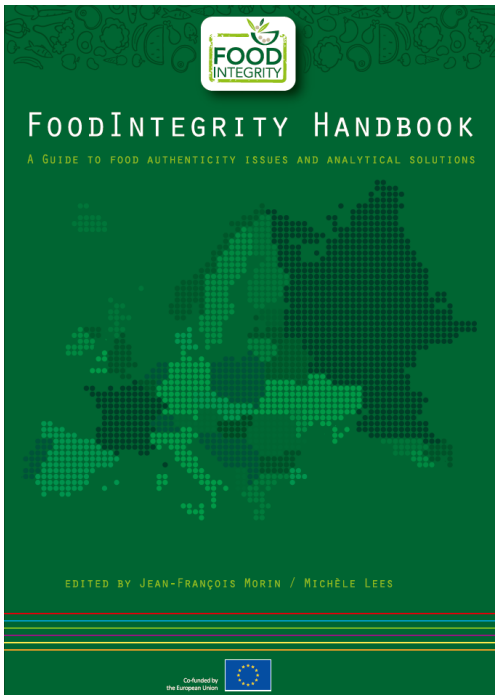
FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

Editors: Jean-François Morin & Michèle Lees, Eurofins Analytics France



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Cereals and cereal-based products

Jean-François Morin*, Michele Lees
Eurofins Analytics France, Nantes, France
**E-mail corresponding author: JeanFrancoisMorin@eurofins.com*

Philippe Vermeulen*, Vincent Baeten
Walloon Agricultural Research Centre, Gembloux, Belgium
**E-mail corresponding author: p.vermeulen@cra.wallonie.be*

Elena Maestri*, Nelson Marmiroli
SITEIA.PARMA, University of Parma, Italy
**E-mail corresponding author: elena.maestri@unipr.it*

General overview of the products

According to the FAO's definition the term cereals refers only to crops harvested for dry grain. Crops harvested green for forage, silage, or grazing are classified as fodder crops. Cereal products are defined as derived from the processing of grain by mechanical or chemical processes, or from the processing of flour, meal or starch. All together the FAO definitions cover 17 primary cereals, the major ones being wheat, barley, maize (or corn), triticale, rye, oats and rice. In 2014, in Europe (EU-28), all these grains (excluding rice) represented in the food, feed, industry (including fuel) and seeds sectors, 24 %, 61 %, 11 % and 4 % respectively [1].

Cereals are generally from the gramineous or Poaceae family and identified according to their genus (see Figure 1 for the phylogenetic relationships of the cereal species and subspecies mentioned in this chapter). With carbohydrates comprising 65-75 % of their total weight, cereals and cereal-based products constitute the main source of energy for the majority of human populations and are therefore important staple foods. Different cereal species have different uses with a wide range of qualities often linked to specific varieties. These perceived differences in quality in the final consumer product can lead to substantial differences in price, with the potential for cheaper varieties to be passed off as the more expensive kind. Hence the need to establish the authenticity of cereals.

The main authenticity issues for cereals generally involve wheat and wheat-based products and rice, making these the main focus of this chapter, with other cereal types mentioned only where relevant.

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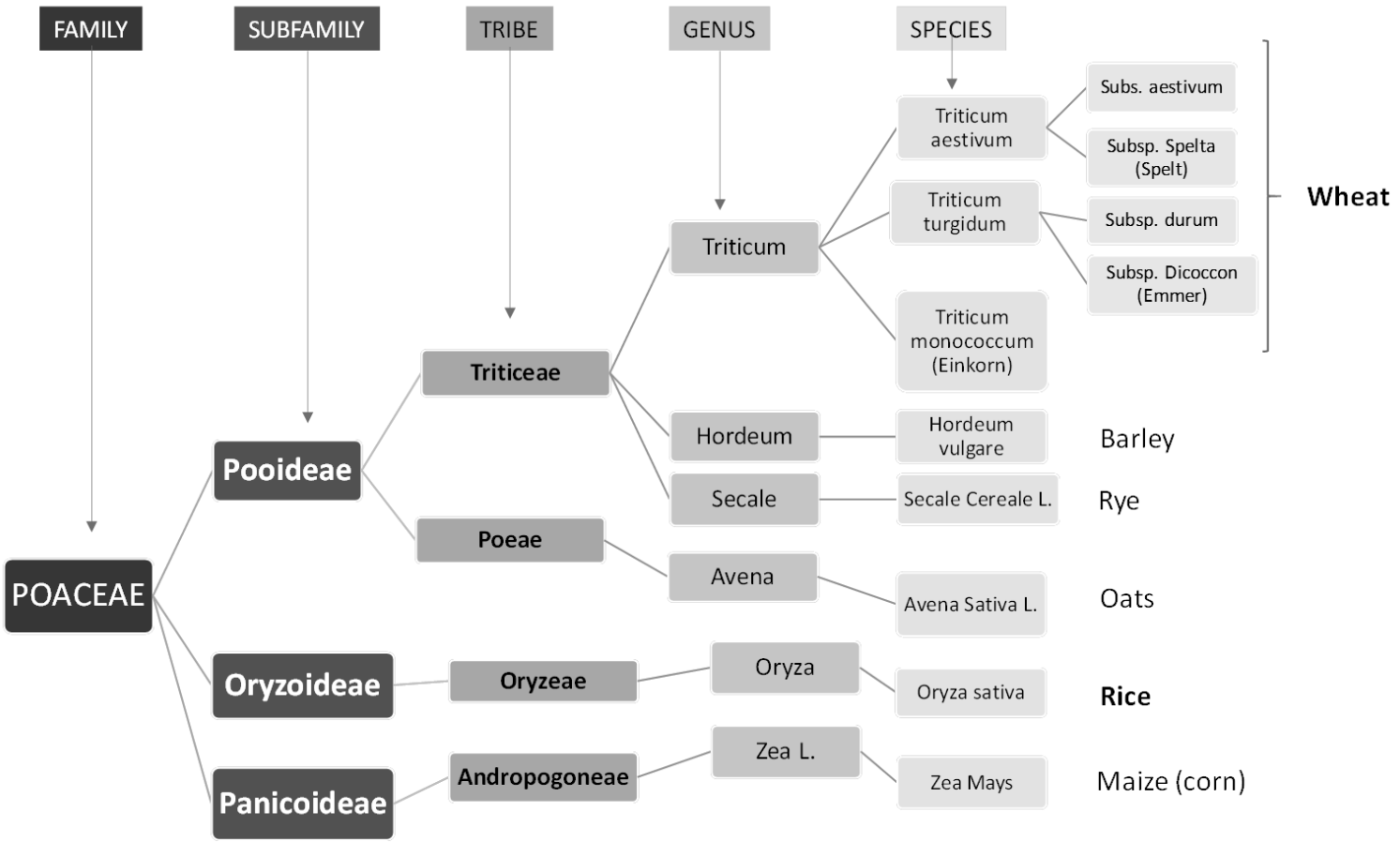

Figure 1: Phylogenetic relationships of the cereal species and subspecies mentioned in this chapter (nomenclature reported according to http://www.ars-grin.gov)

Wheat and related products

1. Product identity

1.1. Definition of the product and manufacturing process

Wheat is widely grown around the world under diverse climatic conditions and has been the staple food of the major civilisations in Europe, Asia and North Africa for 8000 years. Of the many species of wheat that make up the genus *Triticum,* the most widely grown is common wheat, *Triticum aestivum*. The second most cultivated species after common wheat is durum wheat, also known as pasta wheat (*Triticum turgidum* subsp. *durum).*

Within each species there are a number of cultivars or varieties that can be placed into a number of groups or types; these may be acceptable botanical groups based on grain or plant characteristics, e.g. red and white grained, hard and soft grain textures, spring and winter types, or groups based on other attributes such as baking performance or gluten characteristics. The harder the wheat, the higher the protein content in the flour. Soft, low protein wheats are used for cakes, pastries, biscuits and oriental noodles, whereas hard, high protein wheats are used in making bread. Durum wheat is used for pasta and noodles.

Wheat production in 2016 accounted for 672.7 million tonnes worldwide [2]. In 2017, production by the 28 EU Member States was 152.6 million tonnes, approximately 22 % of the worldwide production. Of this European production, 93.6 % (142.8 million tonnes) was soft wheat with durum wheat accounting for the remainder (6.3 %; 9.6 million tonnes) [3]. Production of soft wheat was concentrated in France (25.3 %), Germany (17.0 %) and the UK (10.3 %). Poland, Romania and Hungary produced 8.0, 6.9 and 4.4 % respectively. In the case of durum wheat, Italy accounted for 45.4 % of total production; other major producing countries were France (21.7 %), Greece (13.0 %), and Spain (12.6 %).

1.2. Current standards of identity or related legislation

1.2.1. In the European Union

Different European and national regulations apply to cereals depending on whether they fall into the food, feed, or seed sectors.

For the food sector, Regulation (EC) No 742/2010 of 17 August 2010 [4] establishes the eligibility criteria to be met by cereals for public intervention and the methods to be used for carrying out tests to establish such eligibility. For the feed sector, the regulation (EC) No 767/2009 of 13 July 2009 [5] lays down rules on the placing on the market and use of feed for both food-producing and non-food producing animals within the Community, including requirements for labelling, packaging and presentation. Regulation (EC) No 1829/2003 of 22 September 2003 [6] lays down Community procedures for the authorisation and supervision of genetically modified food and feed as well as provisions for their labelling.

There are also specific regulations for cereal products, particularly for those destined for consumption by infants. Commission Directive 2006/125/EC [7] on processed cereal-based foods

and baby foods for infants and young children lays down requirements for the composition of such products, including cereal, protein, carbohydrate, mineral and vitamin contents. Further compositional and labelling rules for processed cereal-based food in the EU Regulation on food for specific groups [8].

At national level, regulations, directives, recommendations are in application in each European country. They concern the production and sale of cereals, milling products, bread and pasta. Some of them, dedicated to authentication, can be found in the regulations section of the FARNHub tool [9]. For example, in Italian regulations, the presidential decree N° 187, dated 9 February 2001 [10], stipulates that durum wheat milling products may contain up to three per cent of soft wheat flour. More general regulations at national level can also be found on the EU-N-Lex website [11].

The upstream cereals sector concerning the seeds is also legislated by regulations defining the production of new varieties, their registration and varietal purity. Council Directive 66/402/EEC of 14 June 1966 on the marketing of cereal seeds [12] establishes rules, amongst others, on the production, packaging, sampling, sealing and marking in order to ensure the identity of the certified seeds. This Directive has been amended several times and in particular by Commission Directive 2009/74/EC of 26 June 2009 [13] as regards certain Annexes to Directive 66/402/EEC in the light of developments of scientific and technical knowledge regarding seed purity.

From the point of view of the general public, consumers are showing increasing interest for different qualities of bread produced from cereals such as spelt (*T. spelta*), emmer (*T. dicoccum*), einkorn (*T. monococcum*). In order to preserve quality food products coming from particular geographical areas and to protect consumers against imitations and false information, the European Commission has defined, via Regulations [14] several quality labels, among which are the Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI) labels. Products such as Farro di Monteleone di Spoleto (emmer) produced in Italy-Umbria (PDO), Farro della Garfagnana (emmer) produced in Italy-Tuscany (PGI) and Petit épeautre de Haute Provence (einkorn) produced in France (PGI) are protected by these European labels. Other cereal products such as Epeautre d'Ardennes (spelt) produced in Ardennes in Belgium are protected by regional labels based on specifications defined by the spelt sector [15].

In addition to the legislation, the cereal sector is managed by standards defining the best practices in cereal sampling and quality analytical control. These are described in section 3.1.1. below.

1.2.2. In the United States

The Department of Health and Human Services of the US Food and Drug Administration (FDA) has published requirements for specific standardized cereal flours and related products in the Code of Federal Regulations (CFR) Title 21, Part 137. These regulations provide the definitions and standards of identify of a wide range of cereal-derived flours. Further up-to-date information on FDA regulations are available on the Government Publishing Office's e-CFR at reference [16].

1.2.3. At the international level: Codex Standards for cereals

The Codex Alimentarius [17] has published a comprehensive document that includes all texts adopted by the CA Commission up to 2007 for Cereals, Pulses, Legumes and Vegetable Proteins. These standards provide accepted definitions for each cereal or cereal product, including certain quality attributes such as moisture content, an important parameter used in the trading of cereals. The relevant standards are summarised in Table 1.

Table 1: Codex Standards for cereals and cereal products

Cereal / cereal product	CODEX STAN	Definition	Moisture content
Couscous	202-1995	Product prepared from durum wheat	<13.5 %
Durum wheat semolina and durum wheat flour	178-1991	Products prepared from grain of durum wheat by grinding or milling processes.	<14.5 %
Maize (corn)	153-1985	Shelled grains of *Zea mays indentata L.* or *Zea mays indurata L.*	<15.5 %
Oats	201-1995	Grains of *Avena sativa* and *Avena byzantina*	<14.0 %
Wheat and durum wheat	199-1995	Wheat is obtained from the varieties of the species *Triticum aestivum L.*	Wheat <14.5 % m/m
		Durum wheat is obtained from varieties of the species *Triticum durum Desf.*	Durum wheat <14.5 % m/m
Wheat flour	152-1985	Product for human consumption preparer from grain of common wheat *Triticum aestivum L.* or club wheat *Triticum compactum Host* or mixtures thereof by grinding or milling processes	<15.5 % m/m

2. Authenticity issues

2.1. Identification of current authenticity issues

2.1.1. Species substitution, varietal identification

One of the main authenticity issues for cereals and cereal products is the deliberate substitution with cheaper species or varieties. Different varieties or class of varieties have different end-use qualities, some being more suitable than others for certain types of food industrial processing or animal feed, and this may lead to significant differences in food and feed market prices. Effective species/variety discrimination of cereals based on product composition is increasingly vital for the needs of the food processing industry.

2.1.1.1. Case of common wheat in durum wheat

Quantifying the degree of adulteration of durum wheat flour with common bread wheat flour is of particular interest in the Italian, French and Spanish markets, where semolina is the only allowed constituent for pasta, while in the north European countries both bread and durum wheat are permitted. The use of common wheat in durum wheat, is considered as fraud according to current Italian legislation [10] with only a maximum of 3 % common wheat allowed to account for any cross-contamination that may occur during the agricultural process. However, mixtures of both wheats can be found due to delivery problems or to reduce prices. For this reason, efficient methods for the detection of accidental or intentional contamination of durum wheat with common wheat are required at the entrance to food operators' premises.

2.1.1.2. Case of common wheat in spelt

A growing interest in foods delivering high nutritional value and health benefits has encouraged breeders to develop new grain species that meet consumer expectations. The grain of hulled wheats (spelt - *Triticum spelta*; emmer - *T. dicoccon*; and einkorn - *T. monococcum*) and the resulting products meet the requirements set for functional foods. To give added value to the genetic and breeding efforts as well as guaranteeing the differentiated quality of bread obtained from these new grain species, efficient methods are needed to assess quality based on the composition [18].

2.1.2. Geographical origin

Most countries have their own specific grain varieties, suited to their own environmental conditions and agronomic practices. Being able to verify the geographical origin of cereal to ensure full traceability from the food to the production location is important when grain from a specific area commands a higher price, or to ensure that the grain does not originate from a region known to be contaminated.

2.1.3. Certification of organic production

Today's consumers are increasingly concerned by the quality and safety of the food they eat, with more and more of them turning to organically grown products. For a cereal-derived product to be labelled as organic, the producer must follow and comply with specific rules laid down in international regulations. This will inevitably lead to higher costs for producing organic products compared to conventional ones, followed by higher prices in the market. An authenticity issue will arise when cheaper non-organic product is passed off as organic.

2.1.4. Gluten-free products

Coeliac disease is caused by a reaction of the immune system to gluten, a protein found in wheat, barley, rye and oats. It can be a serious disease if undiagnosed and can only be treated by following a gluten free diet for life. Food products labelled "gluten-free" are usually prepared using cereal species which naturally do not contain gluten such as rice, maize, amaranth [19,20]. However, both intentional and unintentional contamination can occur leading to an authenticity issue for such products.

2.2. Potential threat to public health

Probably the most serious potential threat to human health concerns the potential contamination of gluten free cereals as described above. For food companies involved in the processing of several species of cereals, accidental contamination of gluten free cereals with wheat, for example, can occur and such products can cause illness or severe reactions for individuals with wheat allergies or coeliac disease [21]. Separate production lines and good traceability are required to reduce the risk and the impact on the public health.

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. General methods for quality control

In order to identify and discriminate varieties, a large number of analytical methods have been developed, including visual examination of kernel morphology (colour, size, shape, texture); simple laboratory tests and measurements (yield, Thousand Kernel Weight [TKW], specific weight, kernel size, germination analyses).

The International Association for Cereal Chemistry (ICC)[1] also provides a number of standard methods for general quality control of cereals. Its compilation of standards includes guidelines for sampling of grain, and methods for the determination of moisture, protein, starch, fat and dietary fibre contents [22].

The American Association of Cereal Chemists International (AACC Intl.) also provide a collection of approved methods for cereals laboratories and companies involved in grain processing, available at [23]. Together AACC Intl. and the ICC have developed a sub-set of harmonized methods for the analysis of key constituents and parameters that are frequently tested on an international basis.

As regards the specific case of wheat varieties, the International Union for the Protection of New Varieties of Plants (UPOV) [24] provides guidelines for the "examination of distinctness, uniformity and stability and the development of harmonized descriptions of new varieties of plants", including specific tests for wheat.

At the European level, the European Committee for Standardization through the technical committee on cereal and cereal products (CEN/TC 338) establishes norms on cereal quality [25]. National agencies of normalisation such as the French agency of normalisation (AFNOR) or the Belgian bureau of normalisation (NBN) define/adapt norms at the national level.

As regards the determination of parameters such as moisture and protein in cereals by NIR spectroscopy, international standards and guidelines have been developed recently by the NIR spectroscopy community [26].

3.1.2. Protein-based methods

Many methods for authenticity studies in cereals use grain storage proteins, which often represent the most important features for the quality of the processed products. In the case of wheat, the bread- and pasta-making properties depend on specific storage proteins, the prolamins [27]. Wheat proteins can be classified into two types: gluten and non-gluten proteins. Gluten protein makes up the bulk of total wheat protein, composed mainly of two fractions: gliadins and glutenins (with high and low molecular weight respectively) which affect the visco-elastic properties of dough. Non-gluten proteins include albumins and globulins [28].

The most established method for the identification of wheat varieties uses polyacrylamide gel electrophoresis (PAGE) to separate the wheat proteins extracted from grain [24]. The high molecular weight glutenin sub units are used for the identification of varieties. ICC Standard 143 [29] specifies a method for the identification of the variety of a given lot of soft or hard wheat, in

[1] The ICC is an international network of cereal scientists and technologists dedicated to the improvement in safety and quality of cereal-based foods, one of its missions in the validation and standardization of suitable test methods.

the form of individual ground kernels, flour, farina or semolina, by the separation of gliadin proteins. The separated protein components are visible from the stained polyacrylamide gels and compared to a variety catalogue established for major wheat varieties. This method is in common use in many countries.

3.2. Other commonly used methods

3.2.1. Biomolecular methods

More elaborate methods such as DNA detection based on the differences in genetic background of the wheat species or varieties are beginning to be more widely used for authenticity purposes. These methods can be used both to discriminate between species and to identify varieties. A thorough description of these methods and their advantages is given in reference [27].

3.2.1.1. Identification of different cereal species in food products

The identification of cereal species in food products can be performed by targeting species-specific genomic information and analysing the nucleic acids extracted from food products. A marker for species identification should be a reference gene showing no allelic variation, with a low number of copies in the genome. The study described in reference [30] has proposed specific marker genes for barley, rice and wheat, respectively: γ-hordein, gos9 and acetyl-CoA carboxylase. A different set of species specific-markers has been proposed [31] for detecting adulteration in chestnut flour by: barley, bread and durum wheat, oat, rye, maize and rice. Amplified fragments were of different dimensions and could be analysed in duplex PCR reactions.

A different approach based on microarrays was proposed for simultaneous detection of several species: wheat, rye, barley, oat, rice and maize [32]. The target, common to all species, was the intron of the chloroplast transfer RNA gene, trnL, which can be amplified with universal primers from all plant species. The application of species-specific probes then allows discrimination among different cereals without cross-hybridization.

To discriminate cereal species within a mixture, another study [33] looked at the same target sequence with a padlock probe approach on microarrays: the trnL target sequence is linked to a unique labelled cZIP-code sequence. It was applied to detection of adulteration in the Italian PGI (Protected Geographical Indication) cereal “Farro della Garfagnana”, emmer wheat.

3.2.1.2. Detection of gluten-containing cereals in “gluten-free” products

Methods based on the Polymerase Chain Reaction (PCR) can detect the presence of traces of material derived from gluten containing cereals [34]. This PCR approach exploited primers specific for wheat, barley and rye. The test showed specificity and sensitivity of 100 %; it recognized all wheat cultivars tested, and it did not recognize all the non-gluten species tested. The sensitivity allowed identification of contamination at 0.1 % (w/w). The test described in [35] based on wheat glutenins, components of gluten: a 135-bp specific fragment of the low molecular weight glutenin gene could be amplified from the Triticum species, but not from barley, rye and other cereals, with a Limit of Detection (LOD) of about 1 copy.

A quantitative competitive PCR system (QC PCR) has also been described as a suitable indicator of contamination of gluten-free food with gluten-containing cereals. This system simultaneously detects Wheat- Barlery-Rice-DNA on the basis of a non-coding region of chloroplast trnL gene. The

method has been favourably compared with the more commonly used ELISA method. A positive QC-PCR signal and a negative ELISA result indicates a possible gliadin-free wheat starch addition whereas the opposite situation indicates a possible addition of wheat-free gliadin as a food additive [36].

3.2.1.3. Identification of Triticum aestivum in pasta products

DNA-based methods are used to detect and quantify the presence of common wheat (*Triticum aestivum*) in durum wheat (*Triticum durum*) pasta and other products. DNA is extracted from the sample and four sections of the nuclear genome are amplified using universal primer pairs for both species of wheat. The amplicons are analysed for their species-specific fragment lengths by capillary electrophoresis. Fragment lengths are compared to a previously-established database which enables the identification of durum or common wheat in the sample. By calibrating the system, it is possible to quantify both species in the sample. This method is applicable to pasta, as well as noodles, semolina, couscous, cracked wheat.

3.2.2. Near Infrared (NIR) - Mid Infrared (MIR) spectroscopy

Several studies have also shown the potential of Near Infrared Spectroscopy (NIRS) to identify and discriminate varieties.

In the wheat sector, NIR technology is nowadays considered as an essential analytical tool that greatly contributes to enhancing the quality and safety of agricultural products. Moreover, it has been implemented with success at different stages of the production chain, making it possible to carry out larger numbers of analyses, thus saving time and money. NIR technology is currently used for the quality control of raw materials and end products, for the detection of undesired products and also for the detection of fraud in the both the food and feed chains.

As such, near infrared (NIR) spectroscopy can be considered as a potential powerful tool to detect wheat species such as common wheat in durum wheat [37]. The protein content and the vitreousness of durum wheat is generally higher than that of common wheat. Both criteria can be assessed by NIR spectroscopy. This technique is often used for authentication and traceability of agricultural and food products [38–40]. Mid infrared (MIR) can be also used to discriminate wheat species in particular hulled wheat such as spelt, emmer and einkorn [41]. Differences on cellulose/hemicellulose and lipid contents can be observed between those species.

3.2.3. NIR hyperspectral imaging

To meet the quality product specifications required by the world grain markets and by the agro-food industries, NIR technology has been adapted for the analysis at the kernel level. To achieve this, NIR hyperspectral imaging has been developed in order to detect contamination and fraud in cereals. One particular case-study can be cited to illustrate this kernel by kernel analysis: the detection of common wheat kernels in durum wheat [42]. The macroscopic and microscopic morphological features are important criteria to discriminate wheat species. RGB (red, green and blue model) images can be used to discriminate between durum wheat and common wheat kernels [43]. NIR is also used to assess amongst other protein content and hardness [44]. NIR hyperspectral imaging combines imaging and NIR. It has been used to classify kernels and to simultaneously determine protein content, moisture content, oil content, and hardness, as well as to detect sprouted, insect-damaged, and fungal-infected kernels in wheat [45,46]. NIR hyperspectral imaging has also been assessed as a fast method for the at-line and on-line discrimination between durum wheat and common wheat at the single kernel and bulk sample

level according to the morphological profile, the NIR spectral profile, the protein content and vitreousness [39].

This NIR technology has also been explored on other species such as barley, maize, and rice to identify and discriminate varieties [38,39,44].

3.2.4. Stable isotope ratio analysis

Stable isotope analyses of both heavy (strontium) and light isotopes (C, N, S, O) provide an isotopic signature that can used to verify the geographical origin of a plant. The light isotopes are incorporated into plants during metabolism, linking the plant to specific features of the environment of provenance [47]. The heavy isotopes like Sr also provide geographical information as their content depends on the geology of the plant's growing area.

Building up a database of isotopic signatures from samples taken around the world can be used to verify specific provenance claims. The availability of authentic samples to establish such a database remains a major limitation to the widespread use of this method. Work was undertaken in the FP6 TRACE project[2] to study how geochemical markers and the relationships between these markers could be used to determine the provenance of food products. The study looked at wheat and other cereals from all over Europe and investigated the potential of stable isotope ratio measurements ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$) together with strontium isotope ratio measurements ($n(^{87}Sr)/n(^{86}Sr)$), and 5 elements (Na, K, Ca, Cu and Rb). Samples were classified in different categories, comparing cultivation regions in the north and south, and near the Atlantic Ocean or the Mediterranean Sea [48].

Stable isotope ratio analyses ($\delta^{13}C$, $\delta^{15}N$ and δD, alone or with $^{87}Sr/^{86}S$) have also been used to identify the geographical origin of winter wheat in China [49]. A further study by the same authors determine $\delta^{2}H$ values for soil water in three growth periods, and rainwater, groundwater, and defatted wheat in the maturity stage, in order to provide a potential indicator for tracing wheat geographical origin [50].

The geographical origin of Indian wheat has also been studied using isotopic composition ($\delta^{13}C$, $\delta^{15}N$) wheat samples collected from adjacent states of India. Results obtained using $\delta^{13}C$ showed good potential; the difference in the $\delta^{15}N$ values from different states were not significant [51].

[2] FP6 TRACE Project. Tracing the origin of food. 2005-2009. Funded by the European Commission under the 6th Framework Programme.

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
PAGE (Polyacryamide gel electrophoresis)	Wheat protein glutenin	Identification of wheat varieties
DNA detection (various techniques)	Species-specific markers	Detection of various species (barley, bread and durum wheat, oat, rye, maize, rice)
Polymerase Chain Reaction (PCR)	Specific primers for wheat, barley, rye	Detection of gluten-containing cereals in "gluten free" products
NIR spectroscopy	Protein content, hardness	Discrimination of species/ varieties
MIR spectroscopy	Lipid, cellulose/hemicellulose content	Discrimination of species/ varieties
NIR hyperspectral imaging	Morphological and spectral information	Discrimination of species/ varieties
Stable isotope ratio analysis	Light element isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$)	Geographical origin
	Heavy element isotopes ($^{87}Sr/^{86}S$)	

5. Conclusion

Potential authenticity issues in the future are likely to come from new products becoming available in the market. The "pseudo-cereals" such as quinoa (*Amaranthaceae*), buckwheat (*Lamiaceae*) and chia (*Lamiaceae*) are becoming increasing popular amongst consumers due to perceived health benefits [52,53]. As these products command a higher price, there is the possibility that adulteration or mislabelling will occur [54].

Fraud on wheat seed coating can also be cited as a potential issue. At the current time, no rapid method exists that is able to assess the coating of cereals seeds. Kernel by kernel analysis by NIR could be a way to address this potential fraud [55].

As regards the future of analytical methods, improvement is likely to be seen in the progress in technology and instrumentation. Biomolecular methods remain the most powerful for differentiating between the different cereals or different varieties of cereals. As the technology surrounding DNA-based methods progresses, moving toward rapid throughput screening and efficient instrumentation at an accessible cost, these techniques will be the methods of choice for unambiguous discrimination. New tools based on proteomics can improve the application of protein-based identification of species, cultivars or genotypes. Proteomic analysis of glutenins can be used to detect allelic variants and quality-related issues in durum wheat flours [56].

Over the last few years, a growing number of handheld instruments based on near-infrared spectroscopy including imaging systems, have appeared on the market. They are particularly characterised by their compact appearance, ease of use, the ability to be controlled using a wireless connection via a tablet or a smartphone. It is expected that innovative technology will be used in order to get integrated NIR systems (spectral information) combined with imaging analysis techniques (morphological information), sampling systems (representative information), and GPS

devices (geolocated information). Some of them include predictive models for the simultaneous determination of different quality parameters of the products. Other are connected through the cloud to a central database and software making remote prediction of these parameters.

Beside the NIR sensors for solid/liquid measurement, new NIR sensors for gas analysis or other sensors based on alternative spectroscopic techniques (mid-infrared, Raman, terahertz, nuclear magnetic resonance etc.) are emerging on the market.

These new, smaller and low-cost instruments compared to conventional infrared devices should answer the forthcoming challenges, in terms of precision agriculture, quality control and fraud detection to improve authenticity and processing issues on food always more sophisticated [57].

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Rice and related products

1. Product Identity

Estimated world production of paddy rice in 2016 was 741.0 million tonnes [1]. Of this, Asia accounted for over 90.1 %, Americas for 4.9 %, Africa for 4.4 % and the 28 EU Member States for about 0.4 %. The three main producers, China, India and Indonesia, produced more than 60 % of the world's rice. Within the EU, Italy, Spain, Greece, Portugal, France, Bulgaria, Romania and Hungary are rice producers. Italy accounts for 52.6 % of the total European production in 2017, followed by Spain with 28.0 %, Greece with 6.5 % and Portugal with 5.6 % [2].

The majority of the world's paddy rice is consumed in Asia where it is produced. In the international rice trade, a relatively small number of exporting countries, notably Thailand, Vietnam and India, interacts with a large number of importing countries in Asia, in Africa and also in Europe [3].

1.1. Definition of the product and manufacturing process

1.1.1. General taxonomy

Rice belongs to the genus *Oryza* and the tribe Oryzeae of the Poaceae family. Of the different species belonging to the *Oryza* genus, *O. sativa* is the most widely grown cultivated species making up the majority of the approximately 100 000 different varieties held by the International Rice Gene Bank (http://knowledgebank.irri.org).

O. sativa includes two main cultivars, Indica, which are grown predominantly in tropical and subtropical regions and Japonica, which are grown in temperate regions. Generally, Indica rice grains are longer and retain their shape after cooking, whereas Japonica grains are shorter, and softer when cooked.

1.1.2. Rice variety classification

In international trade, rice variety classification is primarily based on its grain size and shape. The simplest system groups the varieties into three groups: long, medium and short grain (see examples below), using kernel length and/or width. Other classifications exist, such as that used by the Indian government which provides for 5 groups based on the length/length-width ratio of the kernel.

Three main chemical characteristics are used to measure the quality of rice. These include:

- Starch gelatinisation temperature – this determines the time required for cooking the rice
- Gel consistency – this indicates the tendency of the rice to harden on cooling
- Amylose content. A low amylose content is associated with sticky, moist rice.

In general, these characteristics are available across the different groups of grain types and account for differences in consumer preferences around the world. The IRRI's publication on *Grain quality evaluation of world rices* [4], gives a full review of selected grain quality characteristics of

milled rice from all countries producing more than 0.1 % of the world's rice. However, consumer tastes are changing, and in a highly competitive market with stringent quality requirements and some varieties prized above others, problems of adulteration can occur.

1.1.3. Examples of commonly-encountered rice types

Some commonly encountered rice varieties are described below.

Basmati rice: this is a long-grain, aromatic, non-glutinous rice. It is mainly grown in India and Pakistan. The approved varieties are detailed in section 1.2.1.3 below.

Jasmine rice: this is long-grain variety of fragrant rice. Also known as Thai fragrant rice, it is grown primarily in Thailand. Its fragrance results from the rice plant's natural product of aromatic compounds, of which 2-acetyl-1- pyrroline is the most abundant.

Italian rice: the most commonly used cultivar is **Arborio,** a short grain rice with a high amylopectin content making it ideal as a risotto rice. Other risotto rices include **Carnaroli**, **Vialone Nano**. The latter has been granted a Protected Geographical Indication under the EU, which stipulates that it can only be grown within the 24 municipalities of Verona.

Valencia rice: or "Arroz de Valencia" is a short grain rice is traditionally used in paella. It is grown in the autonomous community of Valencia and protected by a PDO quality label, which includes the rice varieties Senia, Bahia and Bomba. Other areas of rice production in Spain are Delta de Ebro and Calsparra.

Black rice: also known as purple rice is a range of rice types some of which are glutinous. It owes its colour to its high level of anthocyanins. It is used in a number of traditional cakes and desserts particularly in China.

Wild rice: is not in fact a rice but the seed of a type of wild grass (*Zinania aquatica*) which grows in the shallow lake area of North America.

Other rice descriptions that consumers may encounter include **brown rice**, the rice which has not had the bran layers and germ removed. It can apply to all grains whether short, medium or long.

1.1.4. Rice by-products

The main by-products of rice are rice straw, rice husks or hulls, and rice bran. Some of these are used as animal fodder and fuel in power stations. Rice bran, produced from the outer layer of brown rice grain, is used in vitamin mixes and cereals due to its high content in vitamin B6, iron and other minerals. Rice bran oil is also becoming a popular cooking oil.

1.1.5. Other rice-derived products

In addition to direct consumption, rice can also be further milled into rice flour, both brown and white, and is used in many Asian dishes and for making rice noodles. Other components such as starch and protein can also be extracted from the rice. Rice starch has a unique starch granule size [5] and is becoming increasing used as a natural, "gluten-free" ingredient in a number of food products including baby and infant foods. Rice protein or protein concentrate is obtained by separating the protein portion from the starch portion of the rice and used in the formulation of many pet foods. Rice "milk" is considered an alternative to cow milk for vegans or for intolerant people.

1.2. Current standards of identity or related legislation

1.2.1. ISO Standards

According to the ISO Standard 7301 [6], the following definitions apply:

Paddy/Rough rice: freshly harvested rice. The rice is first dried from approximately 20 % moisture content to about 14 %, and then cleaned of foreign material.

Husked rice: paddy rice from which the husk only has been removed. Also known as brown rice, it may be consumed as is or milled into white rice for consumption.

Milled rice: rice obtained after milling which involves removing all or part of the bran and germ from the husked rice. Milled rice is also referred to as polished rice.

Parboiled rice: rice, the starch of which has been fully gelatinized by soaking paddy or husked rice in water followed by a heat treatment and a drying process.

Glutinous rice, waxy rice: special varieties of rice (*Oryza sativa* L. glutinosa) the kernels of which have a white and opaque appearance. The starch of glutinous rice consists almost entirely of amylopectin. It has a tendency to stick together after cooking.

The ISO Standard also provides specification for physical and chemical characteristics, including accepted moisture content and the maximum contents of extraneous matter, defective kernels and other kinds of rice in husked and milled rice.

1.2.2. Codex Alimentarius

Codex Standard 198-1995 [7] applies to husked rice, milled rice and parboiled rice, all for direct human consumption, providing similar definitions to those in ISO Standard 7301 above. It also provides guidance on general quality factors, contaminants, labelling and packaging.

In particular, Codex Stan 198 provides specifications for long, medium and short grain rice, depending on whether the kernel length or kernel length/width ratio is used for the classification.

1.2.3. EU Regulations

1.2.3.1. Common Market Organisation, import tariffs and quotas

European Parliament and Council Regulation (EU) No 1308/2013 [8] provides the Common Market Organisation for rice, including market intervention and trade measures. It applies to the following products:

- Rice in the husk (paddy or rough)
- Husked (brown) rice
- Semi-milled or wholly-milled rice
- Broken rice
- Rice flour
- Rice groats and meal
- Rice pellets
- Flaked rice grains
- Rolled grains of rice
- Rice starch

Commission Regulation (EU) No 1272/2009 [9] lays down common detailed rules for buying-in and selling of agricultural products under public intervention. However, rice is only accepted into intervention if it complies with certain eligibility criteria (quality specifications), related to moisture content, milling yield, defects in the grains, miscellaneous impurities, grains of other rice varieties.

As regards trade with third countries, Commission Regulation (EC) No 1342/2003 [10] lays down specific rules for the system of import and export licences for cereals and rice. Following international agreements under WTO or bilateral negotiations, various Tariff Rate Quotas (TRQs) allow rice imports at low or even zero duty. These are detailed in Commission Regulation (EU) No 1273/2011 [11] which are reopened on 1 January each year and apply specifically to the country of origin of the imported rice.

In addition, for broken rice used in the production of infant foods, a specific tariff quota for 1000 tonnes at zero duty is available through Commission Regulation (EU) No 480/2012 [12].

1.2.3.2. Geographical origin labelling

Although Regulation (EU) 1169/2011 [13] establishes rules relating to the origin of foods in general, the labelling of rice in the EU is currently not mandatory. In 2014, FERM, the European Federation of Rice Millers, undertook a survey of major retailers in six Member States (Belgium, Germany, Netherlands, Portugal, Spain, UK) to assess the level of voluntary country of origin labelling. Of 678 products investigated, 41 % had some form of origin labelling, with 23 % of products specifically mentioning the country of origin [14].

1.2.3.3. Specific case of Basmati Rice from India and Pakistan

Commission Regulation (EC) No 972/2006 (last amended by Commission Regulation (EU) No 706/2014) [14] lays down special rules for imports of Basmati rice and a transitional control system for determining their origin.

A zero rate of import duty is granted to husked Basmati rice of the following 9 varieties originating from India or Pakistan:

- For India (8 varieties): Basmati 370, Basmati 386, Type-3 (Dehradum), Taraori Basmati (HBC-19), Basmati 217, Ranbir Basmati, Pusa Basmati and Super Basmati.
- For Pakistan (4 varieties): Kernel (Basmati), Basmati 370, Pusa Basmati and Super Basmati.

1.2.4. India (Approved Basmati rice varieties)

So far 29 varieties have been notified under the Indian Seeds Act 1966 and subsequent amendments. A detailed list of notified Basmati varieties as of 2017 are available on the APEDA (Agricultural and Processed Food Products Export Development Authority) at reference [15].

1.2.5. Thailand (Thai Hom Mali Rice)

The Thai National Committee on Agricultural Commodity and Food Standards have established a specific quality standard for Hom Mali Rice, the main rice crop grown in Thailand (Thai Agricultural Standard TAS 4000-2003) [16]. Varieties that have been certified by the Department of Agriculture, Ministry of Agriculture and Cooperatives, are the Khao Dawk Mali 105 variety and its derivative Gor Khor 15.

2. Authenticity issues

2.1. Identification of current authenticity issues

Both cultivar and cultivation area are major factors in determining the market price of rice. Hence, the main authenticity issues are the substitution of one variety or cultivar with another, or the mislabelling of the geographical origin of the rice.

2.1.1. Substitution or dilution of premium rice with cheaper varieties

Premium rice varieties such as Basmati and Thai Hom Mali have been the subject of adulteration with cheaper varieties.

The authenticity of Basmati rice depends on both geographical origin and cultivar. Basmati is the name used for a class of rice comprising a few defined varieties grown in the Haryana, Punjab and Uttar Pradesh regions of India and Pakistan. The highly favoured properties of Basmati such as its fragrance and flavour give it the status of one of the premium varieties of rice enabling it to sell for a premium price. Since it is difficult to visually distinguish different types of rice from each other, the adulteration of Basmati rice with other varieties has occurred.

As mentioned in section 1.2.5 above, the Thai government has protected two cultivars Kao Dawk Mali 105 and its derivative Gor Khor 15 of Thai Fragrant rice. A possible adulterant, Pathumthani 1 (another Kao Dawk Mali 105 derivative to be commercialised) and for which cultivation is not restricted to a certain region or season, is sold as a much cheaper price.

2.1.2. Mislabelling of risotto rice

A poor harvest of Arborio rice in Italy in the early 2000, which pushed prices up, led to the adulteration of this premium rice with cheaper varieties [17].

2.1.3. Other authenticity issues

Other issues include the addition of paraffin to rice [18] to give it its desirable translucent appearance and the use of artificial dyes to pass cheaper white rice off as black rice.

2.2. Potential threat to public health

Although passing off cheaper rice varieties as more expensive ones does not pose a particular risk to the consumer, the addition of adulterants such as the paraffin and synthetic dyes described above are obvious potential health hazards.

A particular case of public health concern involved the case of synthetic rice found in China, Indonesia, the Philippines, Singapore, India and Vietnam. The product causes serious disruption to the gastrointestinal tract and is potentially lethal if large quantities are consumed. The counterfeit material looks almost identical to rice grains but is generally made of potato starch mixed with a plastic that is generally found in packaging. In some cases, the plastic rice is mixed with regular grains, making it harder to detect [19]. The use of a handheld Raman spectroscopic device was proposed to the authorities in the Philippines to screen for plastic rice [20].

The most well-known case of adulteration involved rice-derived products and the addition of melamine and melamine-related products in rice protein concentrate. This occurred in 2007, when melamine and cyanuric acid were found in products labelled as rice protein concentrate being used in the production of pet food. These products had been added to increase the apparent protein content. The contaminated pet food led to the sickness and death in some cases of pet dogs and cats in the USA [21].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

3.1.1. Standard methods for quality control

The standard methods for the general quality control of cereals published by the International Association for Cereal Chemistry (ICC) are also applicable to rice (see section of wheat and related products above).

AACC International Approved methods for cereals are also applicable. In addition, AACCI has published methods to measure the gelatinisation and paste viscosity characteristics of milled rice flour [22], and to determine the apparent amylose content of milled rice [23], a rapid screening method applicable to milled raw, parboiled and precooked rices.

Rice flour and rice-derived products are often used in the manufacture of “gluten-free” food products. The AACCI also provides a standard method for the detection of gluten in rice flour [24]. The method uses a sandwich enzyme-linked immunosorbent assay (ELISA) kit with proprietary antibodies optimised to determine gluten levels less than 200 mg/kg in samples and is intended for the evaluation of samples with respect to a 20 mg/kg regulatory decision level.

3.1.2. Methods for the detection of melamine and related products

Following the serious incidents in which pet food and some of its ingredients were found to be contaminated with melamine and a related compound, cyanuric acid, a number of analytical methods were developed including both selective quantitative methods and rapid screening techniques. In 2009, the World Health Organisation (WHO) in collaboration with the FAO (Food and Agriculture Organisation, supported by Health Canada published an overview of methods for the analysis of melamine in foods and animal feed [25]. Of the many methods available, the US FDA’s Laboratory Information Bulletin describes an analytical procedure using GC-MS specifically for dry protein materials including rice protein [26].

3.2. Other commonly used methods

3.2.1. Biomolecular methods

One of the most important authenticity issues for rice is the mislabelling of premium varieties, or their substitution or dilution with cheaper ones. Analytical techniques based on DNA based markers are therefore the most suitable techniques for rice variety authentication.

3.2.1.1. Authentication of traditional Basmati rice

Traditionally Basmati adulteration was detected through the analysis of specific aromatic compounds, sometimes by simply smelling the rices after immersion in boiling water [27], or by more sophisticated chromatographic analysis [28]. However, techniques based on molecular markers have been shown to provide a far more accurate discrimination of Basmati, either from other varieties or from other cheap Basmati varieties obtained by crossing with Indica rice.

Of the methods developed for this purpose, work has focused on exploiting DNA based markers ([29] and references therein). Amplified Fragment Length Polymorphisms (AFLPs) have been found to be the most effective, with the maximum discriminatory power. A database of microsatellites for discrimination of different Basmati varieties has also been produced [30]. A more recent development makes use of HRM in Real-Time PCR to allow the analysis of microsatellites without capillary electrophoresis [31]. With this approach, melting curves of the amplified products can be differentiated and identification of heterozygote is possible, and two amplified products of the same length can be distinguished if different in the proportion of GC bases composition.

A recent review of methods for the detection and quantification of adulteration of rice using Basmati as a case study is given in reference [32].

As detailed in section 1.2.1.3. above, EU Regulations specify the Basmati rice varieties from both India and Pakistan that are granted a zero rate of import duty on presentation of an authenticity certificate based on DNA analysis. The UK-based Rice Association published a revised version of their Code of Practice in 2017 which provides an updated list of rice varieties that can be labelled as “Basmati”, with a tolerance not exceeding 7 % of non-Basmati varieties to take into account problems of seed impurity and other segregation issues at origin [33]. The Code of Practice refers to PCR-based methods described on the Food Authenticity Network website [34] designed to detect permitted Basmati varieties.

3.2.1.2. Authentication of Thai Fragrant Rice

As described above Thai Fragrant Rice can be adulterated with cheaper, non-approved varieties. Approved Hom Mali can be distinguished from Pathumthani using DNA Microsatellite fingerprinting, which can determine the quantity of each variety as well as the quantity of any other rice varieties present [35]. Adulteration with non-fragrant rice varieties can be confirmed by testing for a defect in the gene coding for the enzyme betainaldehyde dehydrogenase. Due to this mutation 2-acetyl-1-pyrolline in enriched, which is the characteristic aromatic compound found in Jasmine rice. This technique is also applicable to Basmati rice.

Other molecular markers have also been investigated as a means of authenticating Thai rice. For example, Sequence Characterized Amplified Regions (SCARs) based on previously identified Random Amplified Polymorphic DNA (RAPD) markers, have been shown to discriminate between aromatic and non-aromatic rice varieties [36]. The two SCAR fragments chosen for identification are present in DNA from non-jasmine rice. This can be useful when testing pure jasmine rice samples because the detection of the marker fragments indicates contamination.

3.2.1.3. Authentication of Italian rice varieties

RAPD markers have also been proposed to distinguish between Italian rice varieties [37]. An interesting feature of methods based on molecular markers is the low quantity of DNA required for analysis, which can be extracted even from a single seed. However, in this case the method was not applicable to parboiled rice samples, which undergo thermal treatment and which lacked the amplified fragments of high molecular weight required for the DNA analysis.

3.2.2. Stable isotope ratio analysis

The use of natural stable isotope abundance is becoming increasingly used as a geographical indicator to determine the provenance of food. The main requirement for using these methods to determine geographical origin is the existence of a comprehensive data base of authentic samples from the regions being authenticated. However, the number of studies, and associated data available in the literature, not to mention proprietary databases, have made these methods suitable for determining provenance in routine quality control.

A number of studies have investigated the potential of the stable isotopes of light elements carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$) and sulphur ($\delta^{34}S$), which reflect the plant's metabolism and its environment. Others have included data on heavy elements such as strontium ($\delta^{87}Sr$), which is linked to the geology of the cultivation area. C, N O and S stable isotope ratio measurements have been investigated to discriminate between the same rice cultivars, grown in China, Korea and the Philippines [38]. The same authors also showed that the parameters $\delta^{13}C$ and $\delta^{15}N$ could be used to distinguish between organic and conventionally grown rice in Korea [39].

The potential of stable isotope ratio analyses to verify geographical origin has been shown to be improved, not only by assessing the isotope fingerprint of several elements, but also by measuring isotope ratios in different parts of the plant. This approach has been taken in order to the increase the resolution of light element stable isotopes by investigating the superior spikelets (SS) and inferior spikelets (IS) in the rice panicle. The study involved a rice cultivar Daohuasiang from adjacent sites in Fujin and Wuchang in the Heilongjiang Province in China, with interesting results [40].

3.2.3. Multi-element analysis

Another approach to verify the geographical provenance of rice varieties is multi-element analysis. An example of this is the study to address the geographical traceability of "Arroz de Valencia", a specific rice variety covered by a Protected Denomination of Origin (PDO) label in Europe. The authors looked at thirty-two different elements determined in rice grains from Spain, Brazil, Japan, and India [41]. Linear Discriminant Analysis grouped the Spanish rice samples apart from samples from the other areas with a correct classification of 91.3 %.

A trace element approach together with a data mining technique known as Support Vector Machine (SVM) has been used to authenticate organic rice produced in Brazil [42]. The study looked at 19 different elements, resulting in a correct classification of 98 % of the organic rice samples. Interestingly, a correct classification of 96 % was obtained when only Ca and Cd were used.

3.2.4. Combined multi-element and stable isotope ratio analysis

Best results for geographical origin verification are obtained by combining multi-element and stable ratio analyses. An early study looked at rice samples cultivated in the USA, Europe and Basmati regions using nine key variables (carbon-13, oxygen-18, boron, holmium, gadolinium, magnesium, rubidium, selenium and tungsten) to discriminate geographical origin [43].

A more recent study of geographic authentication of rice has used combinations of elemental/isotopic composition analysis and chemometric techniques to distinguish between rice grown in six Asian countries. The major common variables responsible for differentiation in these models were $\delta^{34}S$, Mn and Mg [44].

Two major targets for geographical authentication are Basmati and Thai rice varieties. For the former, a combination of 10 rare earth elements ((La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Er, Yb) and the isotope ratio of strontium ($^{87}Sr/^{86}Sr$) were used as tracers for differentiating Indian Basmati rice from the other countries of origin[45]. Discrimination of Thai jasmine rice (Khao Dawk Mali 105) cultivated in five different regions was achieved using 9 elements (As, Mg, Cl, Al, Br, Mn, K, Rb and Zn) and stable isotopes $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ with 100 % correct classification [46].

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, analyte or parameter	Authenticity issue / information
Amylograph (AACCI Method 61-01.01)	Gelatinisation and paste viscosity	Characterisation of milled rice flour
Colorimetric determination (AACCI Method 61-03.01)	Apparent amylose content	Cooking and processing qualities
ELISA (Sandwich enzyme-linked immunosorbent assay)	Prolamin and glutelin proteins	Detection of gluten in rice flour
DNA based methods	DNA markers (AFLPs, microsatellites, SCARS, RAPD)	Discrimination of rice varieties
Stable isotope ratio analysis	Light element isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$)	Geographical origin
	Heavy element isotopes ($^{87}Sr/^{86}S$)	
Multi-element analysis	Various elements	Geographical origin

5. Conclusion

The main authenticity challenge in the future will be likely to concern the differentiation of rice varieties. There is currently a huge diversity of rice varieties available, with new varieties being developed all the time. Some of these varieties command premium prices because of their particular cooking characteristics or flavour profile. Others fall under negotiated trade tariff categories granting low or zero duty on imports. GM technology is being investigated for the biofortification (enhanced folate, zinc and iron content). A further example is Golden Rice [47], a variety of *Oryza sativa* that has been genetically engineered to biosynthesis beta-carotene, a precursor of vitamin A, in the edible parts of rice. This variety has been accepted as safe by a number of governments around the world but because of the stiff resistance to GM technology, the product is not yet available.

Today the most complete and comprehensive analytical tool for rice authentication is the combination of DNA-based methods to confirm variety together with stable isotope ratio analysis with multi-element analysis to verify provenance. In the future, technological progress particularly in the instrumentation used will greatly improve the ease-of-use of these techniques. A current

example is the use of modern sequencing, known as Next-Generation Sequencing (NGS), or high-throughput sequencing, which enables the analyst to sequence DNA and RNA much more quickly and cheaply than before.

Today's focus is shifting more and more towards this type of untargeted approach. Other techniques investigated in this respect include ^{1}H NMR, which has been studied as a means of discriminating rice from different regions in China [48], multi-platform MS-based metabolomics and multivariate analysis for the geographical origin [49] and a LC-MS untargeted approach to distinguish between organic and conventional rice [50]. These approaches offer the potential of rapid authentication methods or of a short-cut to identifying suitable markers for use in authenticity testing.

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