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Analytical authentication of organic products: an overview of markers

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Abstract

Consumers' interest in organic foods is increasing and so is the need for robust analytical tools for their authentication. This review focuses on the most promising biomarkers/analytical approaches that are available for the authentication of organic produce. Food products have been subdivided into two groups: foods of plant origin (crops) and foods of animal origin (meat, milk and dairy products, eggs and fish). For each food category the most suitable biomarkers are presented and their potential for authentication is discussed. In the light of current knowledge, it is unlikely that the authentication of organic food products can be attained by the measurement of a single marker. Analytical approaches based on the measurement of multiple markers and/or complex chemical or physical profiles/fingerprints supported by multivariate statistical analysis seem considerably more promising in this respect. For the development of robust classification models, well-designed experimental studies must be performed that rely on data sets that are both well balanced and of sufficient size to ensure that all relevant sources of variation for the target biomarkers are included in the reference database.

Keywords: authentication; biomarkers; fingerprint; organic; biological; sustainable

INTRODUCTION

Consumers' interest in the authenticity of the foods they purchase is increasing, especially where it concerns more expensive 'value-added' products such as organic foods, fairtrade products or products with a protected designation of origin (PDO). 'Value-added' is a term used to characterise food products that have incremental value in the marketplace by differentiating them from similar products based on product attributes such as geographical location, environmental stewardship, sensory quality, artisanal production or functionality.

Considerable attention is being paid to organic farming.[1] The interest of consumers in organic products mainly stems from health and environmental considerations. Consumers are concerned about the safety of what they eat and about the use of pesticides, hormones and other veterinary drugs in farming practice. Furthermore, they are increasingly aware that cultivation and farming methods able to conserve the soil, to preserve the rural countryside as such, to take advantage of local resources and to contribute to sustainability are necessary. Organic farming seems to be a relevant tool with the potential to solve simultaneously a range of problems related to food production, environment, animal welfare and rural development.[2]

In the last few decades the demand for organically produced foods has increased constantly, so that in some countries the market share for organic produce is a significant percentage of the total market. Although organic market growth has slowed down in the last two years, it has remained positive. Despite this success, there are no worldwide standards for organic agriculture and farming. Organic production is regulated within the European Union by a set of laws such as Regulation (EC) No. 1804/99, Regulation (EC) No. 834/2007 and, the most recent, Regulation (EC) No. 889/2008 with detailed rules on production, labelling and control. According to this legislation, organic production systems for plant products are severely restricted in the use of synthetic fertilisers, pesticides and insecticides. It is based on (1) frequent crop rotations and extensive soil tillage, (2) the use of compost or organic waste rather than chemical-synthetic pesticides and readily soluble mineral fertilisers and (3) biological pest control. Organic farming is characterised by an animal diet that comprises at least 85% organic feed (100% for beef and dairy cattle), better if produced by the farm itself, and special care is devoted to animal welfare, with the requirement of adequate access to an outside area and the absolute prohibition of practices that may cause animal suffering.

Organic products tend to retail at a higher price than their conventionally grown/produced counterparts, mainly because of lower yield and certification costs. This premium price and the increasing demand thus make organic food products susceptible to fraud. Although the 'added value' of these products is guaranteed by a system of certification that should warrant their complete traceability at all stages of production, processing and marketing, such paper trail-based systems can be falsified. The problem mainly concerns small shops, farmers' markets and internet sales rather than big retailers and supermarkets, the traceability of whose organic products can be more easily

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ascertained. For consumer confidence it is therefore important that, in addition to certification, the acclaimed specifications of foods can be verified in an objective and independent way.

From an analytical point of view, however, the authentication of organic food products is a challenging problem. It has been the focus of much attention worldwide, as witnessed by the activities of the working group on authenticity established within the EU-funded Network of Excellence MoniQA, the EU CORE II Organics project 'AuthenticFood' and the many scientific studies that have accumulated over the past years.

An overwhelming literature is available on the compositional differences between organic and conventional products. Those differences and their nutritional impact have been extensively reviewed.[3–8] However, to the best of our knowledge, a comprehensive review of the analytical strategies that can be implemented for the authentication of organic products is lacking at present. This review provides a summary of the biomarkers/approaches that have been proposed and/or whose potential has been investigated in the scientific literature. Food products have been subdivided into two groups, i.e. those of plant origin and those of animal origin, the latter being further subdivided into four food categories: meat, milk and dairy products, eggs and fish.

FOODS OF PLANT ORIGIN

Crops

Stable isotopes

Stable isotope analysis of the light elements H, C, N, O and S has been applied for more than 20 years in food authentication control.[9,10] These methods are based on the measurement of stable isotope ratios of a product or of a specific component such as a fraction, an ingredient or a target molecule (or group of molecules) of the product. The determination provides information on the botanical and geographical origin of produce.

The use of nitrogen isotopes for the authentication of organic crops is based on the assumption that the $^{15}N/^{14}N$ ratio ($\delta^{15}N$) of a plant reflects that of the soil in which the plant has grown; this in turn depends on the fertilisation strategies implemented, which are markedly different between organic and conventional farming. In fact, synthetic nitrogen fertilisers are not permitted in organic farming; instead, soil fertility is mainly maintained through the application of manure and the use of crop rotations, although the application of selected fertilisers may be permitted where the need is recognised by an inspecting authority. Synthetic nitrogen fertilisers tend to have $\delta^{15}N$ values close to zero,[11,12] since their nitrogen is derived from atmospheric nitrogen ($\delta^{15}N_{atm} = 0‰$) and there tends to be little fractionation during the production process. On the other hand, nitrogen compounds released by the decomposition and transformation of animal manure show much higher $\delta^{15}N$ values.[13] This enrichment is mainly due to the preferential volatilisation of ^{14}N-depleted ammonia from the manure. It has therefore been suggested that plants grown under a conventional regime will exhibit lower $\delta^{15}N$ values than plants grown under an organic regime.[14]

Indeed, previous studies have demonstrated that grain crops grown in soils to which synthetic nitrogen fertilisers have been added have lower $\delta^{15}N$ values than plants grown in the same soils but to which manure has been added[15–17] and that different plant organs respond in different ways to the isotopic signature of fertilisers, with old leaves and fruits being more sensitive to the addition of synthetic fertilisers.[17] However, it has been reported that both the rate and mode of application of a synthetic fertiliser are also important. For instance, $\delta^{15}N$ values of lettuce (*Lactuca sativa*) tissues can reveal the use of synthetic fertilisers only when these are applied in a high single dose, and it is much more difficult to detect the addition of synthetic fertiliser to basal organic fertilisation.[18]

The potential of the nitrogen isotope ratio for the authentication of organic crops has been thoroughly investigated. Camin *et al*.[19] evaluated a number of markers for the authentication of organic potatoes (*Solanum tuberosum* cvs Vivaldi and Hermes). The tubers were grown in four separate field trials and, within each field trial, organic and conventional tubers were subjected to the same soil and climatic conditions. The authors reported a significant enrichment of ^{15}N in organic crops. When crops from all four field trials were considered, a threshold value of 4.3‰ could be established, which allowed the correct classification of all organic samples and the misclassification of only 15% of conventional crops. Rogers[20] explored the feasibility of $\delta^{15}N$ values as a direct screening tool for the discrimination of off-the-shelf organic and conventional fast-growing vegetables (tomatoes, peas, broccoli, cucumber and zucchini) and slow-growing vegetables (pumpkin, eggplant, potatoes and corn). The author concluded that nitrogen isotopes can be used to differentiate between organic and conventional produce as long as the vegetables are not nitrogen-fixing (e.g. legumes) and that the discrimination is more successful for fast-growing crops such as zucchini, tomatoes and broccoli (growing time <80 days), because the difference in $\delta^{15}N$ values between organic and conventional samples is greater than for slow-growing produce such as potatoes (growing time >80 days). In a survey conducted on 14 different commercially available vegetables from the Slovenian market, Sturm and Lojen[21] concluded that complete discrimination was possible based on $\delta^{15}N$ values for endive, rocket, leek, potato and two chicory cultivars (Palla Rossa and Pan di Zucchero), whereas for tomato, sweet pepper, garlic, onion and carrot, despite the significant difference in mean $\delta^{15}N$ values, differentiation was not possible because of the considerable overlapping of the $\delta^{15}N$ values for the organic products and their conventional counterparts. Rapisarda *et al*.[22] measured the $\delta^{15}N$ values in pulp proteins and amino acids of organically and conventionally grown orange (*Citrus sinensis* cvs Navelina and Tarocco) fruits. They reported significantly higher $\delta^{15}N$ values in organic fruit juice, but no threshold value could be established in this case. The effect of different organic fertilisers on the quality parameters and $\delta^{15}N$ values of orange (*C. sinensis* L. cv. Osbeck) fruits was also investigated.[23] Significantly higher $\delta^{15}N$ values in organic oranges compared with conventional oranges were reported irrespective of the type of organic fertiliser applied. Even though no threshold values could be proposed, the observed differences were such that the authors indicated $\delta^{15}N$ as a suitable tool for the authentication of organic oranges. Bateman *et al*.[24] as the result of a survey on authentic commercially available produce in the UK, reported significantly higher $\delta^{15}N$ values in tomatoes (8.1 and -0.1% for organic and conventional crops respectively) and lettuces (7.6 and 2.9% respectively), but no difference was found in carrots. Overlap between the $\delta^{15}N$ values of the organic and conventional data sets (for both tomatoes and lettuces) means that it is necessary to employ a statistical methodology to try and classify a randomly analysed off-the-shelf sample as organic/conventional. Finally, Schmidt *et al*.[25] reported that the $\delta^{15}N$ values of organic lettuce, cabbage, onions and Chinese cabbage from field cultivation were significantly higher than those of their conventional counterparts. Even more remarkable were

the differences in the δ^{15}N values for organic and conventional paprika and tomato samples cultivated in a greenhouse. However, these differences do not allow an unequivocal classification of the produce according to the farming system based on the δ^{15}N values alone.

From the overview of the literature available, it can be concluded that the use of nitrogen isotopes, although promising, has some limitations, and its potential is highly variable depending on the produce considered, the use of organic manure in conventional agricultural production systems, the use of leguminous plants for enhancing the nitrogen fertility of soils, the δ^{15}N of synthetic nitrogen fertilisers (used recently or in the past), the form of nitrogen in the applied synthetic fertiliser (NO_3^-/NH_4^+/urea), the variability in the δ^{15}N of non-synthetic nitrogen fertilisers, the timing of the fertiliser application, the temporal and spatial changes in soil δ^{15}N and the changes in plant δ^{15}N over time during the growth period. An additional complication derives from the fact that legislation allows (partly) other types of fertilisation in organic production, which may lead to false negatives. Furthermore, even if plant δ^{15}N can be used to distinguish satisfactorily between crops that have been grown with and without the application of synthetic nitrogen fertilisers, it does not follow automatically that the crop can be described as organic, since it may not have been grown in compliance with all requirements of organic cultivation, which may result in false positives.[16] Nevertheless, nitrogen stable isotope ratios can be used as a valuable supporting tool for the authentication of organic production, particularly if combined with other suitable markers in a multivariate approach (see 'Major and trace elements' and 'Other multi-marker approaches' for specific examples).

Major and trace elements

The elemental uptake of a specific crop and thus its chemical composition are influenced by many factors, including plant species, cultivar, physiological age, soil fertility, climate, crop rotation and fertilisation strategy as well as pest and weed control management.[26,27] Organic and conventional farming differ strongly in the last two factors. In particular, organic farming severely restricts the use of artificial fertilisers; instead, use is made of organic fertilisers and nitrogen-fixing plant species. The extent and rate of release of nutrients are thus different in the two farming practices, and this has an impact on the overall crop chemical composition.

A considerable number of papers have compared the concentrations of major and trace elements between organically and conventionally grown crops.[1,28–30] It appears that the findings are not consistent across crop species or even across different varieties of the same species. Ordonez-Santos *et al.*[31] for instance, demonstrated that the most important factor of variation in microelements in tomato is variety. In particular, it is unlikely that generic tracers that can be applied to all crop species exist. However, there is a growing body of evidence suggesting that systematic differences in the concentrations of certain elements such as Mn, Ca, Cu and Zn occur between crops cultivated under organic and conventional practices. This may be a result of the presence of elevated levels of arbuscular mycorrhizal fungi (AMF) in 'organic soils'.[32]

Discrimination between agricultural practices using the elemental profile has been attempted. Gundersen *et al.*[33] managed to discriminate between organic and conventional onions (*Allium cepa* cv. Hysam) and peas (*Pisum sativum* cv. Ping Pong) based on major and trace element profiles combined with multivariate analysis. Principal component analysis (PCA) applied to the data of all measured elements (63 elements in onions and 55 in peas) discriminated between organic and conventional crops. However, it must be noted that no systematic differences were highlighted between differently grown crops when they were compared across plant species. Laursen *et al.*[34] investigated the potential of the multi-element profile for the authentication of organic winter wheat (*Triticum aestivum* cv. Tommi), spring barley (*Hordeum vulgare* mixture of cvs Simba, Smilla and Power), fava bean (*Vicia faba* cv. Columbo) and potato (*S. tuberosum* cv. Sava). The crops were obtained from a controlled field study that excluded the effects of season, climate and soil characteristics. The authors demonstrated that no single element allowed discrimination between conventional and organic crops across locations, years and crop species. The quantitative analysis of 14 elements allowed only a partial discrimination between farming systems, the variability being mainly dominated by the location factor. To improve their results, the authors measured 11 additional trace elements by semi-quantitative inductively coupled plasma mass spectrometry (ICP-MS) analysis. The new multi-element profile based on 25 elements allowed the discrimination between organic and conventional samples for barley, wheat and potato, whereas the discrimination was still incomplete for fava beans. The authors underlined that the inclusion of non-essential elements in the profile greatly improves the discrimination, likely because of the impurities present in the artificial fertilisers typical of conventional farming.

Kelly and Bateman[35] analysed samples of organic and conventional tomatoes (*Lycopersicon esculentum*) and lettuces (*L. sativa*) for their trace element concentrations and reported significantly higher concentrations of Ca, Cu, Zn and Rb in organic tomatoes and of Cu and Rb in organic lettuces and a significantly lower concentration of Mn in organic tomatoes (the Mn concentration was numerically but not significantly lower in organic lettuces). The results for tomato are in line with a pattern already reported and attributed to the presence of AMF in 'organic soils'.[32] The authors reported that, when the trace element concentrations are combined with δ^{15}N isotope data and subjected to multivariate analysis (canonical discriminant analysis, CDA), the correct classification of organic and conventional tomato samples is improved (from 94 to 100% of cases from each group correctly classified by cross-validation). However, no further improvement in the success rate was observed for lettuces. Finally, De Nadai Fernandes *et al.*[28] investigated samples of Brazilian green coffee (*Coffea arabica*) for their elemental composition by instrumental neutron activation analysis (INAA). The elements Br, Ca, Cs, Co, Mn and Rb were found to be suitable markers for discrimination of organic from conventional coffees. Bivariate analysis suggested that discrimination was possible considering different pairs of elements. Furthermore, the authors developed a Bayesian neural network based on the database of the 11 elements analysed, which was tested on additional 23 coffee samples with a very good success rate (one misclassification).

Metabolites and proteins

The metabolite profile of a food sample can be regarded as an analytical signature of that food product and thus can help in discriminating between crops grown under different agricultural practices. The comprehensive and quantitative analysis of wide arrays of metabolites in biological samples is known as metabolomics, whereas that of the proteome is

referred to as proteomics. The potential of metabolomics and proteomics for the authentication of organic crops has been explored mainly for cereals. Zorb *et al*.[36] determined the levels of 52 metabolites in wheat (*T. aestivum* L. cv. Titlis) grains grown under comparable organic and conventional conditions by a high-throughput gas chromatography/mass spectrometry (GC/MS) technique. The metabolite profile included amino acids, organic acids, sugars, sugar phosphates, alcohols and nucleotides. The authors found significant differences in only eight of the 52 metabolites measured and concluded that the farming system has a small or negligible impact on the metabolite profile of wheat grain. However, no multivariate statistics was applied to the analytical data. In a subsequent study, Zorb *et al*.[37] investigated the levels of 62 metabolites, including lipids, cations, starch and proteins, in wheat (*T. aestivum* L. cv. Runal) ears and mature grains measured by various techniques. They found significant differences for many metabolites in wheat ears, but this difference disappeared or became marginal in mature grains. Even in this case, no multivariate statistics was applied to the metabolite profile. An untargeted analytical approach was explored by Zorb *et al*.[38] who recorded the levels of 1049 proteins in conventional and organic wheat (*T. aestivum* L. cv. Titlis) grains. Twenty-five proteins showed significantly different levels between the two farming systems. Sixteen were selected based on the calculation of a numerical 'consistency score', which is a measure of the impact of agricultural effect on the parameter variability compared with that of seasonal effect. These diagnostic proteins should constitute a sort of protein signature that would be of help for the authentication of organic wheat, but again no multivariate statistical analysis was applied.

The metabolomics approach seems to be promising, because plant metabolite profiles are potentially a very rich source of biomarkers. However, Rohlig and Engel[39] could not differentiate maize crops of three different varieties (*Zea mays* cvs Amadeo, Lukas and Flavi) grown under organic and conventional conditions in two different locations based on PCA of the metabolite profile obtained by GC/MS. Only three compounds were found to be significantly different between farming systems when samples from all three varieties and both locations were considered, suggesting that the genetic differences and seasonal effects are stronger than the differences due to farming practice. On the other hand, an untargeted approach based on MS fingerprint measured by flow injection electrospray ionisation ion trap mass spectrometry (FI-ESI-IT-MS) and flow injection electrospray ionisation time-of-flight mass spectrometry (FI-ESI-TOF-MS) of organic and conventional grapefruits (*Citrus paradisi* cv. Rio Red) grown under strictly controlled conditions and PCA showed a complete separation of samples based on farming practice.[40] However, owing to the analytical set-up, it was not possible to identify the compounds corresponding to the *m/z* ratios most responsible for the discrimination.

Phenolic compounds

Phenolic compounds are secondary metabolites that are widely distributed in plant foods such as fruits and vegetables but also in cereals, legumes, tea, coffee and chocolate. A number of studies have shown that, because of their antioxidant properties, phenolic compounds are able to exert beneficial effects on human health, such as protection against aging, coronary heart diseases and arteriosclerosis.[41,42] The phenolic content of organic products relative to their conventional counterparts has been extensively investigated. For instance, higher levels of flavonoids (a major class of phenolic compounds) are reported in organic tomatoes,[43,44] collard greens (*Brassica oleracea*), Chinese cabbage (*Brassica rapa*), spinach (*Spinacia oleracea*), garlic (*Allium sativum*) and green bell pepper (*Capsicum annuum*),[45] a higher content of anthocyanins is reported in organic blueberries (*Vaccinium corymbosum* cv. Bluecrop)[46] and higher concentrations of total phenolics are reported in organic eggplants (*Melanus melongena*),[47] beet (*Beta vulgaris*),[48] peach (*Prunus persica*) and pear (*Pyrus communis*),[49] marionberries (*Rubus* subgenus *Rubus*)[50] and miscellaneous plants.[51] On the other hand, other studies have found no significant effect of cultivation practice on the phenolic content of eggplants[52] and strawberries (genus *Fragaria*).[53]

The phenolic content of organic and conventional processed vegetal products has also been investigated. Higher levels of polyphenols are reported in organic Croatian wines,[54] whereas no significant differences in polyphenol content are reported in organic and conventional Monastrell grapes and their corresponding red wines.[55,56] Moreover, significantly higher concentrations of phenolic compounds have been reported in organic tomato-derived products (ketchups).[57] Food processing adds an extra level of complexity to the authentication issue because of the potential effect of any of the process steps on the occurrence of the biomarker in the final product. This effect varies according to the product and the process. It will be minimal for wine, whose polyphenol content would mainly reflect that of the starting grape (unless a long maturation period strongly affects the phenolic content). On the other hand, it might be significant during ketchup manufacture because of the heat treatment of the tomato paste and the mixing with other ingredients.

The different phenolic content in organically grown crops has been ascribed to the absence of synthetic pesticides in organic farming, resulting in higher exposure to stressful situations, which would lead plants to produce secondary metabolites as a defensive mechanism.[1,58] The availability of inorganic N, which depends on the type of fertilisers, can also modulate plant biosynthetic pathways, resulting in more limited formation of phenolic compounds in organic crops.[59] Finally, organically produced plants generally have longer ripening periods compared with conventional plants.[60,61] Secondary metabolites form in the ripening period and thus are expected to accumulate in higher concentrations in organic plants. The amount of phenolic compounds occurring in plants varies according to plant species and cultivar, climate, soil and growing conditions and maturity stage.[62] At present, although there are still controversies in the scientific literature, it appears that organically grown crops have a higher content of phenolic compounds, although the genetic effect is also important, so this conclusion cannot be generalised to all crops. A possible option for the authentication of organic produce would be to consider the profile/fingerprint of (a specific class of) phenolic compounds rather than the overall phenolic content (or the content of a class of phenolic compounds). However, this option has not been explored so far.

Copper chloride crystallisation patterns

Copper chloride crystallisation is an analytical method based on the evaluation of the crystallisation patterns that occur when an aqueous cupric chloride solution ($CuCl_2 \cdot 2H_2O$) is crystallised on a glass dish in the presence of a plant extract.[63] The crystallisation patterns are characteristic of the sample material that is analysed. However, the evaluation of the crystal patterns has long been a major limitation of this method because of the lack of standardised objective criteria for their description. Recently, a standardised visual evaluation approach based on a trained panel[64] and

a computerised image analysis (applied computerised image analysis, ACIA)[63] have been presented. Several authors claimed that the copper crystallisation method would be particularly suitable for the authentication of organic crops.[1,65] Organic products would clearly differ from their conventional counterparts because they form highly organised crystals pattern.[66,67] Szulc *et al*.[68] performed a computerised image analysis of the crystallisation patterns of farm pair samples of organic and conventional winter wheat (*T. aestivum* cv. Cubus) grains collected in two harvesting seasons. A total of 15 texture variables were evaluated on the crystallograms using computerised image analysis and subsequently subjected to discriminant analysis (DA). When samples from the same harvesting season were considered independently, the DA models were able to correctly classify a variable percentage ranging from 70 to 100% of the analysed samples depending on the region of interest (ROI, the circular portion of the crystallogram that was included in the image analysis) and the harvesting year. However, an attempt to pool the data from the two harvesting seasons resulted in a DA model with a rate of correct classification lower than 60%. Similarly, Kahl *et al*.[69] applied the copper chloride crystallisation method to wheat (*T. aestivum* cvs Tamaro, Titlis and Runal) samples from a long-term trial (DOC trial) that used a field plot design where conventional and organic farming systems with identical crop varieties were run together so as to rule out climatic and soil-related sources of variation. The authors performed both a computerised image analysis of the crystallograms and a visual inspection by means of a trained panel. The data collected were subjected to linear discriminant analysis (LDA). It was reported that the percentage of correctly classified samples varied strongly with the year of harvesting and the type of pattern evaluation (computerised or visual). The highest success rate was achieved for samples from 2003 and visual inspection of the crystallisation patterns, which allowed 100% correct classification.

The crystallisation method is the most recognised of those holistic methods that aim to assess the food as a whole rather than its constituents. It represents an interesting fingerprint approach to organic food authentication, but the sparse literature available on the subject and the high variability of the method are major drawbacks of this approach.

X-ray spectra

X-ray spectroscopy is a technique based on the analysis of the emission spectra of test samples after X-ray excitation. The characteristics of the spectra provide information on the chemical composition of the test sample. Bortoleto *et al*.[70] investigated the potential of energy-dispersive X-ray fluorescence (EDXRF) combined with chemometrics analysis (PCA) of the spectral data to discriminate between organic and conventional coffee and tomato samples. The results of their study suggest that this approach has good potential for the discrimination of crops according to farming practice. The K-peaks of potassium proved to be the most responsible for the discrimination of tomato and coffee samples according to management practices. It must be highlighted, however, that at the moment no significant difference in potassium content between organic and conventional produce is apparent from an overview of literature data. The approach appears to be promising for routine quality analysis since it can be carried out by portable equipment and thus can be implemented *in situ*, but more robust classification models have to be built based on a larger database.

Infrared spectra

Infrared spectroscopy is based on the measurement of the intensity and wavelength of absorption of infrared light by a sample.[71] Depending on the region of the infrared spectrum that is used, a distinction can be made between near-infrared (NIR), mid-infrared (MIR) and far-infrared (FIR) spectroscopy. Infrared spectroscopy is a non-invasive, non-destructive, rapid and relatively inexpensive technique that can be easily applied for the online analysis of food samples. Only one study published so far has investigated the potential of this approach for the authentication of organic produce. Cozzolino *et al*.[72] applied MIR spectroscopy and multivariate analysis to discriminate between Australian organic and conventional wines. The authors applied different statistical treatments to the spectral data. The partial least squares discriminant analysis (PLS-DA) model showed classification rates of 100 and 88% for organic and conventional white wines and 73 and 85% for organic and conventional red wines respectively.

Other multi-marker approaches

Some other approaches have been reported in the literature. The authentication of organic orange juice based on profiles of volatile compounds has been explored. The volatile profile of orange juice comprises various compounds such as aldehydes, alcohols, ketones, esters and hydrocarbons. The present authors obtained the volatile profile of orange juices by proton transfer reaction mass spectrometry (PTR-MS).[73] Mass spectral fingerprints constituted by the masses of the volatile compounds and corresponding signal intensities were recorded and used as patterns for comparison between samples of different types. PCA performed on the spectral data showed a clear distinction between conventional and organic orange juice samples.

Rapisarda *et al*.[22] performed LDA on the data set composed of 11 analytes measured in the juice of 64 organic and conventional orange samples of two different varieties. The model resulted in 86.9% of organic samples and 93.9% of conventional samples correctly classified in their groups. Vitamin C and Synephrin in juice and the δ^{15}N value in pulp proteins were the most relevant variables to discriminate between the two groups according to the standardised canonical discriminant function coefficients. Camin *et al*.[74] applied CDA to several physical-chemical and isotopic parameters (δ^{13}C, δ^{15}N, δ^{18}O, δ^{2}H and δ^{34}S) to verify the possibility of improving the discrimination between organic and conventional oranges, strawberries, peaches and clementines of different cultivars from both experimental fields and certified organic and conventional farms. For each fruit, CDA was applied to different sets of analytes, namely those that proved to be significantly different in the analysis of variance (ANOVA) test. A variable success rate ranging from 75 to 98% of correctly classified samples was obtained for oranges, strawberries and peaches. Ascorbic acid, δ^{15}N value and total soluble solids were the most significant parameters for the discrimination between conventional and organic oranges, peaches and strawberries, whereas further markers were needed for the successful discrimination of clementines. Finally, Raigon *et al*.[47] measured the levels of dry matter, proteins, total phenolics and eight minerals in two landraces and one commercial hybrid of eggplant in two successive years. They found higher mean contents of K, Ca, Mg and total phenolics in organic eggplant samples from the first year and higher mean contents of K, Mg and Cu in organic eggplants in the second year. PCA was performed and showed a clear discrimination between cultivars, years and cultivation methods.

Transcriptomics

Given that nutrients regulate plant metabolism via gene expression, transcriptomics is another option for the discrimination between fertilising strategies and thus for the authentication of organic produce. At present, the number of studies investigating the effect of farming practices on gene expression is very low. One study has been carried out regarding the identification of gene markers for wheat grown under organic and inorganic nitrogen fertilisers.[75] The study demonstrated that several genes related to nitrogen metabolism and storage protein synthesis are differently expressed in grain endosperm according to whether the nitrogen is supplied in either organic or inorganic conditions. More recently, a study has been performed to assess the potential of transcriptomics to detect differences in the gene expression in potato tubers of different varieties grown under different environmental conditions (fertilisation and crop protection strategies).[76] Microarray analysis together with quantitative reverse transcriptase principal component regression (Q-RT-PCR) analysis was used. The results showed that the differences in gene expression due to the different growth conditions were less pronounced than those due to the different varieties employed in the study. In another study, Van Dijk *et al*.[77] investigated the potential of transcriptomics to discriminate between organic and conventional farming practices. Potato tubers of the cultivar Sante were grown under four distinct farming conditions (organic and conventional fertilisation strategies combined with organic and conventional crop protection strategies). The microarray and Q-RT-PCR data were subjected to multivariate analysis (PCA). Several metabolic pathways were identified where the gene expression permitted a clear separation of the samples according to the type of fertilisation/crop protection strategy, among which were the lypoxygenase, the starch synthase and the biotic stress pathways. The paper showed that transcriptomics combined with chemometrics can be a tool for the authentication of organic farming, but its potential is still underexplored and should be investigated in a wider range of produce.

Pesticide residues

The prohibition on using synthetic pesticides is one of the main features differentiating organic from conventional agriculture. However, although synthetic pesticide levels are significantly lower for organic products compared with their conventional counterparts, residues of synthetic pesticides can occur in organic produce because of field cross-contamination or contamination during packaging and storage.[78] Furthermore, pesticide analysis is not a conclusive strategy, since the absence of such compounds does not imply compliance with other rules of organic farming. Nevertheless, pesticide analysis is a well-established technique and can provide additional evidence in suspected cases of organic misdescription.

FOODS OF ANIMAL ORIGIN

Meat

Stable isotopes

The carbon, nitrogen and sulfur isotopic composition of animal tissues depends both on the isotopic composition of the diet and on tissue-specific fractionation. The stable isotope composition of animal tissues is a potent tool for the traceability of animal diets and thus for the authentication of those production systems that rely on feed sources with distinct isotopic compositions.[79] According to their differences in photosynthesis, plants are classified into three types, C3, C4 and crassulacean acid metabolism (CAM) plants, having different δ^{13}C values.[80] These values are lowest for C3 plants such as grass, hay and soybean and highest for C4 plants such as maize and sorghum, while CAM plants have intermediate values. Since pasture is almost exclusively made up of C3 plants, δ^{13}C levels are expected to be lower in meat from pasture-fed or organically reared cows. Piasentier *et al*.[81] investigated the potential of carbon and nitrogen stable isotope ratios for the authentication of lamb meat. The authors applied CDA to fat δ^{13}C and protein δ^{13}C and δ^{15}N values measured in lamb meat samples from animals on different diets and were able to correctly classify 91.7% of the meat samples according to the type of diet. Boner and Förstel[82] reported values of δ^{13}C in German organic beef lower than those in conventional beef and never exceeding −20‰. They concluded that δ^{13}C is a potential biomarker for the authentication of organic meat, but no advanced statistics was used by the authors to support their conclusion. The difference was explained by the fact that conventional farms use maize more intensively and consistently than organic farms do to obtain rapid growth and consistent meat production. Higher δ^{15}N values in conventional meat compared with organic meat have also been reported.[81,82] The higher level of δ^{15}N expected in organic meat samples because of the effect of fertilisers was not observed. This has been explained by the preferential loss of ^{14}N from conventional, more 'open' farm systems.[83]

Schmidt *et al*.[83] succeeded in differentiating organic from conventional Irish beef by means of the combined carbon, nitrogen and sulfur isotopic composition. The two groups differed significantly (MANOVA $F_{3,28} = 10.3$, $P < 0.001$). Organic Irish beef had a more negative and somewhat less variable δ^{13}C value (-26.0 ± 0.2‰) than conventional beef (-24.5 ± 0.7‰). This can be explained by the higher proportion of fresh or preserved grass compared with maize and concentrates in the organic practices. The approach worked well for Irish beef, but it should be kept in mind that feeding practices may vary greatly among different countries and that the discriminative power of this approach might be reduced when less contrasting feeding conditions are applied.

However, it has been reported that δ^{13}C of conventional beef showed a marked seasonal positive shift of >2‰ between December and June, while δ^{13}C in organic beef was less variable.[84] The implication for the authentication of organic meat is that this approach may work provided that the temporal dynamics of isotopic composition is taken into account. It has been also reported that the turnover of light elements (C, N and S) in bovine skeletal muscles is a slow process and that skeletal muscles contain isotopic information on dietary inputs integrated over a long period of time (months to years).[85] No information on fraudulent changes in animal diets shortly before slaughter (e.g. a diet rich in concentrate that does not comply with the minimum amount of 60% roughage laid down in EU regulations) can be obtained from the isotopic composition of beef. To obtain short-term information on animal diets, the analysis of bulk skeletal muscles is inadequate and information on additional meat fractions or tissues is necessary.

Fatty acids

Diet is known to markedly affect the composition of meat (and milk) fat.[86–88] Fatty acid profiles may thus be a fruitful source of information on animal diets. The effect is related to the transfer of specific compounds from the feed to the meat and/or to the

formation of specific compounds by rumen microflora due to the effect of a particular diet. Feeding regimes are usually quite different between organic and conventional farms. Organically raised cattle have, on average, more access to outside areas and their diet is richer in fresh or preserved forage, whereas the diet of conventionally raised cows is richer in concentrates or cereal crops. According to EU regulations, a minimum amount of roughage has to be fed to animals in organic farming. Fresh pasture and forage are very rich in α-linolenic acid, which is the most abundant fatty acid.[89] Although part of the α-linolenic acid in ruminants is biohydrogenated in the rumen, a variable amount escapes ruminal metabolism and is incorporated in fat tissues. In general, a diet based mainly on pasture relative to concentrates has been reported to increase the concentration of ω-3 fatty acids in beef[90,91] and lamb.[92] Literature data suggest that there are systematic differences in fatty acid composition between organic meat and conventional meat. In particular, organic meat is significantly richer in α-linolenic acid and total ω-3 polyunsaturated fatty acids (PUFA) and has a more favourable ω-3/ω-6 ratio.[93–95] Perez-Palacios *et al*.[96] also concluded that α-linolenic acid is a potential marker for pasture feeding along with arachidonic acid, neophytadiene and γ-tocopherol. Husak *et al*.[97] reported higher levels of PUFA and ω-3 fatty acids in the breast and thigh of organically raised broilers compared with free-range and conventionally raised broilers. The access to outdoor organic feed and grass can explain the difference between organically and conventionally raised broilers, since the latter are confined indoors, but cannot explain the difference between organically raised and free-range broilers, since both groups have access to outside areas. Finally, higher levels of PUFA have been reported by Castellini *et al*.[98] in the breast and drumstick of organically raised broilers.

Based on the strong effect that the feeding regime has on meat fat composition and the marked differences in feeding practice between organic and conventional farming, it is likely that a multivariate analysis of the fatty acid profiles would prove useful for the authentication of organic meat. Such an approach has already proved successful in discriminating meat samples as a function of their feeding background. Perez-Palacios *et al*.[96] applied PCA to the fatty acid profiles measured independently on subcutaneous fat, on intramuscular fat of the *biceps femoris* muscle and on intramuscular fat of the *semimembranosus* muscle and were able to discriminate meat samples of Iberian pigs as a function of their feeding background (acorn and grass diet *versus* oleic acid-enriched concentrate diet). Arce *et al*.[99] also succeeded in discriminating Iberian pig samples according to the feeding regime (outdoor *versus* indoor) by means of infrared spectroscopy. Although the discrimination was based on the infrared spectra, a strong correlation with the fat profile was highlighted. Presently, only one study has explored, although in an indirect way, the potential of the fatty acid profile for the authentication of organic meat. Pla *et al*.[94] used NIR spectroscopy to analyse the fatty acid content of rabbit meat. Calibration equations were developed for each fatty acid, with variable prediction rate. To discriminate between conventional meat and organic meat, PLS-DA was performed on the NIR spectra originating from the two groups. Only one organic sample was misclassified (98% success rate).

Volatiles

A study on dry-cured hams showed the potential of fingerprinting of volatile compounds.[100] PTR-MS was used to discriminate artisanal organic hams from conventional hams. The volatile organic compounds in the mass range *m/z* 21–150 were subsequently subjected to PLS-DA to authenticate groups of samples. Discrimination between organic and conventional ham production systems and origins of the samples was achieved with high success rates and thus few misclassifications. This study showed that, although the volatile profile of hams is a very complex attribute that develops mostly during the storage/ripening stage, the volatile fingerprint of the starting meat can still provide suitable information for the authentication of processed meat.

Veterinary medicines

In the organic farming system, for the proper control of animal diseases, phytotherapeutics, homeopathic products and trace elements are preferred to chemically synthesised allopathic veterinary medicines. However, the regulations do permit sick animals to be treated with conventional medicines (under strict control) provided that the animals are not sold as organic within a certain withdrawal time or if the animals have been treated more than once and their productive cycle is less than 1 year. Prophylactic treatment with antibiotics is specifically forbidden under organic regimes. The historical pattern of veterinary drug administration can thus help in verifying compliance with the rules of organic farming. Kelly *et al*.[101] developed a method to distinguish between a single therapeutic dose of a tetracycline (permitted under the standards) and both multiple therapeutic dosing and prophylactic dosing (not permitted). The method uses bone sectioning and observation under ultraviolet light (either direct observation or fluorescence microscopy). The method is at present applicable to pigs and chickens and to a selected array of tetracyclines. Despite its usefulness, this approach obviously cannot be considered conclusive, since the absence of tetracyclines does not imply that the meat has been produced organically.

Milk and dairy products

Stable isotopes

In animals, stable isotope ratios are primarily determined by diet,[102,103] although further enrichment of the heavier isotopes can occur during metabolism.[104] Milk has been shown to rapidly reflect changes in isotope signature of feeds. The δ^{13}C in feeds depends strongly on the relative contribution of C3, C4 and CAM plants. Since pasture is almost exclusively made up of C3 plants, δ^{13}C levels are expected to be lower for milk from pasture-fed or organically reared cows. In fact, it has been demonstrated that the δ^{13}C values in grass-fed cows' milk are significantly lower than those in maize-fed cows' milk[105] or crop-fed cows' milk.[106]

Molkentin and Giesemann[107] reported a substantial difference between organic and conventional milk samples in their all-year variation in δ^{13}C in milk fat, with a maximum value for organic milk of −28‰ and a minimum value for conventional milk of −26.6‰. This difference could be explained by the different percentage of maize in the feed. The basic feed on the conventional farm was made up of 60% maize silage during the whole year, whereas the organic farm used only small amounts of maize silage during the pasture period. In a subsequent study, Molkentin[108] analysed 268 retail milk samples from three organic and three conventional milk brands over 18 months. Based on the results of this study, an exclusion criterion for organic milk of >-26.5‰ could be laid down. This would exclude >99% of the conventional milk samples. Conventional milk can exceed this threshold value under atypical and rare conditions, but differentiation from organic milk can be improved by time-resolved comparison of data and

knowledge of the production date of the milk sample. The author warned that the results of the study were primarily applicable to unadulterated milk, mainly to German milk samples, and might be unsuitable for milks of foreign origin or containing substantial amounts of dairy ingredients of foreign origin. In their most recent study, Molkentin and Giesemann[109] reported the seasonal variation in $\delta^{13}C$ values in milk protein obtained from organic and conventional retail milk samples. A clear year-round difference existed between organic and conventional milk, with no overlap. $\delta^{13}C_{protein}$ values ranged from −27.03 to −23.75‰ in organic milk and from −23.29 to −21.16‰ in conventional milk. The authors established a threshold value of −23.50‰ for the authentication of organic milk and concluded that $\delta^{13}C$ values in milk protein and $\delta^{13}C$ values in milk fat are equally suited for authentication of organic milk, as both depend similarly on the proportion of maize in the milk cows' rations. Moreover, owing to the relatively constant fractionation of carbon isotopes between milk protein and milk fat, the simultaneous determination of $\delta^{13}C$ values in milk protein and $\delta^{13}C$ values in milk fat may be used to unveil fraudulent addition of non-organic fat to organic milk.

With regard to nitrogen isotopes, the heavier ^{15}N is enriched in proteins along the food chain in terrestrial or aquatic ecosystems.[110] According to the standards for organic farming, all feed must be obtained from organic farming, preferably from the same farm. Since artificial fertilisers are forbidden in organic farming, and organic manure contains relatively more ^{15}N than artificial fertilisers, which are commonly produced by the fixation of atmospheric N_2, $\delta^{15}N$ would be higher in organically produced feed, which would be reflected in milk protein. However, Molkentin and Giesemann[107,109] and Molkentin[108] observed a higher level of $\delta^{15}N$ in conventional milk, with a substantial overlap for the all-year variation in $\delta^{15}N$ in retail and farm samples of both organic and conventional milk. They hypothesised that conventionally reared cows might have been fed a high amount of leguminous plants (which directly fix atmospheric N_2 and thus have lower $\delta^{15}N$) and/or that a significant amount of liquid manure from animal husbandry (which has significantly higher $\delta^{15}N$) might have been used in conventional farms. The authors fixed a threshold value for $\delta^{15}N$ of 5.50‰, which is not exceeded by organic retail milk samples. On the other hand, conventional milk samples can show $\delta^{15}N$ values below this threshold value.

Phytanic acid

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a branched-chain fatty acid that is produced by bacterial oxidation and biohydrogenation of the chlorophyll side chain phytol in the rumen. Phytanic acid cannot be *de novo* synthesised by mammals and thus is exclusively derived from feed.[111] For this reason, phytanic acid and its main degradation product pristanic acid have been proposed as potential markers for pasture-fed milk/dairy products. Since the proportion of grass in animal ration is higher in the case of organically raised cattle, phytanic acid has been proposed as a marker for organic milk/dairy products as well.

Schröder *et al.*[112] explored the day-to-day variation in phytanic and pristanic acids in cows' milk. Milk samples from one organically and one conventionally raised cow taken on subsequent days were analysed. In the organic milk, phytanic acid reached on average 3.14 g kg^{-1} fat, with a lowest level of 2.48 g kg^{-1} fat. On the other hand, the highest concentration of phytanic acid in conventional milk fat was 1.77 g kg^{-1} fat. Similarly, Vetter and Schröder[113] found that levels of phytanic acid in organic milk/dairy products exceeded those in conventional products by 50% and that concentrations of pristanic acid were also significantly higher in organic milk/dairy products. However, the concentration ranges of the two fatty acids in the organic and conventional milk overlapped substantially. The highest content in conventional products surpassed the lowest concentration measured in organic products. Thus an unequivocal discrimination was not possible based on the concentration of either phytanic or pristanic acid. Nevertheless, the authors proposed a threshold limit for phytanic acid concentration in organic milk and dairy products of 2.00 g kg^{-1} fat. Only two of the 14 samples of organic cheese the authors analysed fell below that target value, whereas all samples of organic milk, cream or butter were above that value. On the other hand, four samples of conventional cheese exceeded the threshold value. Although significantly higher in organic dairy products, no threshold was proposed by the authors for pristanic acid because of the less marked differences between organic and conventional products. The authors suggested monitoring manufacturers whose organic dairy products show concentrations below the proposed threshold value in order to confirm the application of organic principles.

To further explore the usefulness of phytanic acid as a marker for organic milk, Schröder and Vetter[114] studied the distribution of the two potential stereoisomers of phytanic acid, *RRR* and *SRR*, produced by microbial activity in the rumen. The authors found that the amount of *SRR* stereoisomer of phytanic acid never exceeded 50% of the total phytanic acid in milk samples taken from one organically raised cow, whereas this amount ranged between 60 and 85% for samples taken from one conventionally raised cow. The authors suggested that the stereoisomer ratio might be used as a potential tool for the authentication of organic or grass-fed milk samples. It is known that the microbial population in the rumen changes according to animal diet.[115,116] It is likely that the rumen bacteria dominant under dietary conditions characterised by a large amount of grass in the forage ration would favour the production of *RRR*-phytanic acid, whereas the bacteria dominant under a concentrate-rich diet would favour the production of *SRR*-phytanic acid. The validity of this hypothesis, however, needs to be verified in future with a larger set of samples. It is interesting to notice that the diastereomer composition of phytanic acid is not a suitable marker for the authentication of organic cheese. Indeed, although the proportion of *SRR* diastereomer was generally higher in conventional samples, the difference between organic and conventional cheese was less pronounced than for milk and the groups could not be differentiated by statistical methods. The authors suggested that post-milking changes might be involved, with the two diastereomers of phytanic acid being degraded at different rates possibly because of differences in cheese-specific microflora. This underlines the necessity of checking the reliability and reproducibility of the target biomarker when processed foods are considered.

α-Linolenic acid

Several studies report higher levels of α-linolenic acid in both retail[107,117,118] and farm[119–122] organic milk samples compared with conventional milk samples. A higher amount of fresh grass relative to concentrates increases the level of α-linolenic acid in milk and dairy products. This effect of feeding regime on α-linolenic acid is also evident from the significant effect of season on α-linolenic acid levels. α-Linolenic acid is indeed lower in winter than in summer when most of the cows turn from a pasture-based diet to an indoor diet based mainly on forage/silage/concentrate.

Based on the results of a large survey of German retail and farm milk samples, Molkentin and Giesemann[107] proposed a threshold

value for α-linolenic acid in milk of 0.56% of total fatty acids that would enable the complete differentiation between all-year ranges of organic and conventional milks. To further prove the feasibility of α-linolenic acid for the authentication of organic milk, Molkentin[108] analysed 268 retail German milk samples from both organic and conventional brands. If the all-year variation in α-linolenic acid is considered, the ranges for organic and conventional milk overlap to a certain extent. Based on these results, the author proposed a new threshold value of 0.50% that was exceeded by all organic milk samples but also by a significant number of conventional milk samples. Even in this case the author pointed out that the results are applicable only to German milk samples and might be unsuitable for foreign milk samples or dairy samples containing substantial amounts of dairy ingredients of foreign origin. However, similar data for α-linolenic acid content in organic and conventional milk samples were also reported for retail milk samples in the UK.[118,123] It is worth pointing out that the addition of rather small amounts of vegetable oils rich in 18:3 ω-3 (e.g. linseed oil) to the feed of conventionally raised cows would increase the α-linolenic acid concentration in milk, which would thus mimic the content in organic milk or that in milk from pasture-fed cows.[124]

Other fatty acids

Many studies have reported that the contents of several fatty acids are significantly different between organic and conventional milk. For instance, organic milk is frequently higher in vaccenic acid (C18:1 t11)[117,118,122] and branched-chain fatty acids.[124] Organic milk is also reported to be significantly richer in conjugated linoleic acids (CLAs) than conventional milk.[117,118,122–127] In a recent study, Butler *et al.*[127] reported that levels of total CLAs and seven CLA isomers (including C18:2 c9,t11) were significantly higher in milk from both organically certified and non-certified low-input systems compared with milk from conventional high-input farms. Their multivariate analysis showed a strong correlation of CLA isomers with the proportion of fresh grass in the diet. CLAs are a mixture of positional and geometric isomers of linoleic acid (C18:2) with conjugated unsaturated double bonds. They are the products of incomplete biohydrogenation of 18:2 fatty acids in the rumen but are also synthesised from C18:2 t11 derived from both C18:2 and C18:3. The predominant CLA in foods of ruminant origin is the c9,t11 isomer, normally accounting for some 80% of the total CLA content.[128] Several health-beneficial effects are attributed to CLAs, such as anticarcinogenic, antidiabetic, antiadipogenic and antiatherogenic effects.[129,130] The content of CLAs has also been shown to respond quite quickly to changes in feeding regime.[131] However, the CLA content of milk has been reported to increase also after the addition of a mixture of fish and sunflower oils to a high-concentrate diet, so that under certain feeding conditions even conventional milk may exhibit high levels of CLAs.[132]

The content of total ω-3 fatty acids is also significantly higher in organic milk than in conventional milk. This holds both at retail level[117,123] and at farm level.[121,125,126,133,134] The ω-3 content is lower in winter, but the difference between organic and conventional milk is still significant. The higher content of ω-3 fatty acids is ascribed to the higher proportion of grass forage in the diet, which is richer in ω-3 fatty acids. Among individual ω-3 fatty acids, eicosapentaenoic acid (EPA) is frequently reported as being significantly higher in organic milk.[112,117,126] It has been proposed as a marker for organic milk by Molkentin and Giesemann,[109] who demonstrated that a complete differentiation is possible for German milk samples even when all-year data are used (α-linolenic acid is also an ω-3 fatty acid and has been discussed in the previous subsection).

These differences in fatty acid composition depend on the different feeding regimes that are typical of organic and conventional farms. On organic farms a diet richer in PUFA-rich fresh grass and roughage leads to the development of specific bacterial populations with more intense activity and therefore increases the content of α-linolenic acid in milk fat and the resulting fermented fatty acids, CLAs and *trans*-vaccenic acid (C18:1 t11).[135,136] However, the differences in fatty acid concentrations between organic and conventional samples are reduced in those countries where organic and conventional farms adopt very similar feeding regimes, as reported by Collomb *et al.*[125] in the Swiss highlands and by Fall and Emanuelson[126] in Sweden during the winter indoor periods when the feeding strategies are much more similar.

Given the strong effect that diet exerts on the fatty acid profile and the notable differences highlighted between organic and conventional milk, a multivariate approach taking into account the entire fatty acid profile/fingerprint or the content of some selected fatty acids might prove much more useful than a univariate strategy based on the assessment of just one fatty acid for discrimination between organic and conventional milk.

Triacylglycerol (TAG) profile

The present authors carried out a study on organic milk authentication in spring 2008. They collected 150 milk samples from 75 organic and 75 regular farms spread over the Netherlands when cattle were outside in the fields. The samples were subjected to TAG analyses. The TAG profiles are presented in Fig. 1. For 12 of the 16 TAGs measured as well as cholesterol, relatively small but significant differences between the two groups were observed (Student's *t* test, $P < 0.05$). The full data set was mean centred and subsequently subjected to PLS-DA in order to predict the identity of the milks (organic or regular) from their TAG fingerprints. The PLS-DA score plot presented in Fig. 2(a) shows two distinct clusters, one for the organic and one for the regular milk samples. The underlying TAGs contributing to the separation are presented in Fig. 2(b). Organic milk is characterised by higher levels of TAGs C42, C52 and C54 and by lower levels of C34, C36 and C38. The performance of the model was evaluated by a leave-10%-out cross-validation procedure ($r^2 = 0.65$, standard error of

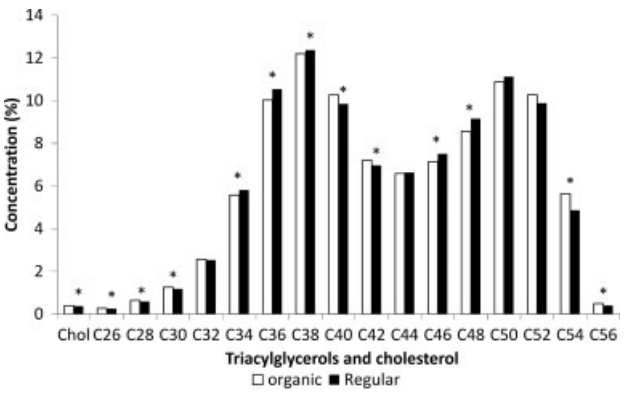

Figure 1. Mean triacylglycerol profiles (% of total chromatogram peak area) of organic and regular milk (C*nn*, triglyceride carbon numbers). *Significant difference ($P < 0.05$, Student's *t* test).

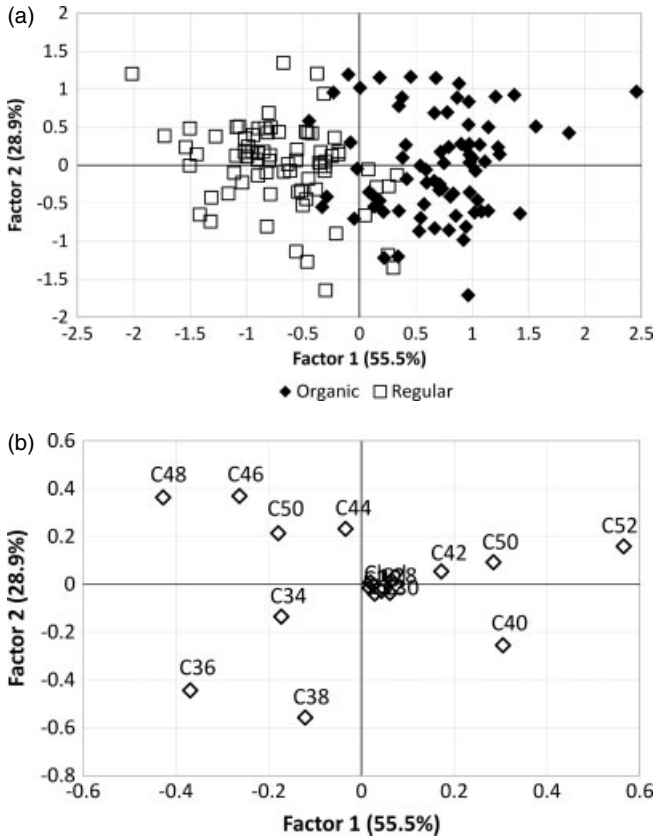

Figure 2. First two dimensions of PLS-DA carried out on mean-centred triacylglycerol data of organic (full symbols, $n = 75$) and regular (open symbols, $n = 75$) milk samples: (a) score plot; (b) loading plot (C*nn*, triglyceride carbon numbers).

cross-validation (SECV) = 0.2972). Sixty-four of the 75 organic milk samples (85%) and 67 of the 75 regular milk samples (89%) were classified correctly. The results show that fat fingerprints have potential for authentication of organic milk, but more research will be required to optimise models, to add more variables and to include seasonal and annual variations.

Eggs

Stable isotopes

The isotopic composition of egg constituents can provide valuable information about the feeding regimen administered to hens and their housing system. Commercial feeds (laying pellets, mash and chick crumbles) are expected to show generally low δ^{15}N values, likely because of the minimal contribution from animal proteins and because the plants are grown in soils fertilised with mineral fertilisers.[20] Conventionally raised chickens fed exclusively on such products would produce eggs with low δ^{15}N values. On the other hand, in addition to commercial feed sources similar to those fed to caged and barn-raised hens, free-range and organically raised hens are expected to have access to alternative food sources with a higher contribution of animal protein (insects) and organic matter derived from soils and vegetation fertilised with chicken manure. Therefore free-range eggs would be expected to have more positive δ^{15}N values than eggs from caged or barn-raised hens. Furthermore, according to EU regulations, organically raised chickens' diet should comprise at least 95% organic feed, which is expected to have higher δ^{15}N values than conventional feed.[1,23] The carbon isotope ratio can be used to determine if the feed is derived from C3 or C4 plants (or marine biomass). For instance, it has been shown that most eggs from small farms in Bavaria have low δ^{13}C values (from −26 to −28%) consistent with a C3 diet, in contrast to larger commercial farms that have mixed C3 and C4 plant diets.[10]

There is only one study exploring the potential of stable isotopes to discriminate eggs according to the hens' housing system. Rogers[137] analysed the carbon and nitrogen stable isotopic composition of whole yolk, delipidised yolk, albumen and egg membrane from 18 different brands of chicken eggs laid under caged, barn, free-range and organic farming regimes. The author found that the δ^{15}N values were significantly higher in the albumen, membrane and egg yolk (both delipidised and un-delipidised) of organic and free-range eggs compared with barn and cage eggs, suggesting a higher contribution of animal protein and/or organically grown plants in the diet of organic/free-range hens. However, a complete differentiation between those two categories or between organic and conventional eggs was not possible owing to the partial overlap between the corresponding ranges of variation in δ^{15}N values. However, it is worth noting that the author sampled at retail level (supermarkets and farmers' markets) and could not verify compliance with the organic production system for all eggs.

Fatty acids

It is well known that the fatty acid composition of eggs is influenced by both animal metabolism and diet. Thus differences in feeding practices typical of the housing system might be reflected in the egg fatty acid profile. However, studies have highlighted limited differences in the fatty acid profile of organic and conventional eggs. In particular, a higher percentage of saturated fats is reported in organic eggs compared with conventional eggs, mainly due to higher amounts of palmitic and stearic acids.[138–140] Nevertheless, the potential of the fatty acid profile of egg yolk as a fingerprint to verify the organic identity of eggs was explored in the present authors' research group.[141] Egg samples from 48 farms were analysed, including organic eggs, free-range eggs and barn eggs. These data were then combined with chemometrics to develop classification models to discriminate between organic and conventional eggs. PLS-DA was used as a supervised classification technique. The statistical model was validated according to a leave-10%-out cross-validation procedure. After autoscaling of the fatty acid data, the classification results were satisfactory, with 92% of the organic and 87% of the conventional eggs correctly classified.

Carotenoids

Carotenoids are a family of compounds containing over 600 fat-soluble plant pigments.[142] Egg yolk contains approximately 10 mg carotenoids kg^{-1}. Since hens do not produce carotenoids endogenously, egg carotenoids are entirely of dietary origin. Maize is the main source of carotenoids in hen feed, but carotenoid content is affected by the extent to which pasture is included in the diet of hens.[143] Organic and free-range hens are supposed to have access to sources of feed different from those provided through the diet: grass and vegetation, insects, worms and additional organic matter from the soil. EU regulations permit the addition of eight xanthophylls to the feed of poultry and laying hens in amounts of up to 80 mg kg^{-1} feedstuff.[144] These substances are the natural xanthophylls capsanthin (C40), β-cryptoxanthin (C40),

lutein (C40) and zeaxanthin (C40) and the synthetic xanthophylls β-apo-8′-carotenal (C30), β-apo-8′-carotenoic acid ethyl ester (C30), canthaxanthin (C40) and citranaxanthin (C33). Apart from dietary effects, the carotenoid content of eggs and poultry also depends on breed, feed fat content, feed vitamins and gender.[145] However, organic hen feed cannot be supplemented with carotenoids to obtain the desired yolk colouration.

Differences in the content of total carotenoids in eggs have been reported.[143,146,147] In particular, lutein and zeaxanthin were the predominant xanthophylls in egg yolks produced in accordance with ecological husbandry, whereas synthetic carotenoids occurred in higher amounts in non-organic eggs.[147]

The present authors' research group developed a method to verify the organic nature of eggs.[148] The fingerprint of the natural carotenoids (carotenes and xanthophylls) in eggs was used to discriminate between production systems. They analysed organic, free-range and barn eggs collected from 36 different Dutch farms. The carotenoid profile was determined by high-performance liquid chromatography (HPLC) and the resulting 'fingerprint' subjected to multivariate statistical analysis in order to develop classification models that could predict the production system in which the eggs were produced (organic or conventional). The best prediction rates were obtained with *k*-nearest-neighbour (*k*NN) models. *k*NN is a supervised learning algorithm where a sample is classified based on the category of the majority of its *k* nearest neighbours (*k* is a whole number). The prediction success rate for organic samples was 100%, while for non-organic samples (either free-range or barn) it was 92% (22 of 24 samples were correctly classified). A new test set (external validation set) of eggs from New Zealand and the Netherlands was then subjected to the developed *k*NN model based on Dutch eggs. Except for one organic egg sample, which after inspection was found to be fraudulent, the production system of the eggs was predicted correctly for all samples. Recently, the model calibrated with Dutch eggs was challenged with eggs from ten different countries.[149] For the eggs from six EU countries, Austria, Belgium, Germany, Greece, Italy and Portugal, high success rates for the prediction of their identity (organic of conventional) were achieved, with 90–100% of organic eggs and 88–100% of conventional eggs correctly classified. For three of the non-EU countries, Norway, Canada and Israel, good results were obtained, with 75–100% of organic eggs correctly classified. Only organic eggs from Turkey showed a very low success rate (<25% of organic eggs correctly classified) and need more research.

Fish

Stable isotopes and fatty acids

Molkentin *et al.*[150] developed a method for the identification of organically farmed Atlantic salmon (*Salmo salar*) based on a combination of stable isotope and fatty acid analyses. The combined data were subjected to analysis using an artificial neural network (ANN). The discrimination between organic and conventional salmon was not possible based on δ^{13}C and δ^{15}N values only. The unequivocal assignment of all 100 samples was possible by using different combinations of analytes, but the most robust classification was achieved by combining stable isotope data with fatty acid data, i.e. by a procedure based on more than one analytical method. In particular, the combined measurement of δ^{15}N and linoleic acid allowed the correct classification of all samples and even the discrimination of farmed and wild salmon. The levels of δ^{15}N and linoleic acid in salmon depend on the relative contribution of vegetable components to fish meal in fish diet and the different trophic level of fish meal in organic aquaculture (higher in organic aquaculture).

Near-infrared spectra

NIR spectroscopy (NIRS) is a fast and reliable analytical technique that has been used to successfully predict the proximal chemical composition of European sea bass (*Dicentrarchus labrax*)[151] and to discriminate between fish from different rearing systems (extensive lagoons, intensive land-based basins and sea cages).[152] Trocino *et al.*[153] investigated the potential of NIRS for discrimination between organically and conventionally reared European sea bass. A soft independent modelling class analogy (SIMCA) method was used as a chemometric approach to classify samples by rearing system. It appeared that NIRS could only partially discriminate sea bass fillets after a freeze-drying step (65–70% success rate) but was unable to correctly classify fresh samples.

CONCLUSIONS

Recent developments in analytical techniques have provided new options for the authentication of organic products based mainly on profiling/fingerprinting approaches. The biomarkers reviewed in this paper are summarised in Table 1. In the light of current knowledge, it is unlikely that the authentication of organic food products can be attained by the measurement of a single marker. Furthermore, it is unlikely that a single type of analytical strategy will be successful for the authentication of all organic products. Finally, it has to be kept in mind that, although organic production within the EU is harmonised and in the process of being harmonised with organic production in the USA, organic production standards elsewhere in the world may vary. Analogously, conventional farming can encompass many different fertilisation and pest control strategies. The success of analytical approaches may vary accordingly.

Stable isotopes have proven to be valuable indicators of authenticity for both plant- and animal-derived food products, although a complete discrimination is not possible based on the analysis of stable isotopes only. Multi-element profiles and multi-marker strategies based on the independent measurement of several markers are promising techniques for the authentication of organic crops, while fatty acid, carotenoid and volatile profiles/fingerprints are potent tools for the authentication of animal food products, given the strong effect that diet has on animal fat composition. On the other hand, approaches involving metabolomics and proteomics have been much less exploited so far. Given that nutrients regulate metabolism via gene expression, genomic techniques such as transcriptomics might also prove useful in the development of new methods to authenticate feeding or fertilising strategies. Such approaches are quite limited at present and should be further explored in the future. The genetic fingerprint of the contaminant microflora can also be exploited to discriminate between organic and conventional food products, because of differences in pest control and housing strategies between organic and conventional farming.

All in all, it seems that at present the first steps in an analytical methodology for the authentication of organic foods have been completed but that evidence of applicability for general use is required. Many scientific studies are based on relatively small sample set sizes and fairly contrasting conditions. However, sample sets considering real-life variation and collaborative validation of

Table 1. Overview of literature studies exploring potential of several biomarkers for authentication of organic produce

Biomarker	Food product	Analytical technique[a]	Multivariate analysis[b]	Quality of discrimination	Reference
Crops					
$\delta^{15}N$	Potatoes	IRMS	—	Correct classification of all organic samples and misclassification of 15% of conventional crops with threshold level of 4.3‰	19
	Various retail vegetables	IRMS	—	Partial discrimination. Higher for fast-growing vegetables (<80 days)	20
	Various Croatian retail vegetables	IRMS	—	Complete discrimination for endive, rocket, leek, potato and two chicory varieties (Palla Rossa and Pan di Zucchero)	21
Major and trace elements	Onions and peas	ICP-MS	PCA	Clear separation between organic and conventional samples	33
	Wheat, barley, fava beans and potatoes	ICP-MS	PCA	Clear separation between organic and conventional wheat, barley and potato samples. Partial discrimination for fava beans	34
	Brazilian coffee	INAA	Bayesian network	Correct classification of 22 out of 23 samples	28
Metabolites	Wheat grains	GC/MS	—	Unsuccessful discrimination	36
	Wheat ears and grains	Various techniques	—	Unsuccessful discrimination	37
	Maize	GC/MS	PCA	Unsuccessful discrimination	39
	Grapefruits	FI-ESI-IT-MS and FI-ESI-TOF-MS	PCA	Clear separation between organic and conventional samples	40
Copper chloride crystallisation patterns	Wheat grains	Copper chloride crystallisation	DA	Correct classification ranging from 70 to 100% of samples	66
	Wheat grains	Copper chloride crystallisation	LDA	Correct classification highly variable across years of harvesting and type of pattern evaluation (computerised or visual)	67
X-ray spectra	Tomatoes and coffee	EDXRF	PCA	Clear separation between organic and conventional samples	68
Infrared spectra	Australian red and white wines	MIR	PLS-DA	Classification rates of 100 and 88% for organic and conventional white wines and 73 and 85% for organic and conventional red wines respectively	70
Volatile fingerprint	Orange juice	PTR-MS	PCA	Clear separation between organic and conventional orange juice	71
$\delta^{15}N$ and trace elements	Tomatoes and lettuces	IRMS and ICP-MS	CDA	Correct classification of 100 and 80% of tomato and lettuce samples respectively	35
Eleven analytes	Orange fruits	Various techniques	LDA	Correct classification of 90% of samples	22
Several isotopic and chemical/physical parameters	Oranges, peaches, strawberries and clementines	Various techniques	CDA	Correct classification ranging from 75 to 98% of orange, peach and strawberry samples	72
Dry matter, proteins, total phenolic content and eight minerals	Eggplants	Various techniques	PCA	Clear separation between conventional and organic samples	47
Transcript profiling	Potato tubers	Microarray analysis	PCA	Clear separation between conventional and organic samples	75

Table 1. (Continued)

Biomarker	Food product	Analytical technique[a]	Multivariate analysis[b]	Quality of discrimination	Reference
Meat					
$\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotopes	Irish beef	IRMS	—	Partial discrimination	81
Fatty acids	Rabbit meat	NIRS	PLS-DA	Correct classification of 98% of samples	92
Volatiles	Dry-cured ham	PTR-MS	PLS-DA	Correct classification of 96% of organic and 83% of conventional samples	98
Milk					
$\delta^{13}C$ in milk fat	German retail and farm milk	IRMS	—	Almost complete discrimination with threshold value of −26.5‰	105, 106
$\delta^{13}C$ in milk protein	German retail milk	IRMS	—	Complete discrimination with threshold value of −23.5‰	107
$\delta^{15}N$	German retail and farm milk	IRMS	—	Partial discrimination with threshold value of −5.5‰	107
Phytanic acid	German dairy products	GC/MS	—	Good discrimination with threshold value of 2 g kg^{-1}. Applicable also to dairy products other than milk	110, 111
Linolenic acid	German retail and farm milk	GC-FID	—	Good discrimination although not complete	105, 106
EPA	German retail and farm milk	GC-FID	—	Good discrimination	105
Triglyceride profile/fingerprint	Dutch farm milk	GC-FID	PLS-DA	Correct classification of 85% of organic and 89% of conventional samples	This paper
Eggs					
$\delta^{15}N$	New Zealand eggs	IRMS	—	Partial discrimination	135
Fatty acids	Dutch eggs	GC-FID	PLS-DA	Correct classification of 92% of organic and 87% of conventional samples	139
Carotenoids	Eggs from various countries	HPLC-UV	*k*NN	Correct classification of 100% of conventional and 92% of organic samples	146, 147
Fish					
Stable isotopes and fatty acids	Atlantic salmon	IRMS, GC-FID	ANN	Complete discrimination based on $\delta^{15}N$ and linoleic acid	148
Near-infrared fluorescence spectra	European sea bass	NIRS	SIMCA	Correct classification of 65–75% of freeze-dried fillets	151

[a] IRMS, isotope ratio mass spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; INAA, instrumental neutron activation analysis; GC/MS, gas chromatography/mass spectrometry; FI-ESI-IT-MS, flow injection electrospray ionisation ion trap mass spectrometry; FI-ESI-TOF-MS, flow injection electrospray ionisation time-of-flight mass spectrometry; EDXRF, energy-dispersive X-ray fluorescence; MIR, mid-infrared; PTR-MS, proton transfer reaction mass spectrometry; NIRS, near-infrared spectroscopy; GC-FID, gas chromatography with flame ionisation detection; HPLC-UV, high-performance liquid chromatography with ultraviolet detection.
[b] PCA, principal component analysis; DA, discriminant analysis; LDA, linear discriminant analysis; PLS-DA, partial least squares regression discriminant analysis; CDA, canonical discriminant analysis; *k*NN, *k* nearest neighbours; ANN, artificial neural network; SIMCA, soft independent modelling class analogy.

the methods are prerequisites for these methods being ready for use in routine control practice, e.g. in conjunction with certification and inspection systems.

In this respect, it is recommended that, for the development of robust classification models based on chemometrics, well-designed experimental studies are performed that rely on data sets that are both well balanced and of sufficient size so that generalisation of the models to as yet unmeasured data is possible. It is particularly important that all possible sources of variation for the target biomarkers (individual variability, breed/variety, feeding/fertilisation practice, geographical location/climate, etc.) are included in the experimental plan. Whenever a global classification model cannot be built, its validity may be temporally or spatially restrained, e.g. developing models based on annual data rather than on multi-year data and/or restrained to a specific country or region. In any case, the classification model development should include an internal as well as an external validation step, i.e. validation of the models with samples used to build the models as well as with new independent samples.

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