FoodIntegrity Handbook

A Guide to Food Authenticity Issues and Analytical Solutions

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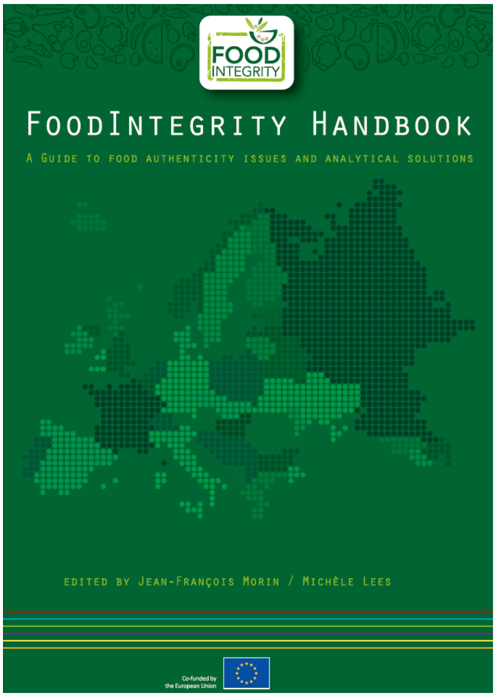

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Nuts, nut products and other seeds

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General overview of the products

Nuts and nut products are a highly heterogeneous category of food, with several applications, consumed roasted, dried, in preparations as ingredients in confectionery, sweets, baking. For the purposes of this handbook, the focus will be on the fruits of plants which are commonly defined as "nuts", which can be sold with or without a hard shell. They are not always nuts in a botanical sense: a nut is in fact defined as a fruit from Angiosperms, dry indehiscent one-seeded, with a hard pericarp, meaning that it does not open spontaneously to release the seed [1]. Considering this, chestnuts and hazelnuts are real nuts, whereas almonds and walnuts are not.

Nuts are highly relevant to some consumers because of the allergy issues linked to their consumption, particularly for almonds, peanuts, hazelnuts. The number of allergic patients worldwide is increasing, and the European Union (EU) has strict requirements for allergen labelling on food products [2].

Recently, nuts have become particularly appreciated in vegan, vegetarian, flexitarian, gluten free diets, and as well as in the paleodiet. They are a source of energy, unsaturated fatty acids and oils, fibre, proteins, vitamins and minerals, including bioactive compounds such as antioxidants, for a healthy diet. As healthy snacks they appeal to people working out of home, in place of sweets and biscuits. They are also marketed as an alternative to meat protein. “Milk” from edible nuts (almond, hazelnut) can replace animal milk. Their production has been increasing in the last years, up to about 4.2 million tons (peanuts excluded), produced mainly in the USA (41 %) followed by China, Turkey, Iran, India. Almonds are the tree nuts with the highest consumption, and Europe is the highest consumer of tree nuts in general, accounting for 40 % of total world imports, with three main importers, Germany, the Netherlands and Italy. Peanut production has reached 41.5 million tons, from China and India. Since many of the nuts are exotic products and healthy snacks, their consumption is expected to grow, if both the market and private means of the consumer increase. The products with rising markets are currently almonds, Brazil nuts, hazelnuts and macadamia nuts.

This chapter will deal neither with oils extracted from nuts, nor with other "seeds" which cannot be classified as nuts: chia, quinoa, amaranth, whose use is more similar to that of cereals.

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1. Product Identity

1.1. Definition of the product and manufacturing process

There is no accepted definition of "nuts" in food commodities. The fruits most commonly considered as nuts include the following (with indication of CN code, cf. Commission Implementing Regulation (EU) 2017/1925 of 12 October 2017 [3]):

Almond (code 0802 11-12): seed of the species *Prunus dulcis*, (or *Prunus amygdalus*) after removal of the fleshy hull (stone fruit). They can be marketed inshell, without shell as kernels, or as blanched kernels after removal of the tegument of the kernel (episperm). There are sweet almond and bitter almonds, containing amygdalin, a glycoside which releases hydrocyanic acid. USA is the major producer followed by Australia and Spain.

Brazil nut (code 0801 21 00-22 00): nuts of the tree *Bertholletia excelsa*, either shelled or after cracking of the shell. The shell is extremely hard and woody, and the kernel is enveloped by a brown seed coat. Bolivia is the major producer, followed by Peru and Brazil; UK is the major importer. The yield is highly dependent on environmental conditions.

Cashew nut (code 0801 31 00-32 00): the nut with the hard shell, also called anacardium, is at the bottom of the fruit (cashew apple) produced by the plant *Anacardium occidentale*. The main producer is Western Africa (Cote d'Ivoire), followed by India and Vietnam.

Chestnut (code 0802 41 00-42 00): these are the fruits of trees from the genus *Castanea,* mainly *C. sativa*. The husk is spiky and breaks open spontaneously, revealing 1-3 fruits; the fruit has a kernel, a thin skin and brown pericarp. The main producer is China, followed by Turkey and Italy.

Hazelnut, or filbert (code 0802 21 00-22 00): nuts of the species *Corylus avellana* and *C. maxima*, free from the husk, sold shelled or after cracking of the shell. Over half of the global production comes from Turkey, followed by Italy, which is also the main importer.

Macadamia nut (code 0802 61 00-62 00): nuts of the species *Macadamia integrifolia*, *M. tetraphylla, M. ternifolia* growing in hot subtropical climates. After drying and shell cracking, the kernels can be dry-roasted or oil-roasted. Used in confectionery, baking, ice cream, snacks. Production is concentrated in Australia, South Africa and Kenya.

Peanut (cod 2008 11): also called groundnuts, they are leguminous fruits of the plant *Arachis hypogaea*, growing underground. They have a thin shell, which is in fact the pod, containing generally two kernels. China is the world major producer, followed by India and Nigeria.

Pecan nut (code 0802 90 10): the seed comes from the tree *Carya illinoensis* and is encased in a husk. It can be consumed fresh or used in cooking. The main producers are Mexico and USA.

Pine nut (code 0802 90 50): decorticated kernels of different species of Gymnosperm, *Pinus*: e.g. *pinea*, *koraiensis*, *sibirica*, *yunnanensis*, *wallichiana*, *gerardiana*, *pumila.* The main producers are in Asia, China, North Korea, Russian Federation. Italy leads the production of Mediterranean pine nut.

Pistachio nut (code 0802 51 00, 52 00): the kernels are in the single-seeded stone fruit of the tree *Pistacia vera*, with a brown seed coat and brilliant green kernel. They are marketed in shell, raw or salted, sugared, flavoured. The major producer is the USA, followed by Iran and Turkey.

Walnut (code 0802 31 00-32 00): nuts of the tree *Juglans regia*, enclosed in a shell made of two halves, free from the outer green and fleshy husk, sold in the shell or after cracking of the shell. China and USA are the major producers.

Nuts are generally harvested by shaking trees, in processes which can be mechanised or performed manually. The fruits are then washed and dried. Some nuts are marketed as such, some undergo bleaching of the shell to improve the appearance, and others are taken out from the shell by cracking. Storage varies, with some nuts being more durable than others. New technologies are actively improving drying and processing, except in cases where natural production requires sun-drying.

They are commonly sold to the food manufacturing industry, as ingredients, to be processed, and repackaged. The chocolate industry is the largest user of many edible nuts, also playing on their health benefits. Breakfast cereals and energy bars involve also edible nuts in their formulations. The snack industry uses large quantities of edible nuts, particularly peanuts. Other industries involved concern bakery, ice creams, nut butter, nut milk, and even pet food. The increase in the use of edible nuts is due to new information about health attributes and claims, the availability of new types of nuts, and new processing and flavouring possibilities.

Increasingly there are requirements for sustainable products in appealing to consumers, particularly when edible nuts come from developing countries.

1.2. Current standards of identity or related legislation

The International Nut & Dried Fruit Council (www.nutfruit.org) is the body of reference for the trade with edible nuts. Statistics on the production and commerce of main nuts are available on the web site.

The standards from the United Nations Economic Commission for Europe (UNECE), Working Party on Agricultural Quality Standards [4] describe the products and the quality requirements at the export control stage, concerning appearance, moisture content, sizing, presence of defects, blemishes and infestation. They also describe packaging requirements. They are available for almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pine nuts, pistachio nuts, walnuts.

The International Standards for Fruit and Vegetables of the OECD also describe nuts in a similar way [5].

Concerning EU legislation, Regulation (EU) 1169/2011 lists nuts in the fourteen groups of allergens that must be declared on the label in a prominent way [6]. Besides allergens, the main concern in EU legislation regarding nuts is the presence of aflatoxins; additionally, the absence of insects and parasites, and of foreign objects. Nuts imported into the EU need a certificate ensuring that they have been sampled for analysis. Aflatoxins of concern, produced by moulds *Aspergillus flavus* and *A. parasiticus*, are B1, B2, G1 and G2 as listed in Regulation (EC) No 1881/2006 [7] amended by the Commission Regulation (EU) No 165/2010 [8]. The main attention is on peanuts from Bolivia, Gambia, Madagascar, Sudan and Senegal, on hazelnuts from Georgia, and on pistachios from the USA. The Rapid Alert System for Food and Feed (RASFF) system periodically informs about instances of aflatoxin contamination in nuts [9].

The standard ISO 1990-1:1982 provides the botanical names of common fruits and vegetables, including nuts [10]. Standard ISO 4125:1991 lists all dry fruits with a low moisture content, and nuts are also included [11].

CODEX has produced a comprehensive document on the Code of hygienic practice for tree nuts, CAC/RCP 6-1972, on the cultivation, processing, shelling etc. A separate document covers practices for peanuts, CAC/RCP 22-1979 [12]. A more recent document concerns the Code of practice for the prevention and reduction of aflatoxin contamination in tree nuts, CAC/RCP 59-2005 [13].

The Transport Information Service [14] provides relevant information and specifications about the transport of nuts and the possible problems which may arise to damage the products. The website Standards Map [15] provides a tool for recovering the information on sustainable trade, linking to the different standards on this subject.

2. Authenticity issues

2.1. Identification of current authenticity issues

The main authenticity issue for tree nuts is probably the country of origin, since this indication is usually mandatory on the label. Indication of the species is also obligatory and relevant, particularly for problems linked to allergies: substitution of nuts from different species can negatively affect allergy patients, if undeclared. The indications on the label can also include the crop year and the variety. The year of harvest is important because older nuts are more prone to infestation and rancidity.

A possibility for fraud exists in the declaration for organic products, which should be produced and processed with specific techniques, including the use of crop rotation, specific crop protection and fertilisation substances.

Additionally, nuts are used in Jewish cuisine and the Kosher certification is another possible source for fraudulent declarations.

The increase in demand, combined with problematic harvests, can lead to the shortage of some edible nuts, and this could open the way to counterfeiting. In the case of Brazil nuts, which are collected in the Amazonian forest, their availability from year to year is not predictable.

Protected denominations (PGI or PDO) for cultivars of some edible nuts are particularly appreciated and can be subject to fraud. One such example is the hazelnut "Tonda Gentile delle Langhe" (Nocciola Piemonte, PGI since 1996) from Italy. Other relevant PDO examples in Europe concern: chestnuts (Portugal and Italy), almonds (Portugal), walnuts (France, Italy).

Some PDO or PGI productions requiring nuts as ingredients are also subjected to fraud. One relevant example is Ligurian pesto, a sauce made with basil containing pine nuts as a highly relevant ingredient. In this case, *Pinus pinea* is the species of origin, but other pine species could be used since recognition by visual inspection is impossible. Mislabelling of pine nuts is relevant for the taste, but also for health effects (see below).

Hazelnut paste is an important ingredient in confectionery, and also the ingredient for appreciated hazelnut spreads for direct consumption (e.g. Nutella). The percentage of hazelnuts in the paste is a critical quality issue and dilution or substitution with artificial compounds or with other ingredients are fraudulent practices.

2.2. Potential threat to public health

In the case of nuts, the main risk to health is represented by allergies. Mislabelling can be very dangerous to allergic patients. On the other hand, precautionary labelling can deprive consumers from enjoying some food products.

The presence of aflatoxins is a relevant threat to health for nuts (cf. Safenut project [16]). The EU RASFF system in 2017 issued a total of 364 notifications for aflatoxins in edible nuts, particularly for peanuts from China, pistachios from Iran and hazelnuts from Turkey. The geographic provenance of nuts is therefore an important component of authenticity issues, since some countries are more susceptible to contamination with aflatoxins.

Pine Nut Syndrome (PNS), also called metallogeusia, dysgeusia, was first described as a disturbance of taste perception, starting with a metallic or bitter taste appearing after consumption of pine nuts and lasting for several days [17]. The cause of the syndrome is yet unknown, with possibilities concerning fatty acids, rancidity, toxins. The prevalence, which is higher in women than in men, has led to considerations concerning genetic polymorphisms and metabolism of bioactive compounds. In particular, the species *Pinus armandii*, from China, has been associated to PNS occurrence; this species is used in industrial applications and not for food production. The uncertainty about the cause of the syndrome and the difficulties in labelling and tracing the provenance of pine nuts are negatively affecting the market.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

Most standards on nuts require visual inspection and morphological evaluation. The U.S. Food and Drug Administration (FDA) has a list of methods for the analysis of nuts and nut products addressing mainly defects, infestation, and presence of foreign material [18].

Other methods for nuts based on analytical techniques are applied mostly in cases where the nuts are not easily recognisable visually, for instance in hazelnut spreads or peanut butter. Identifying the nut species in these cases is essential to protect patients suffering from allergies. These methods can be based on detection of proteins/peptides, DNA markers, or specific metabolites and compounds.

The presence of specific nuts in food products is usually checked with ELISA and lateral flow devices for rapid testing. Several companies have placed on the market tests to detect different species of nuts by recognising specific allergenic proteins as target, with the aim of protecting consumers. However, in some processing conditions proteins can be denatured or degraded, making their detection more difficult. In these cases, analysis of DNA markers can be effective, since DNA as a molecule is more stable than protein.

For the recognition of specific cultivars, for instance to check for PGI or PDO varieties, visual inspection or imaging techniques are often applied [19]. However, the aspect of the nut and of the shell can be affected by several environmental factors. In these cases, DNA typing provides a better way for identification, even if DNA analysis is made difficult by the high lipid content of nuts [20]. DNA analysis can be applied to whole nuts or to products made with nuts. The markers of choice are often the genes encoding for allergens, which are considered to be highly specific for each type of nut.

The presence of lipids and fatty acids in nuts and nut products can be a problem in some analytical techniques, but it is also a distinctive feature of nuts. Methods based on analysis of fatty acids or other metabolites have been developed to check for the authenticity of the species or of the cultivar, even in highly processed foods [21–23].It is widely considered that these kind of markers can provide indications about the geographic origin of food products, whereas DNA markers cannot be used for this purpose [24,25]. They could also provide indications about the year of harvest, but no method has currently been developed.

3.2. Other commonly used methods

In some cases, DNA analysis requires specific adaptation. One such example is the discrimination between marzipan (containing almonds) and persipan (containing apricot or peach kernels), which is difficult because the *Prunus* species are closely related. DNA analysis with specific markers and barcoding has been shown to be effective [26]. Potential DNA markers applicable to discriminate between cultivars can be found in the chloroplast DNA or in genes encoding for ribosomal DNA (rDNA) as in the case of analysis for recognition of *Pinus armandii* [27,28] or hazelnut [29].

Multiplex methods can be useful when looking for different species of nuts at the same time in a food product [30]. Methods based on DNA extraction and amplification of specific diagnostic fragments can be multiplexed in different formats, endpoint PCR, Real time PCR, microbeads [31], etc.

Also, there will be a development of methods for performing biomolecular analyses in a quick and rough way, for instance with no requirements for lengthy DNA extraction and no need for costly equipment. An example is Loop-Mediated Isothermal Amplification (LAMP) PCR which does not require a thermal cycler [32]

Similarly, screening methods using chemometrics with no extraction of the sample, such as with spectroscopic techniques, could be interesting additions, when coupled with portable instruments for *in situ* quick non-destructive analyses.

Non-targeted analyses performed with spectroscopic techniques are being developed, to detect differences in lipids, proteins, carbohydrates with fast analyses. The chemical profile could discriminate nuts according to the geographic origin [33].

4. Overview of methods for authenticity testing

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

Analytical technique	Indicative data or analyte	Authenticity issue / information
ELISA	Allergens	Hazelnuts, etc.
Fourier transform Infrared Spectroscopy, portable	No particular analyte (lipids, proteins)	Variety discrimination
Gas chromatography	Fatty acids	Geographical origin
GC/MS, gas chromatography mass spectrometry	Filbertone	Hazelnut spread
HPLC	Tocopherol	Chestnut varieties
Imaging techniques	Identification of cultivars	Hazelnuts cultivars
Loop mediated isothermal amplification (LAMP)	Allergen marker	Peanut in food
Microbead fluorometric PCR	Allergens	Nuts in foods
PCR	DNA markers	Species identification, e.g. *Pinus armandii*
PCR	Chloroplast markers	Species identification, e.g. *Pinus armandii*
TaqMan real time PCR	ITS marker	Hazelnut allergen

5. Conclusion

The worldwide trend in consumption of nuts, because of their health benefits, supports a sustainable growth for this market. New literature reporting on the beneficial effects of unsaturated fatty acids and other components of some nuts are leading to their introduction in diets for patients or people at risk of cardiovascular diseases. Milk substitutes from nuts, and nuts substituting meat, will be appealing to vegans. Savoury snacks are already widely used, but they are constantly increasing because of health effects, new processing ideas, and availability of several nut types. In view of this, the increased consumption could lead to fraud opportunities. Additionally, since the chemical composition of nuts depends on species and geographic origin, fraudulent labelling for provenance could be envisaged. Current methods can effectively recognise species and cultivars, but recognition of provenance is more difficult.

Consumer interest in sustainability of provenance and fair trade are particularly increasing in the EU market, and this also applies to fruit and nuts. Certification of these schemes will probably become more common, creating potential opportunities for frauds. However, methods to establish the correctness of such claims are not yet available.

In the case of nuts, climate change is expected to have highly negative and unpredictable effects. The areas for production, often in tropical countries, will be heavily affected by changes in growing seasons, temperature and rainfall. Additionally, new pathogens and infectious diseases are expected to appear. Currently, pine nuts are defined as the “caviar of plants” because they are becoming increasingly rare, mainly due to an increase in pathogens worldwide, and to deforestation and changes in climate conditions. The yield and safety of the products will therefore be affected in a negative way, and this will probably require additional methods for traceability and inspection. In Ligurian pesto, for instance, the pine nuts required by the original recipe are sometimes substituted with cashews. Fraud control will therefore require methods for species recognition, but also methods for provenance recognition, which are not completely developed.

The certification of organic cultivation will also become more important. This market is growing continuously, and the production cannot keep up with the pace. This will also be an opportunity for fraud. Unfortunately analytical controls on organic cultivation are not well developed at the moment.

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