FoodIntegrity Handbook

A guide to food authenticity issues and analytical solutions

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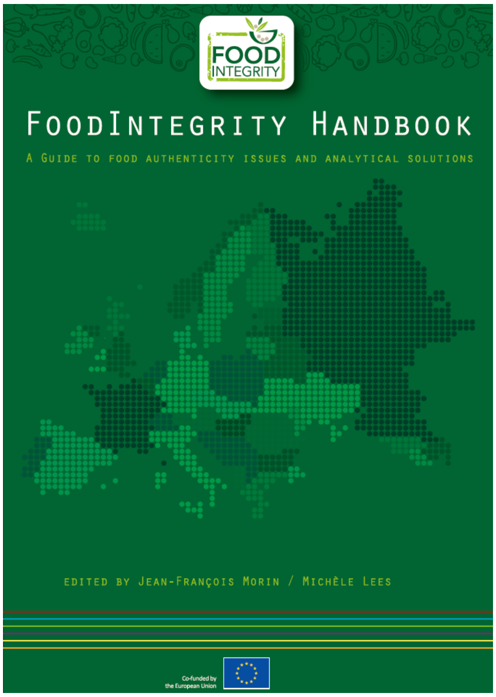

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Eggs and egg products

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General overview of the product

Eggs are a key component in human and animal diets thanks to the large content of high-quality proteins and vitamins. For this reason, eggs are widely used as ingredients in the industrial preparation of different types of foods (foams, emulsions, pastry and bakery products); these mixtures falling under the denomination "egg products".

The eggs sector is one of the most important agricultural industries all over the world since, unaffected by weather, it is suitable for all different climate regions.

Figure 1: Global distribution of egg production in 2013 on country development base (Source: FAO database; design: Ursula Welting)

The main producing countries are the European Union (EU), China and North-Central America. Given the perishable nature of both shell eggs and liquid egg products, the flow of trade in these goods between different countries is limited and most of the production is fully dedicated to internal markets. This may vary in case of big issues such as the outbreak of Avian Influenza in the

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US that led the entire country to import liquid products from the EU. Powder products are a different case, especially albumen which has specific properties for use in bakery products. These can be commercialised without concern for their expiry dates, but may face some trade restrictions between countries (i.e. not all European countries can export to the US).

Most suppliers operate a supply chain in which the different steps are vertically integrated: the feed-mill, the shelling and heat treatment plant and farms (either fully owned or under an agistment contract). There has been a steady decrease in spot market purchasing of eggs due to traceability requirements.

The increasing importance of animal welfare has put the entire sector under even more pressure and it has had to adapt to new regulations especially in the EU (2012) and also to consumer demand to eradicate the use of cages by suppliers. In response to these issues, the market has reacted in order to tackle the increasing demand for suitable farms with better animal welfare, and in addition to ensure a more transparent supply chain and thus prevent any other possible scandals that have affected the market in the past.

1. Product Identity

1.1. Definition of the product and manufacturing process

The concept of egg products is related to all the forms of presentation of the egg: yolk, albumen or a mix of both. The term “Egg Products” refers to processed or convenience forms of eggs obtained by processing shell eggs: egg products include whole eggs, egg whites, and egg yolks in frozen, pasteurised and refrigerated liquid and dried forms available in a number of different product formulations. In particular, the food industry is interested in high quality egg products in a liquefied form, obtained from eggs shelled within 4 days and which have undergone homogenisation and pasteurisation: their use is mainly related to the preparation of egg pasta and bakery products [1].

1.1.1. Farming systems

Eggs used in the production of egg products for the food industry come from farms where an “intensive” farming of hens is usual, following different modalities depending on the structure of the farms and their management. Battery cages, Cage-free (Free-range, Barn) and Organic are the most common types of farming systems utilised.

1.1.1.1. Battery cages

These comprise a housing system used for various animal production methods, but primarily for egg-laying hens. The name comes from the arrangement of rows and columns of identical cages connected together, in a unit, as in a battery. Although the term is usually applied to poultry farming, similar cage systems are used for other animals. Battery cages are the predominant form of housing for laying hens worldwide, but have generated controversy between advocates for animal rights and industrial producers. These housing systems reduce aggression and cannibalism among hens; on the other hand, they are barren, restrict the hens’ movements preventing natural behaviour, and finally, increase rates of osteoporosis. The introduction of the European Union Council Directive 1999/74/EC which banned conventional battery cages in the EU from January 2012 for welfare reasons, has meant that the number of eggs from battery cages in the EU Member States is decreasing.

1.1.1.2. Cage-free

In the EU, this type of egg production includes barns, free-range, organic (in the UK, systems must be free-range if they are to be labelled as organic) and aviary systems. Non-cage systems may be single or multi-tier (up to four levels), with or without outdoor access.

In free-range systems, hens are housed to a similar standard as the barn or aviary. In addition, they have constant daytime access to an outside range with vegetation. Each hen must have at least 4 m^2 of space.

The European Union Council Directive 1999/74/EC stipulates that non-cage systems must provide the following:

- A maximum stocking density of 9 hens/m^2 of "usable" space
- If more than one level is used, a height of at least 45 cm must exist between the levels

- One nest for every seven hens (or 1 m^2 of nest space for every 120 hens if group nests are used)
- Litter (e.g. wood shavings) covering at least one-third of the floor surface, providing at least 250 cm^2 of littered area per hen
- 15 cm of perching space per hen.

In addition to these requirements, free-range systems must also provide the following:

- One hectare of outdoor range for every 2,500 hens (equivalent to 4 m^2 per hen; at least 2.5 m^2 per hen must be available if rotation of the outdoor range is practiced)
- Continuous access during the day to this open-air range, which must be "mainly covered with vegetation"

1.1.2. Transformation process

After laying, the eggs are sorted in order to separate out damaged, dirty, or broken ones and to classify them (in function of size) according to the characteristics defined by law (A category, B category).

A classification of the eggs also in terms of freshness, in other words the time from laying to the transformation into egg products or the shipping to the retail market, is done. According to this classification, "extra-fresh" eggs or "fresh" eggs are clearly distinguished from "conventional" eggs.

Eggs for industry use, entering the process for transformation to liquid egg products, are destined to management by food transformation factories where from eggs in shells, they turn into pasteurised refrigerated egg products. The flow diagram of this transformation process is in Figure 2.

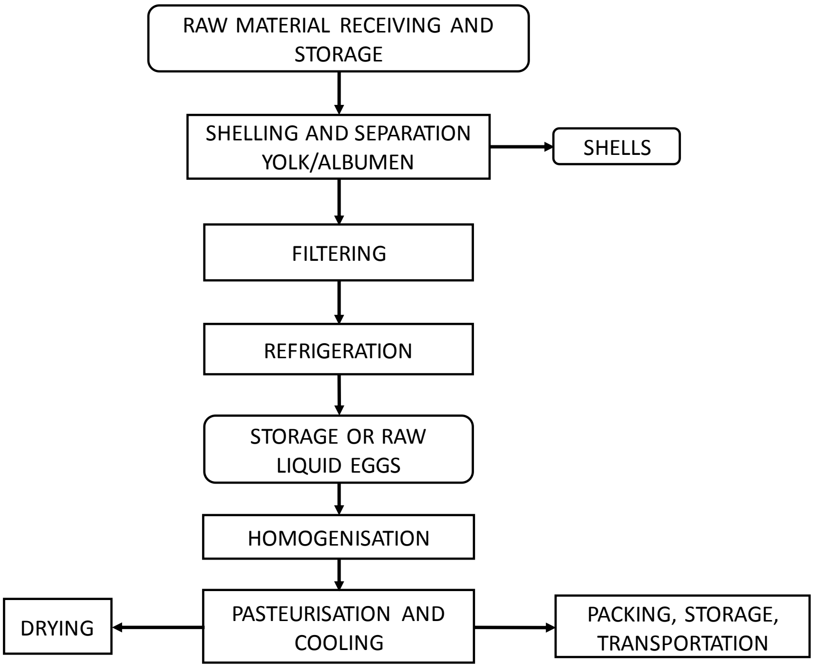

Figure 2: Flow diagram whole eggs

Liquid egg products coming from this process are commonly delivered to food companies in refrigerated tanks and their quality and food safety characteristics (chemical, physical parameters included in related technical specifications) are carefully controlled by the producers before their release and by the customers on reception and before use.

1.2. Current standards of identity

The following legislation in the EU relates to eggs and egg products.

178/2002 [2] – This regulation lays down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

852/2004 [3] – This regulation and its annexes define a set of food safety objectives that food operators must meet.

853/2004 [4] – This regulation aims to ensure a high level of food safety and public health. It complements Regulation (EC) No 852/2004 on the hygiene of foodstuffs, whose rules mainly cover the approval of operators in the sector. Its rules apply to unprocessed and processed products of animal origin. They generally do not apply to food that contains both products of plant origin and processed products of animal origin. EU countries must register and, where necessary, approve establishments handling products of animal origin.

2073/2005 [5] – This regulation concerns the microbiological criteria applied to foodstuff.

1881/2006 [6] – This regulation lays down the maximum limits for certain contaminants in food in particular to protect the health of the most sensitive population groups, i.e. children, the elderly and pregnant women.

589/2008 [7] – This regulation lays down detailed rules for implementing Council Regulation (EC) No 1234/2007 as regards marketing standards for eggs:

- Describes the characteristics for CAT A and CAT B (shell and cuticle, air space, yolk, albumen, germ, foreign matter and smell)
- Grading Cat A by weight defining the classification in different sizes
- Defines shelf life and timing to grading, marking and packing eggs
- Defines how to handle industrial eggs
- Defines the code to mark the eggs
- Indicates which records to be kept by producers, collectors and packing centres
- Checks
- Non compliances and tolerances

2. Authenticity issues

2.1. Identification of current authenticity issues

This section concerns pasteurised eggs used in food preparations, starting from liquid eggs already shelled, provided by suppliers located in the EU with integrated supply chains for farms, feed mills and transformation plants.

2.1.1. High risk issues

The **highest risk factors** that can impact egg authenticity include:

Different farming systems for hens with an impact on animal welfare

There is an increasing market demand for eggs from barn hens or from cage free farming systems. However, existing facilities need to be converted and it is evident that a number of uncertainties remain as to whether all the volumes are/will be satisfied within the animal welfare requirements.

Currently, there are no available analytical methods able to categorise different farming approaches (barn hens or cage free farming system) and this fact increases the opportunity for fraud. In addition, intermixing of eggs is possible at the farm level, during transportation and at the transformation steps.

Fresh eggs

Eggs can be declared as fresh eggs within 28 days shelf life. However, eggs over 28 days shelf life can be found on the market still declared as “fresh” in order to fraudulently exploit a higher price compared to the others.

Albumen and yolk contain enzymes, and if eggs are not stored at a sufficiently low temperature, the proteins can be altered. The optimal temperature for correct egg storage is normally about 6-8 °C. Enzymatic alteration of the albumen modifies its viscosity, which can be used to recognise the freshness of the egg: in fact, when the egg is not fresh the albumen tends to liquefy and the yolk breaks easily.

Immediately after the laying phase, the contents of the egg with its entire shell are practically sterile and can be contaminated from environmental microorganisms only if the shell is broken.

Egg categories

Over the last few years, there has been an increasing demand for cat A eggs, with quality parameters as described by the regulation currently in force. There are insufficient farms able to keep up with the demand, and Cat B eggs are cheaper than Cat A ones.

This intermixing of categories is possible at farm level, during transportation and at the transformation facility.

Dilution with incubated eggs

The eggs that come from the incubation process must be sent for destruction or use as animal feed. It is not possible to use them for human consumption. The price of these eggs is very low and can lead to the illegal use in some periods (when the egg offer on the market is low or when availability from the incubators is high). There are parameters regulated by law in order to avoid the use of these eggs for human consumption.

Artificial colorants

Artificial colorants are allowed but some supply chains claim to be free from artificial colorants. Eggs intermixing is possible at farm level, feed mill and at the transformation facility.

2.1.2. Lower risk issues

Lower risk factors, but still possible, include the following:

Use of eggs from different animals

This type of fraud is not always economically viable and there are some technical and mechanical restrictions in the shelling lines.

Dilution with water

The opportunity of this type of fraud is decreased by its detectability with current analytical methods (dry matter analyses).

2.2. Potential threat to public health

The microbial contamination of eggs could be due to: (1) endogenous factors, due to contact with microorganisms present in the cloacae which go up in the oviduct and contaminate the egg during its own formation process, and (2) exogenous factors, that is, microorganisms in certain conditions could enter through the shell which is highly porous. This contamination frequently happens in pasteurised and hulled eggs.

Egg microbial contamination could be due to pathogen and/or alterative microorganisms responsible for organoleptic changes (colour and odour). Among the most well-known pathogens are: *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Campylobacter jejuni* and *Salmonella Enteritidis*; the latter is the most frequent and feared. Amongst the most well-known alterative microorganisms are: *Pseudomonas spp*., *Aeromonas spp*., *Alcaligenes spp*., *Escherichia coli*, *Proteus spp*. and *Serratia spp*.

During the last ten years, the sector has faced many different issues regarding fraud (i.e. utilisation of incubated eggs that is forbidden by law) and the Fipronil incident (many different EU countries have knowingly or unknowingly used this pesticide for the treatment of red mites despite the law declaring this molecule illegal for livestock usage). In addition, different cycles of Avian Influenza that, without affecting human health, have led to huge modifications in the sector's infrastructure and the supply and demand equilibrium.

3. Analytical methods used to test for authenticity

An overview of methods for authenticity testing is reported in a table format in section 4. Methods are divided into "officially recognised", "other commonly used" and "future analytical approaches".

3.1. Officially recognised methods

The residual quantity of shells, of egg membranes and other possible particles in the egg products must not exceed 100 mg/kg of egg product.

The significant microbial growth that occurs in a shelled egg, before the pasteurisation process, causes the formation of different microbial metabolites and leads to a significant alteration of the original enzymatic properties.

Egg products that have been restored through thermal pasteurization must respect in particular these two microbial parameters: total viable mesophilic bacteria, max 5 log CFU g^{-1}; *Enterobacteriaceae* count, max 2 log CFU g^{-1}.

Microbiological analysis can be performed following specifications reported in ISO and AOAC official documents [8–10].

In general, total viable mesophilic bacteria are enumerated using spread plates of plate count agar incubated at 30 °C for 72 h; Enterobacteriaceae counts are determined by using violet red bile glucose agar with a double layer, incubated at 37 °C for 24 h.

The egg contains a series of organic acids such as succinic and lactic acids, the presence of which is directly correlated to microbial quality and which cannot be altered through thermal restoration actions [11].

3-hydroxybutyric acid is a specific indicator of fertilised, incubated eggs. Succinic acid is used to evaluate microbial spoilage. Lactic acid is increased in both cases, and can be used to screen egg products for suitability for human consumption.

The amounts of lactic and succinic acids in high quality liquid fresh egg products are usually not higher than 200 and 5 mg kg^{-1} dry egg, respectively [12]. Currently, the legal European Union limit is: lactic acid ≤ 1000 mg kg^{-1} dry egg [4,13–15].

The level of 3-hydroxybutyric acid, again following European legislation, must not be higher than 10 mg kg^{-1} dry egg.

A gas chromatographic approach can be used as analytical method for routine testing of these egg carboxylic acids to indicate pre-pasteurisation spoilage of egg products: NaOMe is used for methylation; the carboxylate esters are separated by gas chromatography on a 5% dimethyl siloxane column under gradient temperature [16].

Also available on the market are several enzymatic tests designed to carry out the quantitative determination of lactic, succinic and 3-hydroxybutyric acids in egg products. Sample preparation and analysis can be done using an UV–vis spectrophotometer and following kit instructions / recommendations and other corresponding studies reported in literature [17].

3.2. Other commonly used methods

Alternative analytical methods have been presented in the literature for different type of egg derivatives: egg products, shelled eggs and egg powder.

Simple but effective tests such as solids content and dry matter can be used to detect illegal water addition to liquid egg products.

In any case one of the most critical issues is eggs freshness: this parameter makes a major contribution to the value of the product, for obvious safety reasons and also because consumers may perceive variability in freshness as a lack of quality [18].

Non-destructive methods to determine egg freshness, including optical and spectroscopic measurements on shell or yolk colours, have been proposed in the past [19]. Scientific literature presents several rapid non-destructive methods able to assess this parameter: both NIR [18,20,21] and Vis-IR [22,23] spectroscopies coupled with chemometric data treatment are able to detect this fraud directly on the shell egg.

At the same time, researchers have attempted to identify volatile components that contribute to the egg's unique flavours and aromas, working with different extraction and analytical techniques (steam-distillation, solvent extraction, purge and trap, etc.): several aldehydes, aromatic compounds and sulphur compounds have been identified in greatest concentrations [24]. In particular, methyl-sulphide compounds are strictly related to deterioration and the perception of unacceptable odours in whole eggs [25]. In most cases these methods are interesting to demonstrate all the potential compounds that can be emitted from eggs, but often the corresponding necessary heating procedures may produce an excess of volatiles which is not representative of the real situation of an egg product which is refrigerated and evaluated by a sensory panel at room temperature or after a short treatment at 30–40 °C.

An alternative strategy to sensing the global profile of organic volatiles emitted by eggs can potentially be achieved by using artificial olfactory systems (AOS), also called "electronic noses". Currently, the application of AOS has been encouraged thanks to the outstanding developments which sensor technology and data processing systems have undergone over the last 20 years [26,27].

Furthermore, in the last years other tests based on hyperspectral imaging are presented as other non-destructive ways to solve the same problem [28].

Recently, a fast-GC electronic nose was presented as a rapid way to collect the volatile fingerprint of hen eggs; subsequently, thanks to chemometric data treatment, eggs were clearly separated according to their storage time, and a prediction of this factor was calculated and validated with a PLS model [29].

Other interesting approaches for freshness evaluation use the intrinsic fluorescence of thick albumen and egg yolk [30] or the quantification of S-Ovalbumin [31]; in addition, a rapid colorimetric test based on the reaction between albumen and 3,3',5,5'-tetramethylbenzidine is mentioned in the literature [32].

Different confirmatory techniques are presented, for instance the evaluation of albumen freshness combining the results obtained with NIR and NMR spectroscopies [33].

Another important issue is the discrimination between organic and conventional eggs and the common approach reported in literature is the evaluation of the carotenoids fingerprint with HPLC analysis, usually followed by chemometric elaboration [34].

Stable isotope analysis has been explored in the past and it seems that the isotopic composition of egg components depends on the diet consumed by the laying hen [35] and on the farming conditions [36]; on the contrary, ratios are not influenced by the pasteurisation process [37]. However, further studies are required before considering this technique a robust tool for egg traceability.

Incubated eggs fraudulently added to fresh egg products are usually detected by exploiting enzymatic assays with 3-hydroxybutyric acid as specific molecular marker [38], as previously indicated. However, it seems that the combination of this legislated marker with the presence of uracil (generated as a consequence of high microbial contamination) could provide a more robust evaluation of the hygienic quality of the products [39].

The analytical method used up to now for uracil determination in eggs is the one presented by Morris [40].

Another possible adulteration is the undeclared addition of dyes to eggs or derivatives which can be detected using liquid chromatography coupled to mass spectrometry [41].

A growing issue of the last years is the introduction of melamine (that results in an apparent increase of the protein content) in eggs. The literature presents portable instruments that, thanks to an approach based on surface-enhanced Raman spectroscopy [42], are able to detect this fraud; however, chromatographic techniques are also widely used [43–45].

3.3. Looking to future analytical perspectives

Future analytical methods for fraud detection in eggs and egg-derived products will continue to explore both rapid and confirmatory approaches. Industries require fast and robust methods for the acceptance or rejection of a batch before its introduction in the production chain but, in parallel, confirmatory methods are required for quality and authenticity certifications, ensuring an improved value to the raw material itself and to the food products.

The creation of predictive models with electronic noses or similar tools able to collect the global fingerprint of the products is the emerging approach for a rapid detection of specific frauds. These methods are fast, easy to use and cheap; instruments are “trained” with pure and adulterated samples and a predictive chemometric model specific for the target fraud is created. Subsequently, unknown samples are analysed and their “authenticity” is predicted by the model. On this topic, a recent study for the assessment of egg product freshness with a GC-IMS instrument was presented in the literature [46].

As regards confirmatory approaches, the novel frontiers for fraud detection will be probably explored with HRMS metabolomic studies: the identification of markers responsible for specific frauds could represent the first step that can lead to the development of specific target methods able to certify the authenticity of the eggs. At the moment the European legislation requires that only a few chemical molecules have to be monitored [4] and the increasing of this “target list” could lead to a more robust evaluation of a specific issue. The first scientific papers are starting to appear on this topic, such as a recent study on a metabolomic approach for the identification of some freshness markers in egg products [47].

4. Overview of methods for authenticity testing

The following table provides a summary of the methods and the authenticity issues they address.

Fraud	Short method description	Reference
False Freshness and quality declarations	Tests based on hyperspectral imaging with a combination of analytical techniques to determine the internal quality of eggs	[28]
	NIR spectroscopy with different chemometric techniques for non-destructive freshness assessment on shell eggs	[18,20,21]
	A MOS-artificial olfactory system is described. A correlation with the legal freshness parameters is demonstrated	[27]
	Eggs freshness is evaluated using the intrinsic fluorophores of thick albumen and yolk	[30]
	A Vis or VIS-IR wavelengths range (400-1100 nm) transmittance method allows the evaluation of intact chicken eggs quality	[22,23]
	S-Ovalbumin is presented as a reference index to express commercial shell egg freshness as equivalent egg age	[31]
	A colorimetric test based on the reaction between albumen and 3,3’,5,5’-tetramethylbenzidine is used for freshness evaluation	[32]
	Albumen freshness is evaluated combining the results obtained with Vis-NIR and NMR spectroscopy	[33]
	GC-E nose for freshness discrimination and for prediction of storage times in hen eggs	[29]
	Uracil determination with HPLC-UV detector	[40]
False hen farming declaration	Nitrogen isotope composition of chicken eggs, measured with IRMS techniques, is able to differentiate eggs laid in a caged regimen and eggs laid by free range hens	[36]
Melamine contamination	Portable surface-enhanced Raman Spectroscopy is used for fast detection of Melamine contamination, also at trace levels	[42]
	HPLC-MS/MS methods with specific sample pre-treatments for the simultaneous detection of Melamine and Cyanuric Acid	[43,44]
	GC-MS coupled with UPLC-MS/MS methods	[45]
Conventional eggs declared as organic	Carotenoids fingerprint obtained with HPLC-PDA and KNN elaboration	[34]
Dyes addition	Simple sample extraction procedure and UHPLC-MS/MS analysis	[41]

5. Conclusion

Eggs and egg-derived products are widely used in the food industry. In addition, there is a clear and growing need for a more transparent supply chain in order to reduce the risks involving product authenticity and traceability: in fact, especially during the last ten years the sector has faced many different issues linked to food fraud.

The food industry requires fast and robust methods for the acceptance or rejection of a batch before its introduction in the production chain but, in parallel, confirmatory methods are required for quality and authenticity certifications.

Eggs contain a series of metabolites, such as some specific organic acids, whose presence is directly correlated to the freshness and microbial quality and which cannot be altered through thermal restoration actions: gas chromatographic and enzymatic methods are available for their quantitative evaluation.

Future directions will involve finding and validating new analytical solutions to detect non-declared addition of dyes/additives, to categorise different farming approaches and to discriminate between organic and conventional eggs (through LC-MS, IRMS, etc.) and enlarging the range of non-destructive methods (mainly based on optical and spectroscopic measurements).

Alternative emerging strategies deal with HRMS metabolomics and sensing organic volatiles patterns by using electronic noses/ion mobility or similar instruments, then creating predictive models able to collect the global fingerprint of the products in relation to potential fraud issues.

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