Cereals and cereal-based products

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


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].


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

<table>
<thead>
<tr>
<th>Cereal / cereal product</th>
<th>CODEX STAN</th>
<th>Definition</th>
<th>Moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couscous</td>
<td>202-1995</td>
<td>Product prepared from durum wheat</td>
<td>&lt;13.5 %</td>
</tr>
<tr>
<td>Durum wheat semolina and durum wheat flour</td>
<td>178-1991</td>
<td>Products prepared from grain of durum wheat by grinding or milling processes.</td>
<td>&lt;14.5 %</td>
</tr>
<tr>
<td>Maize (corn)</td>
<td>153-1985</td>
<td>Shelled grains of Zea mays indentata L. or Zea mays indurata L.</td>
<td>&lt;15.5 %</td>
</tr>
<tr>
<td>Oats</td>
<td>201-1995</td>
<td>Grains of Avena sativa and Avena byzantina</td>
<td>&lt;14.0 %</td>
</tr>
<tr>
<td>Wheat and durum wheat</td>
<td>199-1995</td>
<td>Wheat is obtained from the varieties of the species Triticum aestivum L.</td>
<td>Wheat &lt;14.5 % m/m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Durum wheat is obtained from varieties of the species Triticum durum Desf.</td>
<td>Durum wheat &lt;14.5 % m/m</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>152-1985</td>
<td>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</td>
<td>&lt;15.5 % m/m</td>
</tr>
</tbody>
</table>

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

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\(^1\) 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- Barley-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 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 (δ¹³C, δ¹⁵N, δ¹⁸O and δ³⁴S) together with strontium isotope ratio measurements (n(⁸⁷Sr)/n(⁸⁶Sr)), 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 (δ¹³C, δ¹⁵N and δD, alone or with ⁸⁷Sr/⁸⁶Sr) have also been used to identify the geographical origin of winter wheat in China [49]. A further study by the same authors determine δ²H 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 (δ¹³C, δ¹⁵N) wheat samples collected from adjacent states of India. Results obtained using δ¹³C showed good potential; the difference in the δ¹⁵N values from different states were not significant [51].

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4. Overview of methods for authenticity testing

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

<table>
<thead>
<tr>
<th>Analytical technique</th>
<th>Indicative data or analyte</th>
<th>Authenticity issue / information</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAGE (Polyacrylamide gel electrophoresis)</td>
<td>Wheat protein glutenin</td>
<td>Identification of wheat varieties</td>
</tr>
<tr>
<td>DNA detection (various techniques)</td>
<td>Species-specific markers</td>
<td>Detection of various species (barley, bread and durum wheat, oat, rye, maize, rice)</td>
</tr>
<tr>
<td>Polymerase Chain Reaction (PCR)</td>
<td>Specific primers for wheat, barley, rye</td>
<td>Detection of gluten-containing cereals in &quot;gluten free&quot; products</td>
</tr>
<tr>
<td>NIR spectroscopy</td>
<td>Protein content, hardness</td>
<td>Discrimination of species/ varieties</td>
</tr>
<tr>
<td>MIR spectroscopy</td>
<td>Lipid, cellulose/hemicellulose content</td>
<td>Discrimination of species/ varieties</td>
</tr>
<tr>
<td>NIR hyperspectral imaging</td>
<td>Morphological and spectral information</td>
<td>Discrimination of species/ varieties</td>
</tr>
<tr>
<td>Stable isotope ratio analysis</td>
<td>Light element isotopes ($\delta^{13}$C, $\delta^{15}$N, $\delta^{18}$O and $\delta^{34}$S)</td>
<td>Geographical origin</td>
</tr>
<tr>
<td></td>
<td>Heavy element isotopes ($\delta^{87}$Sr/$\delta^{86}$Sr)</td>
<td></td>
</tr>
</tbody>
</table>

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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