An understanding of the reasons for particular classifications in this type of research remains rare. This therefore represents a valuable development because a clear understanding of the basis of a test permits its more general use, for instance on commodities from a country or region which is not represented in the database.

Discovering the reasons for particular classifications of sample sets may be easier using NMR than IR. However, less research has been undertaken using NMR and pattern recognition techniques. The approach undoubtedly holds promise, as has been demonstrated for orange juice and for apple juice by 1H NMR. Colquhoun has recently provided a summary of this approach.

As yet there are no well characterised methods for determining the region of origin of a product with any degree of certainty. However the most promising approach seems to be the use of multi-element data together with a pattern recognition approach. The concept relies on the transfer of trace elements from the soil in the region of interest and hence has so far been best evaluated for fruit products. Baxter et al. were able unequivocally to classify white Spanish wines from three regions. The accuracy of classification fell to 95% when red and rosé wines were included in the database. A similar approach has been adopted for the country of origin of orange juice. It may be that other analytical parameters (e.g., stable isotopes) will extend the possibilities for certifying region of origin.

9 Effects of food fraud

There can be no argument that consumers have a right to accurate, informative labelling. Studies linking sensory perceptions and chemical composition are therefore helpful in ensuring that molecules which have a sensory effect are present in premium quality foods. A unique study investigated the biological effect of mixing peppermint oil with corn-mint oil. Both materials exhibited a wide range of activity against different species of bacteria but both showed consistent spasmolytic activity on guinea-pig ileum suggesting that they are equally effective in treating conditions associated with smooth muscle (e.g., irritable bowel syndrome). Thus admixture of these oils would not necessarily disadvantage consumers who took the oils for this medical purpose.

In most cases, the materials used for extending food are innocuous (e.g., sugar or water). However, if honest labelling is not enforced through legislation then the possibility arises that more harmful practices may ensue. The addition of ethylene glycol to Austrian wine some 10 years ago is a good example. What is less well known is that the incorporation of this material in “elixir sulfanilamide” in 1938 led to 105 deaths in the USA and forced fundamental changes in the operation of the Food and Drug Administration.

The adulteration of food may lead to long term health effects in survivors. A follow up study of survivors of the Spanish toxic oil syndrome found that 58% still suffered symptoms 12 years after the poisoning event. There can therefore be no doubt of the need for continued vigilance in the determination of food authenticity.

References

1. A. H. Hassall, Adulterations Detected or Plain Instructions for the Discovery of Frauds in Food and Medicine, Longman, Green, Longman and Roberts, London, 1861.