Research Signpost
37/661 (2), Fort P.O.
Trivandrum-695 023
Kerala, India

Recent Advances in Pharmaceutical Sciences IV, 2014: 99-117 ISBN: 978-81-308-0554-2
Editors: Diego Muñoz-Torrero, Manuel Vázquez-Carrera and Joan Estelrich

7. Organic chicken product authentication: State-of-the-art and future perspectives

Alba Tres, Rafael Codony and Francesc Guardiola
Nutrition and Food Science Department-Xarta-INSA, Facultat de Farmàcia
Universitat de Barcelona, E-08028 Barcelona, Spain

Abstract. Organic food products are highly susceptible to fraud. Currently, administrative controls are conducted to detect fraud, but having an analytical tool able to verify the organic identity of food would be very supportive. The state-of-the-art in food authentication relies on fingerprinting approaches that find characteristic analytical patterns to unequivocally identify authentic products. While wide research on authentication has been conducted for other commodities, the authentication of organic chicken products is still in its infancy. Challenges include finding fingerprints to discriminate organic from conventional products, and recruiting sample sets that cover natural variability. Future research might be oriented towards developing new authentication models for organic feed, eggs and chicken meat, keeping models updated and implementing them into regulations. Meanwhile, these models might be very supportive to the administrative controls directing inspections towards suspicious fraudulent samples.

Introduction

Food products with high added value are susceptible to fraud. National and international regulations underpin mandatory label information, but

Correspondence/Reprint request: Dr. Alba Tres, Nutrition and Food Science Department, Facultat de Farmàcia, Universitat de Barcelona, Av Joan XXIII s/n, E-08028 Barcelona, Spain. E-mail: atres@ub.edu

unfortunately, they are not sufficient to prevent food fraud [1]. Food fraud can occur intentionally or not, for instance during product cross-over in factories, or mislabeling errors. Products most susceptible to intentional fraud are those of high added value, for which consumers are willing to pay a higher price, but that are rather similar to other lower cost (and lower quality) products. Some examples of food fraud include foods from a particular geographical origin (i.e. foods under Protected Designations of Origin), or products from special production practices (i.e. organic, sustainable, animal welfare friendly products). Fraud committed at the first steps of the food production chain (i.e. in crops or feed) implies fraud in the final product (i.e. meat, milk, eggs). Therefore, it is important to control and to avoid fraud at all steps of the food chain.

All stakeholders are interested in fraud prevention. Fraud implies always an economic prejudice for the end consumer, but also to the retailer and the intermediate consumer/producer when fraud is committed at the first steps of the food chain. Moreover, fraud occurrence diminishes the quality of the sector and the confidence of consumers in the high quality product, which might have as consequence a decrease in the sector competitiveness. Accurate labelling is important to support fair trade [1]. Furthermore, fraud might become a food safety concern when it is committed using non-authorized, unknown or uncontrolled substances.

An authentic product is one which strictly complies with the declaration given by the producer in terms of ingredients, natural components, absence of extraneous substances, production technology, geographical and botanical origin, production year, and genetic identity [2]. Authenticity in most food products is verified by means of administrative controls, certifications and inspections. However, this administrative system is rather expensive and slow because it requires one entire inspection for each product (including all steps in the production chain). This makes that fraudulent products easily scape undetected. The state-of-the-art research on food authentication is to find analytical tools that are able to distinguish authentic from non-authentic products. Such analytical tools would permit to analyze a great number of products in a relatively short period of time (depending on the analytical method involved) increasing therefore the number of inspected products with respect to the administrative system. Despite the evident supportive role of food authentication analytical tools to the administrative system, they are not currently applied in most food products. In most cases, the reason is that research has not been able to find suitable analytical tools to distinguish authentic from non-authentic products due to their similarities and natural variability of their composition. Moreover, implementing these analytical tools into legislation is nowadays a challenge.

1. Organic products

The Food and Agricultural Organization of the United Nations [3] defines 'organic agriculture' as a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system.

Nowadays, there is no common worldwide standard for the production and labeling of organic products. This complicates international trade of organic products. In Europe, the Council Regulation No 834/2007 [4] set the conditions on organic production and labelling of organic products. It was implemented by the Commission Regulation 889/2008 [5].

Organic farming and the organic food market are rapidly growing [6,7]. Consumers purchase organic foods for different reasons, including animal welfare and environmental concerns, believe on an improved human health, and perceptions that organic foods are tastier than their conventional alternatives [8]. However, a recent literature review on health, nutritional and safety characteristics of organic and conventional foods concluded that published literature lacks strong evidence that organic foods are significantly more nutritious than conventional foods [9]. On the other hand, even if consumers believe that organic products taste better than conventional products, they failed in discriminating between organic and conventional products in a number of studies [6,10]. Furthermore, it has been shown that labeling associated with a food can create expectation regarding its sensory properties, and ultimately its acceptability [6,10].

As mentioned, organic produce tends to retail at a higher price than their conventional counterparts. This together with the relatively similar composition between organic and conventional products and the increase of organic food market makes them susceptible to fraud. Therefore, it is important to establish effective control systems to avoid fraud in the organic food sector. Currently, these are based on administrative controls and inspections, and there is no analytical tool routinely applied. However, some authentication models based on analytical determinations have been developed for some specific organic products [11].

Authentication of organic products is complex, and depends very much on the product examined. It is unlikely to find a unique marker that allows a general discrimination between organic and conventional farming [12].

Instead, each type of organic product will require the development of a specific analytical tool, depending on its composition, characteristics and similarities with the corresponding conventional product. For instance, in chicken sector, fatty acid profile has been useful to discriminate organic from conventional hen feed [13], but it does not necessary imply that fatty acid profiling would be useful to authenticate other organic products.

2. Considerations for developing authentication models

2.1. Fingerprinting techniques

The global aim of an authentication model is that it can be used in the future for verifying the identity of unknown or suspicious samples. Apart from finding a suitable analytical marker to authenticate organic products, the statistical approach applied is of importance. Traditional analytical strategies for detecting adulterations have relied on the determination of the amount of a marker compound or compounds in a material and a subsequent comparison of the value(s) obtained with those from authentic products used as references (*targeted approach*). But this approach fails when the natural variability of the compounds in the authentic product is so large that reference values would need to be set so wide that some adulterations would still go unnoticed, which is the case of organic products [12]. Actually, the fewer the number of compounds used to authenticate a product, the easier to hide fraud by keeping the values of these target compounds in the fraudulent product within the limits established for the authentic product.

The state-of-the-art strategy in food authentication consists in finding, in high dimensional analytical data, (untargeted) patterns for the category to be authenticated that are different from those in other lower-quality categories. The pattern of unknown or doubtful samples might then be compared with that of the authentic one (which is considered the fingerprint of the authentic product) to verify its category. Indeed, these patterns might be built using raw analytical signals directly obtained from measuring equipment (i.e. chromatograms, Near Infrared –NIR- data). This *fingerprinting approach* has as advantage that it does not require to identify and quantify the compounds responsible for the discrimination (untargeted approach) to build the authentication model, although it is recommended to do so to be able to biologically understand why models are successful. The untargeted approach saves time invested in identifying and quantifying compounds, permits a faster application of the model, and allows dealing with any raw analytical data, even when the "shape" of this raw data complicates the identification and quantification of compounds (as it happens for instance with NIR data).

Also, the use of the raw analytical signals might imply an improved exploitation of the information included in the data. For instance, in chromatographic data, once peak integration and identification has been conducted a great part of the raw data is not further considered (all not integrated signal); however, these non-considered data might include very small peaks or differences in peak shapes that might contain very useful information on the identity of the samples. Due to the highly dimensional data collected in the fingerprinting approach, the application of chemometrics to build and validate authentication models is necessary. An intermediate approach between fingerprinting and the targeted approach is the application of *selective fingerprinting* in which authentication models are based on a great number of markers, half way between using only one or two single markers and the raw analytical data. This approach still requires the identification and quantification of the marker compounds, but it provides a more reliable and robust authentication tool than the targeted approach.

2.2. Sample set

Classification models need to correctly identify the samples used to develop the model, but also samples in the future during its routine application. Therefore, several aspects need to be considered during model development to test and assure model's usefulness in the future. Here we will address those that might be particularly relevant in developing models for authenticating organic chicken products; more information in this respect might be encountered in previous literature [14,15].

As for the authentication of other commodities, a proper sample set is essential to develop the model because its future applicability will depend on the number and type of samples used in model development. At least two sample sets are needed: one to build and optimize the model (training set) and a second one to externally validate the model by using it to predict the identity of samples in this new set (validation set) [14]. The authenticity of samples in both sets needs to be assured. Sample origin and identity (organic or conventional) need to be known. In this respect, it is important to remind that samples bought in supermarkets and stores cannot be considered authentic. Also, samples from experimental studies are neither valid as authentication samples because their production is so experimentally controlled that it might not reflect the real farming practices. Therefore, even though experimental studies and market surveys might provide some insights on possible authentication markers, they should not be considered valid as authentication studies. Unfortunately, most of the published studies on organic chicken products are market surveys [16–19] and experimental studies [20–22] rather than authentication studies.

Table 1. Possible sources of variability to take into consideration for developing authentication models for organic chicken products

Source	Examples of variability
Regulations	Changes in regulations within a country or region
Product categories	Categories included in the model (organic, free range, barn, cage, specialty eggs…)
Animal	Breed, age
Feed ingredients	Feed formulation (Authorized feed ingredients, price, availability)
	Natural variability of feed ingredients
Season	Differences in climate conditions between seasons
	Grass availability and composition
Geographical origin	Differences in regulations between countries or regions
	Differences in climate, environment, soil…. between regions
	Farming practices in each location

The number of samples in the training and validation sets will mostly depend on the sources of variability included in the model. Consequently, this will condition the future applicability of the model because it can only be applied to authenticate samples belonging to the same sources of variability considered in the training and validation set. Therefore it is interesting that both sets cover as much natural variability as possible. Regarding organic chicken products, several sources of variability might be taken into consideration (Table 1). One of the first sources of variability is the changes in regulations regarding the production of (organic and conventional) chicken products, because they might imply a change the composition of the end products. Therefore, models developed on (feed, meat or egg) samples produced according to past regulations need to be (at least) validated with samples produced according to the present regulation.

Samples need to cover natural variation within the considered categories (Table 1). In organic chicken products, several subcategories exist for conventional products (cage, barn and free range). Therefore, all of them need to be represented in the set. If all categories are not included, models should only be used to verify the identity of samples belonging to the

included categories. For instance, van Ruth et al. [12] and Tres and van Ruth [23] did not include eggs produced by caged hens in their set. Therefore, in these studies, it would be necessary to validate and/or rebuild the models with cage eggs before using them to identify this category.

Another aspect to take into account is sample origin (Table 1). Models developed on sample sets collected in a confined geographical origin should only be used to authenticate samples from this particular origin [13]. Before being broadly used to identify samples from other origins, model performance needs to be validated using it to predict the identity of authentic samples from other origins. Sample origin might have an influence on certain composition parameters that depend for instance on the soil, latitude, altitude, environmental and climatic conditions. Differences in the regulations and farming practices within the organic chicken sector between geographical origins might also have an influence on some parameters. An example of geographical validation is the egg authentication study leaded by Van Ruth [12,24]. First, an authentication model to verify the organic identity of Dutch eggs was developed based on egg carotenoid profiling, and it was validated by authenticating eggs produced in New Zealand [12]. Later on, the model performance in authenticating eggs produced by other countries was evaluated (Austria, Belgium, Germany, Greece, Italy and Portugal), including countries outside Europe (Canada, Israel) [24]. The percentage of correctly classified organic eggs was above 90% for all countries, except for Israel.

Feed ingredient composition might also become a source of variability. As for any other natural product, natural variability makes that the composition of feed ingredients might change within and between years, between geographical origins, farming practices and feed ingredient producers. Moreover, feed formulations are designed depending on the regulations, and availability and price of ingredients; significant changes in any of these aspects might imply a change in the formulation that might affect meat and egg composition. Therefore, it is highly advisable that feed, meat and egg sample sets include samples produced in different seasons and years, as well as samples from animals fed with feeds from various producers. For instance in Tres et al., [13,25] samples from two consecutive years and various feed producers were included. It would also be important to periodically validate the models by testing their performance in identifying samples from different production years. Published studies on the authentication of organic chicken products are so recent that no yearly effects have been detected so far; however, it is possible that in the future this aspect might become relevant, and thus, models will need to be updated.

Seasonal influence on egg composition has been described in various experimental studies [21,22,26], especially for eggs laid by outdoor reared hens. The content of certain grass components, such as tocopherols and carotenoids, varies depending on the season [21,22,26]. This, together with grass availability led to differences in egg composition between seasons. Therefore, it is highly recommended that models based on these parameters are developed on sets that include samples from all seasons.

One of the advantages of fingerprinting models is that even if they are already developed, it is possible to easily test their performance in identifying samples from new origins, years, seasons… If this leads to a substantial reduction of model's performance, then it would be advisable to incorporate the new samples in model's data base, rebuild the model and validate it, so that the new source of variability is considered in model development. If this still does not provide a successful model, it might be necessary to build one specific model for each independent source of variability (i.e. one model per origin, per season…). Both approaches are correct, but model's robustness is improved if one single model could be used to authenticate samples from any source of variability.

3. State-of-the-art in the analytical authentication of organic chicken products

As for other organic products, the production of organic eggs and meat has increased in the lasts years. Since they are products with added-value, they are highly susceptible to fraud [11]. However, research on developing analytical techniques to authenticate organic chicken products has only recently started, and only a few analytical techniques have been developed in this respect. In most cases, these techniques have been selected because comparative experimental (or market survey studies) revealed potential differences in the content of some compounds between organic and conventional chicken products. However, it is important to have in mind, that comparative experimental studies are not useful as authenticating studies but they might be a starting hypothesis to build models to discriminate various chicken product categories. In this section, we will cover the published authentication studies on chicken products. Moreover, we will summarize some of the market surveys and experimental studies on the effects of the rearing system on meat and egg composition.

In Europe, the production of organic chicken products requires that animals are fed with organic feed (that is to say that at least 95% of its dry matter should come from ingredients of organic farming) [5]. Moreover, for

the production of organic eggs hens should have access to an outdoor area (at least 4 m^2/hen), and they cannot be kept in cages when they are indoors (6 hens/m^2). Fraud at the feed level would imply a fraud in the end product, even though all the other conditions required for the production of organic products (such as animal housing) are respected. Therefore, the control of the organic production of chicken products should cover all stages of the production chain, from feed to the end product [13].

Within conventional chicken products, several categories exist in the European market, varying in price, quality and consumer demand. The European Council Directive 1999/74/CE [27] set minimum standards for the protection of laying hens. Conventional eggs include eggs from caged hens, barn and free range eggs [28]. For the production of free range eggs, hens should also have daytime access outdoors (min 4 m^2/hen), and when being indoors, they cannot be kept in cages (9 hens /m^2). Thus, as organic hens, free range hens can forage in soils, picking up grubs, beetles, worms, grass and seeds; they have more exposure to sunlight, and higher chances of physical activity. Hens laying barn and cage eggs do not have access outdoors, but in the case of barn hens, they cannot be kept in cages [28].

3.1. Organic feed authentication

Studies on organic feed authentication are scarce. In several experimental studies in which the composition of organic and conventional eggs or chicken meat were compared feeds were also analyzed [20,21,29]. However, in most of these studies, feeds were experimental, specifically designed for a particular study, and not real market samples. In fact, in most cases the same ingredient composition was used, only differing in the organic or conventional source of each ingredient. However in the real market, strict regulations exist for the composition of organic feed, while a broader range of feed ingredients are authorized for conventional feed. This, together with the price and availability of ingredients, causes that ingredient composition of organic feed is usually different than that of conventional feed [13]. Therefore, these differences in feed formulas need to be covered by the authentication model so that it reflects real farming practices.

As far as we are concerned, only a study on the development of two analytical tools to authenticate organic chicken feed exists on published scientific literature [13,25]. The study was leaded by RIKILT – Wageningen University and Research Centre (The Netherlands), within the Cluster of Authenticity and Nutrients, a research group worldwide known as expert on authentication. Two models were developed to authenticate organic feeds used for laying hens in the Netherlands. One of the models was based on feed

fatty acid composition assessed by gas chromatography [13], and the other one was based on feed NIR spectra [25]. A total of 96 organic and conventional feed samples were collected in the Netherlands during two consecutive years. First, the fatty acid model was developed following a selective fingerprinting approach on 30 identified chromatographic peaks, combined with a PLS-DA algorithm (after data log10 transformation and scaling to unit variance). The classification model (Figure 1) was successful. It correctly identified all organic feed samples, and almost all conventional feed samples (Table 2).

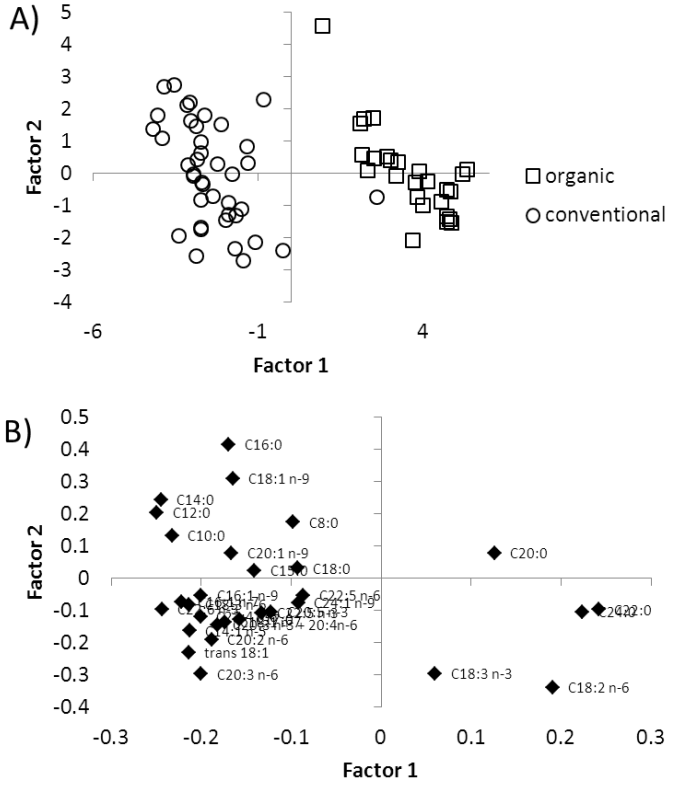

Figure 1. First two dimensions of PLS–DA on the fatty acid profiling data of organic and conventional feeds: (A) scores and (B) loadings plot (data preprocessing: log10 transformation and scaling to unit variance; OSC = 1). (Reprinted with permission from [13]. Copyright © 2011 American Chemical Society).

When examining PLS-DA scores and loadings plots (Figure 1) it was revealed that several fatty acids were responsible for the discrimination of organic and conventional feed. Fatty acids such as C24:0, C22:0 and C18:2 n-6 and C18:3 n-3 were associated with the organic feed class. Fatty acids such as C12:0, C14:0, C16:1 n-9 and C16:1 n-7 were associated with the conventional feed class (Figure 1). These differences in fatty acid composition were attributed to differences in the ingredient composition between organic and conventional feed [13].

The determination of the fatty acid composition requires sample grinding, extraction of the feed lipid fraction by using solvents, and the determination (and quantification) of fatty acid methyl esters by gas chromatography. Therefore, the drawbacks of this method are the use of solvents and the costs related to the reagent expenses, gas chromatography and trained personnel. Also, the procedure, although quite fast, does not allow an immediate answer on the identity of the feed. These drawbacks led to search for alternative more rapid techniques that would provide a faster answer on the authenticity of a feed sample [25].

Near Infrared Spectroscopy (NIRS) is a nondestructive, easily applicable, and fast technique that requires minimal or no sample preparation and that permits the response of certain molecular bonds (such as O-H, N-H or C-H) to NIR radiation. NIRS generates a spectrum that may be characteristic of a sample and may be considered its “fingerprint” [30]. Its use in the feed sector is already quite commonly implemented, even in form of on line or in situ applications [31,32]. Due to these characteristics, Tres et al., [25] decided to evaluate it as a rapid authentication tool to verify the organic identity of feed. Classification models were developed on the same samples used in the fatty acid model [13]. PLS-DA classification models were successful in correctly identifying organic and conventional feed samples (Table 2). Apart from the NIR regions related to fat content, fatty acids and their unsaturation, regions related to the N-H stretch were also revealed as important in the NIR model

Table 2. External validation of authentication models to verify the organic identity of laying hen feed

Model	Approach	Organic (% correctly identified samples)	Conventional (% correctly identified samples)	Reference
Fatty acid composition	Selective fingerprinting	100%	90%	[13]
NIR	Fingerprinting	91%	100%	[25]

[25]. This implied, that apart from fatty acid composition [13], the protein content and / or composition might differ between organic and conventional feeds [25].

Taking into consideration the results on model validation and the characteristics of both techniques, the model based on NIRS data was suggested as a screening model, and the model based on the fatty acid composition data as a confirmation model [25] (Figure 2). This combined approach permits to apply a fast technique that will correctly identify conventional feed. If some supposedly organic feed samples are identified as conventional by the model, their fatty acid composition will be analyzed and submitted to the fatty acid model. Its answer will determine if it is a real organic feed (that was revealed as false negative by the NIR model), or if it is a fraudulent feed (when the fatty acid model confirms it as conventional), reducing time, costs and environmental impact of solvent use [25].

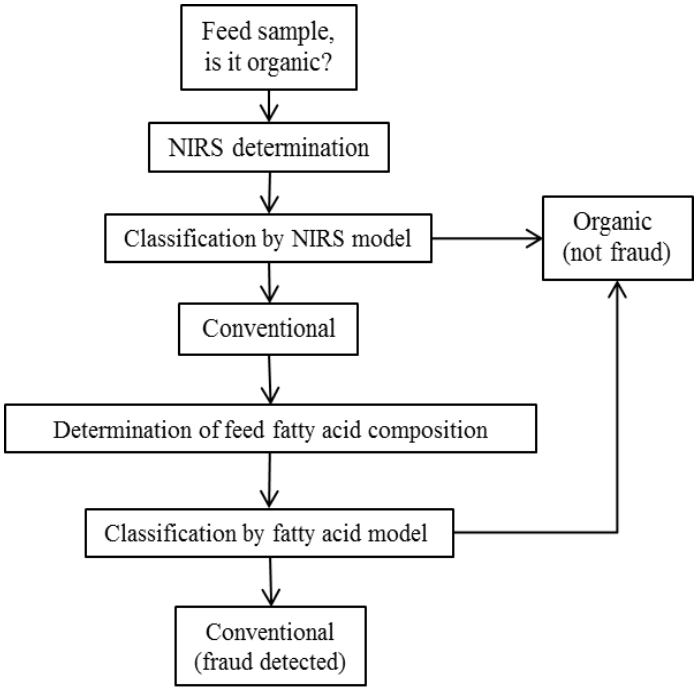

Figure 2. Decision tree combining NIRS and fatty acid models as screening and confirmation tools to verify the organic identity of a laying hen feed sample.

3.2. Organic egg authentication

While there have been numerous investigations on supplementation of layer's diets to enrich eggs in n-3 fatty acids, vitamins A and E and other lipid-soluble nutrients; there has been little work investigating the effects of different production systems on these nutrients [33] and even less work has been conducted with authentication purposes. As mentioned in section 2, markers revealed as different between organic and conventional products by comparative experimental studies and market surveys might be the starting hypothesis to build authentication models. For instance, Rogers [34] claimed that $\delta^{15}N$ values are a promising indicator to differentiate free range and organic eggs from cage and barn eggs; however, a higher number of samples would be recommended to confirm these results (only 18 samples were collected, only 4 of them being organic eggs and 2 out of these were considered outliers). On the other hand, $\delta^{13}C$ values did not lead to any significant separation among egg farming regimens [34].

Higher Se and lower P and Zn contents in organic eggs than in cage eggs have been reported [35,36] even though feeds had similar Se and Zn contents. Differences between farm locations, access to soil and grass and higher physical activity of organic hens would be related with these differences. However, these were comparative studies, for which especial feed formulations were designed. Thus, more studies comparing trace element content between eggs produced by hens fed commercial (conventional and organic) feed, and from various farm locations would be needed to estimate the potential use of trace elements as authentication tools.

Another parameter that showed variations between egg production systems in experimental studies is the content [21,22,26] and composition of tocopherols [20]. Variations between free-range and indoor kept hens were found, especially when available pasture was large. As far as we are concerned, no egg authentication studies have relied on these compounds, but it seems that their utility as authentication tools would mostly depend on the season and on the amount of available grass [21,22,26].

Variations in the fatty acid composition between different egg production systems have been revealed by experimental studies and market surveys [16,18,20,22]. But the number and type of fatty acids varying among rearing systems, and the magnitude of the differences depended on several study design parameters such as feed composition and the number and origin of egg samples. Moreover, laying hens diets on free-range conditions include grass, which has a relative high level of n-3 polyunsaturated fatty acids, tocopherols and some other non-saponifiable lipid components [21,22]. This has been attributed as one of the reasons leading to higher n-3 polyunsaturated fatty

acids in eggs laid by free-range and organic hens [22], especially when the available grass area was larger than the minimum required by current regulations [21,26]. However, it needs to be taken into account that grass composition varies between seasons, and therefore its influence on the fatty acid and tocopherol composition also depends on the season [21,26].

Even if the differences on the fatty acid composition between egg categories were relatively small, an authentication model to discriminate organic vs conventional eggs was developed on the egg fatty acid profile [23]. For this study, 48 authentic egg samples including organic, free-range and barn eggs were collected directly from farms in the Netherlands. The authentication model was based on a selective fingerprinting on the fatty acid composition data and a PLS-DA algorithm. Although results were only internally validated (by leave 10%-out cross-validation), they were quite satisfactory with the 92% of the organic and 87% of the conventional eggs correctly classified. Even if results were not directly comparable with those from the feed authentication study [13] because egg and feed samples from both studies were not related, the fatty acid approach as authentication tool was better for feed than for egg authentication. Reasons behind this fact might include, among others, the influence of hen metabolism on egg fatty acid composition [23]. Still the main fatty acids discriminating organic from conventional eggs were similar: polyunsaturated fatty acids were important for the discrimination of organic eggs, and C16:0 for conventional eggs. Results are partially contradictory with those of some market surveys [16–18] that reported higher contents of palmitic acid in organic eggs. This fact might be explained by differences in the study designs, number of recruited samples, and differences between the origin of samples and thus the farming practices and feed formulations. Regarding conventional egg categories, no differences were encountered between barn and free range eggs for the fatty acid composition [23], although higher contents of n-3 polyunsaturated fatty acids had been revealed by comparative experimental studies comparing eggs laid by indoors and outdoors reared hens [22,26]. Real farming practices, seasonal effects (especially variations on the amount of available grass and its composition) and other natural variability might have masked these variations in the authentication model.

In organic egg production, hen feed cannot be supplemented with carotenoids [4], while it is permitted in conventional eggs. Therefore, in organic egg production, carotenoids need to be originated from the organic feed components such as maize, and from other sources such as grass, vegetation, insects, worms, and additional organic matter from the soil. Differences in the egg carotenoid profile between different rearing systems were revealed in various experimental studies [16,21,37]. In particular, lutein

and zeaxanthin were the predominant xantophylls in organic egg yolks, whereas synthetic carotenoids such as β-apo-8'-carotenoic acid ethyl esther or citranaxanthin occurred in higher amounts in non-organic eggs [37]. Moreover, carotenoid content was higher in eggs laid by hens pasturing in large grass areas, especially in spring and autumn, revealing a seasonal effect depending on the carotenoid content of grass [21,26].

Later, van Ruth et al., [12] developed an authentication model to verify the organic identity of eggs through a multivariate approach on the carotenoid profile determined by HPLC-Diode array detector. Their classification model was based on a K-Nearest Neighbors (KNN) algorithm, and was developed with a first set of eggs, including authentic organic, free-range and barn eggs (but not eggs from caged hens), and validated with a second set of (market) eggs. Using the carotenoid profile, they discriminated organic from conventional eggs, lutein/zeaxanthin being the dominating carotenoids in the organic egg pattern. Interestingly, they encountered some misclassified eggs which belonged to conventional farms in transition to organic practices, but which were not still authorized for producing organic eggs. However, they could not discriminate free-range from barn eggs, although in some experimental studies, carotenoids were higher (in spring and autumn) in eggs laid by pasturing hens (10 m^2/hen) [21,26]. Later on, the model was validated and expanded to the authentication of eggs produced in other countries (Austria, Belgium, Germany, Greece, Italy and Portugal), including countries outside Europe (Canada, Israel) [24]. The percentage of correctly classified organic eggs was above 90% for all countries, except for Israel.

3.3. Chicken meat authentication

As far as we are concerned, while a few studies on organic feed and egg authentication have already been published, no studies on organic chicken meat authentication have been published in scientific journals. Instead, several market surveys [19,38] and experimental studies [39] have been conducted. A study comparing meat composition of chickens reared indoors or outdoors (receiving the same feed) showed that the contents of Fe (total and haem iron) and saturated and n-3 polyunsaturated fatty acids were higher in meat from outdoor reared animals [39]. Unfortunately, in this study, free-range and organic rearing systems were not compared. Chen et al. [40] found that long periods of outdoor access lead to thigh muscles richer in polyunsaturated fatty acids, and lower in several monounsaturated fatty acids. These findings are consistent with those of market surveys [38]. On the contrary, a market study conducted by Jahan et al., [41] led to different results on organic chicken meat fatty acid composition; but it needs to be

taken into account that the number of samples included in this study was very low and samples came from completely different origins.

Another strategy to find suitable markers to develop authentication models for organic chicken meat could be to look into promising markers for the authentication of organic meats from other species. Stable ratio mass spectrometry of carbon, nitrogen, and sulphur isotopes has been successful in differentiating organic and conventional Irish beef [1,11]. These differences are partly due to differences in the feed intake (grass or concentrate). However, the higher content of $\delta^{15}N$ in conventional beef meat compared with organic meat might be a result of the mineral fertilizers applied to the soil where conventionally grown animals are fed [1,11].

4. Challenges and future perspectives

One of the most important challenges in food authentication is to keep models updated and ready to be applied when needed. This requires that their performance is tested periodically with new sets of authentic samples. They might include similar samples to those in the training set, but also samples from other years, seasons or geographical origins, not only to keep models updated but also to expand their possible applicability. Efforts in this respect have already been done for the egg authentication models by the research group headed by Dr van Ruth [24] when they expanded their model to other countries. Updating and validating the models are essential so that models can be routinely used to detect fraudulent samples.

The ultimate aim of authentication models would be to incorporate them into regulations; however, more research is necessary before this can be achieved. For accomplishing this, great efforts would need to be done with regards to model inter-laboratory validation, among others. Moreover, it would be necessary to build and share a common data base compiling the analytical data obtained from authentic samples. Sharing this data base would permit that models can be built on the same data by various laboratories and would contribute to incorporate more natural variability into the models. Moreover, since it is recommendable to build models on the largest number of samples as possible [14], the availability of such a database would be an efficient tool and improved use of resources. Actually, this has already been suggested for other commodities such as olive oil [42], but it does not exist yet, although much more research has been conducted in the authentication of olive oil than in organic products.

Meanwhile authentication models might be very supportive to the current administrative controls and inspections. If analysis cost is not extremely high,

a great number of samples could be collected and analyzed. Only those showing suspicious values would then be submitted to an inspection procedure, focusing the inspection efforts and resources only to suspicious samples. In this respect, fast, easy and cost-effective analytical methods are desirable. In this line, a combination of a rapid screening model based on NIRS, and a confirmation model based on fatty acid profiling has been suggested as an authentication strategy for organic laying hen feed [25].

Apart from this, and according to what has been described in previous sections, it is evident that there is a lack of authentication models to verify the organic identity of feeds and eggs, and especially for chicken meat. Furthermore, the published authentication models have been mainly focused in discriminating organic from conventional products. However, the various conventional categories for chicken products also vary in price, quality and consumer demand (especially because of consumer's concerns on animal welfare). Therefore, it would be interesting to find suitable markers and models able to discriminate these categories. In some studies, differences between the composition of eggs laid by caged hens and other egg categories have been described [16,18]. Others, have found differences in composition between products from free range and indoor rearing [22,26,34]. These studies might become the starting point of future authentication models.

Acknowledgements

The authors would like to acknowledge the *Secretaria per a Universitats i Recerca, Generalitat de Catalunya* (Spain) for the financial support through the EU-COFUND Beatriu de Pinós post-doctoral Program (ref 2011 BP-B 00113).

References

1. Ballin, N. Z. 2010, *Meat Sci.*, 86, 577.
2. Kamm, W., Dionisi, F., Hischenhuber, C., Engel, K. H. 2001, *Food Rev. Int.*, 17, 249.
3. Food and Agriculture Organization of the United Nations (FAO) Organic Agriculture, 1999.
4. The Council of the European Union 2007, *Off. J. Eur. Union*, L 189, 1.
5. European Commission 2008, *Off. J. Eur. Union*, L 250, 1.
6. Bourn, D., Prescott, J. 2002, *Crit. Rev. Food Sci. Nutr.*, 42, 1.
7. Siderer, Y., Maquet, A., Anklam, E. 2005, *Trends Food Sci. Technol.*, 16, 332.
8. Magkos, F., Arvaniti, F., Zampelas, A. 2003, *Int. J. Food Sci. Nutr.*, 54, 357.

9. Smith-Spangler, C., Brandeau, M. L., Hunter, G. E., Bavinger, J. C., Pearson, M., Eschbach, P. J., Sundaram, V., Liu, H., Schirmer, P., Stave, C., Olkin, I., Bravata, D. M. 2012, *Ann. Intern. Med.*, 157, 348.
10. Napolitano, F., Castellini, C., Naspetti, S., Piasentier, E., Girolami, A., Braghieri, A. 2013, *Poult. Sci.*, 92, 820.
11. Capuano, E., Boerrigter-Eenling, R., van der Veer, G., van Ruth, S. M. 2012, *J. Sci. Food Agric.*, 93, 12.
12. Van Ruth, S. M., Alewijn, M., Rogers, K., Newton Smith, E., Tena, N., Bollen, M., Koot, A. 2011, *Food Chem.*, 126, 1299.
13. Tres, A., van Ruth, S. M. 2011, *J. Agric. Food Chem.*, 59, 8816.
14. Berrueta, L. A., Alonso-Salces, R. M., Héberger, K. 2007, *J. Chromatogr. A*, 1158, 196.
15. Marini, F. Chemometrics in Food Chemistry, Elsevier B.V., Oxford, UK, 2013.
16. Hidalgo, A., Rossi, M., Clerici, F., Ratti, S. 2008, *Food Chem.*, 106, 1031.
17. Cherian, G., Holsonbake, T.B., Goeger, M.P. 2002, *Poult. Sci.*, 81, 30.
18. Samman, S., Kung, F. P., Carter, L. M., Foster, M. J., Ahmad, Z. I., Phuyal, J. L., Petocz, P. 2009, *Food Chem.*, 116, 911.
19. Jahan, K., Paterson, A. 2007, *Int. J. Food Sci. Technol.*, 42, 251.
20. Pignoli, G., Rodriguez-Estrada, M. T., Mandrioli, M., Barbanti, L., Rizzi, L., Lercker, G. 2009, *J. Agric. Food Chem.*, 57, 11517.
21. Mugnai, C., Dal Bosco, A., Castellini, C. 2009, *Ital. J. Anim. Sci.*, 8, 175.
22. Lopez-Bote, C. J., Sanz Arias, R., Rey, A. I., Castaño, A., Isabel, B., Thos, J. 1998, *Anim. Feed Sci. Technol.*, 72, 33.
23. Tres, A., O'Neill, R., van Ruth, S. M. 2011, *Lipid Technol.*, 23, 40.
24. Van Ruth, S. M., Koot, A. H., Brouwer, S. E., Boivin, N., Carcea, M., Zerva, C. N., Haugen, J. E., Hohl, A., Koroglu, D., Mafra, I., Rom, S. 2013, *Qual. Assur. Saf. Crop. Foods*, 5, 7.
25. Tres, A., van der Veer, G., Perez-Marin, M. D., van Ruth, S. M., Garrido-Varo, A. 2012, *J. Agric. Food Chem.*, 60, 8129.
26. Mugnai, C., Sossidou, E. N., Dal Bosco, A., Ruggeri, S., Mattioli, S., Castellini, C. 2014, *J. Sci. Food Agric.*, 94, 459.
27. The Council of the European Union 1999, *Off. J. Eur. Union*, L 203, 53.
28. European Commission 2007, *Off. J. Eur. Union*, L 132, 5.
29. Küçükyilmaz, K., Bozkurt, M., Çath, A. U., Herken, E. N., Çinar, M., Bintas, E. 2012, *S. Afr. J. Anim. Sci.*, 42, 360.
30. Woodcock, T., Downey, G., O'Donnell, C. P. 2008, *J. Agric. Food Chem.*, 56, 11520.
31. Fernández-Ahumada, E., Roger, J.M., Palagos, B., Guerrero, J.E., Pérez-Marín, D., Garrido-Varo, a 2010, *Appl. Spectrosc.*, 64, 83.
32. Fernández Pierna, J. A., Lecler, B., Conzen, J. P., Niemoeller, A., Baeten, V., Dardenne, P. 2011, *Anal. Chim. Acta*, 705, 30.
33. Holt, P. S., Davies, R. H., Dewulf, J., Gast, R. K., Huwe, J. K., Jones, D. R., Waltman, D., Willian, K. R. 2011, *Poult. Sci.*, 90, 251.
34. Rogers, K. M. 2009, *J. Agric. Food Chem.*, 57, 4236.

35. Giannenas, I., Nisianaks, P., Gavriil, A., Kontopidis, G., Kyriazakis, I. 2009, *Food Chem.*, 114, 706.
36. Küçukyilmaz, K., Bozkurt, M., Yamaner, Ç., Çinar, M., Çatli, A. U., Konak, R. 2012, *Food Chem.*, 132, 989.
37. Schlatterer, J., Breithaupt, D. E. 2006, *J. Agric. Food Chem.*, 54, 2267.
38. Husak, R. L., Sebranek, J. G., Bregendahl, K. 2008, *Poult. Sci.*, 87, 2367.
39. Castellini, C., Mugnai, C., Dal Bosco, a 2002, *Meat Sci.*, 60, 219.
40. Chen, X., Jiang, W., Tan, H. Z., Xu, G. F., Zhang, X. B., Wei, S., Wang, X. Q. 2013, *Poult. Sci.*, 92, 435.
41. Jahan, K., Paterson, A., Spickett, C. M. 2004, *Int. J. Food Sci. Technol.*, 39, 443.
42. García-González, D. L., Aparicio, R. 2010, *J. Agric. Food Chem.*, 58, 12569.