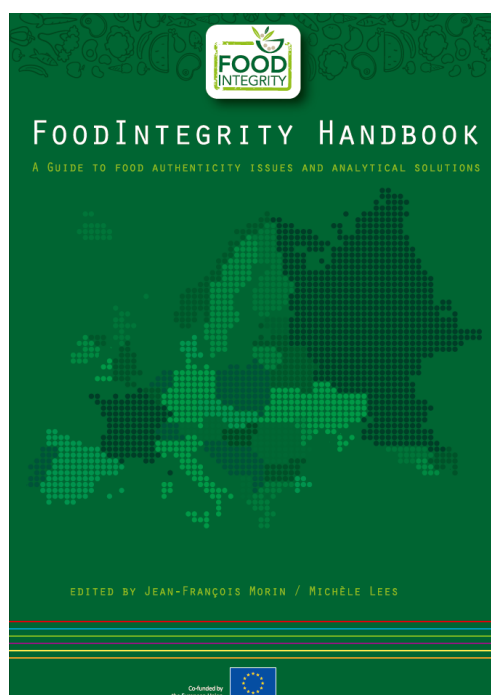# FOODINTEGRITY HANDBOOK

A GUIDE TO FOOD AUTHENTICITY ISSUES AND ANALYTICAL SOLUTIONS

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Coffee

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General overview of the product

The coffee tree is a tropical evergreen shrub classified under the genus *Coffea*, and part of the botanical family *Rubiaceae*. It grows between the Tropics of Cancer and Capricorn. Although more than 100 species exist in this genus [1], only two of them are of real economic importance for the production of the beverage coffee:

- *C. arabica*, called Arabica coffee: production area is mainly South and Central Americas, with the exception of Ethiopia, the country of origin for coffee;

- *C. canephora*, called Robusta coffee: most of the world's Robusta is grown in Central and Western Africa, parts of Southeast Asia and in Brazil.

The other species *C. liberica* (Liberian or Liberica coffee, or Excelsa coffee) is traded to a very limited extent. The share of Arabica fell from about 80 % of world production in the 1960s to around 60 % in the 2010s. Initially this was because of the strong growth of Robusta production in Brazil and parts of Africa, but more recently because of the emergence of Asia as the world's leading Robusta producing region [2].

On the world market, Arabica coffees attract the highest prices. Arabica trees are costly to cultivate because the ideal terrain tends to be steep and access is difficult. Also, because the trees are more disease-prone than Robusta, they require additional care and attention. Robusta is primarily used in blends and for instant coffees. The Robusta tree has the advantage of being able to withstand warmer climates, which enables it to grow at far lower altitudes than Arabica. Compared with Arabica, Robusta beans produce a coffee which has a distinctive taste and more caffeine.

Coffee is grown globally in around 70 coffee producing countries. In 2016/17, coffee production was 159.1 million bags (i.e. 9.5 millions of tonnes, each bag contains 60 kilograms of green coffee), from which 98.8 were Arabica and 60.4 Robusta. Brazil is the largest producer: its coffee sector contributes 35.2 % to the world's total coffee production. Vietnam is the second largest producer of coffee in the world, accounting for 16.8 % of global production. It is the main producer of Robusta. Colombia is the second-largest supplier of Arabica coffee after Brazil, with respectively 15 % and 46 % of the worldwide production. Indonesia is the world's second-largest exporter of Robusta. Ethiopia is the largest coffee producer in Africa. The European Union (EU) is the primary market, accounting for 40 % of the world's coffee bean imports, followed by the United States with 24 % [3].

A great variety of coffee products can now be purchased. International coffee trade is conducted almost exclusively in green coffee. However, consumers are nowadays offered roasted coffee beans, roasted and ground coffee, as well as liquid and dried coffee extracts (soluble coffee). Furthermore, coffee can be mixed with coffee substitutes, and also sold as roasted and ground blends or as dried extracts. Whole-bean roasted coffee may also be soaked with liquid flavouring agents to produce flavoured coffees. Finally, dried coffee extracts already containing milk solids (café au lait, cappuccino) exist on the market. Decaffeinated forms of each of these coffee products are also available.

The different varieties of coffee bean and the region where the coffee is grown may give rise to products of different qualities that are more or less popular with the consumer. This in turns leads to price differences on the market and the potential for adulteration or misrepresentation by a dishonest trader. A popular component of the Western diet, coffee is also an important commodity in international trade upon which the economies of a number of countries are particularly dependent. In 2010 the International Coffee Organization (ICO) estimated total coffee sector employment at about 26 million persons in 52 producing countries[4]. Thus, the coffee industry itself has devoted considerable time and effort to ensuring both the quality and authenticity of its product, and to developing suitable analytical techniques for this purpose.

In the last 30 years, the coffee market has seen the emergence of an increasing number of initiatives related to fair-trade and sustainability. Often marked with a label on the coffee packaging, these labels certify the sustainability of coffee production and the respect of smallholder producers by improving their conditions of trade (e.g. more equitable and more stable prices). In the coffee market, most extended programmes are UTZ Certified and the Rainforest Alliance, which merged early 2018, and the Max Haavelar Foundation. According to Fairtrade International, fair-trade coffee farmers produced an estimated 560 900 tonnes of coffee in 2015 (approximately 6 % of the worldwide production).

1. Product Identity

1.1. Definition of the product and manufacturing process

Green coffee may be produced by either a wet or dry process. The wet process involves washing the coffee cherries and transferring them to depulping machines which remove the outer skin and most of the pulp. This process leaves some of the pulp mucilage on the parchment shells which encase the coffee bean and this remaining mucilage is fermented and washed away with clean water. The beans are then dried and the inner husk known as 'parchment' is broken by rollers and removed. Further rubbing removes the film or 'silverskin' which closely adheres to the coffee bean. The dry process involves drying the fresh ripe cherries in the sun for up to three weeks. The dried coffee cherries are dehulled mechanically to remove the outer skin, pulp, 'parchment' and the 'silverskin' to leave the clean, naked, green coffee beans.

Coffee is usually traded as green coffee beans, a state in which they can be kept without loss of quality or taste. It is roasted and further processed in the purchasing country. Roasting brings out the aroma and flavour that is locked inside the green coffee beans. The roasting process involves the heating of the green beans at about 200 °C, followed by fast cooling to stop the process. Once roasted, coffee should be used as quickly as possible before the fresh roast flavour begins to diminish.

Instant coffee (soluble coffee) is also produced in the coffee growing countries and may be traded packed ready for retail sale or in bulk for re-packaging in the country of receipt for national consumption or for further export. Instant coffee is the dried water-extract of roasted, ground coffee. Roasted, ground coffee is placed into columns known as percolators through which hot water is fed in a counter-current process. The extract is further concentrated and may be traded in bulk as such or dried to produce soluble coffee solids. Instant coffee is sold in three forms, which relate to the drying process of the soluble coffee extract. Instant coffee powder is formed by spray drying the extract; coffee granules are formed by agglomerating this powder with steam; and freeze-dried coffee is formed by removing moisture from the extract under vacuum (sublimation) at much lower temperatures than spray drying. Freeze-drying is more energy expensive but is gentler on the product as less heat is applied to evaporate the water content. Consequently, freeze-drying is used for the finer and more expensive blends of instant coffee.

Decaffeinated coffee is produced from green beans. Three different extraction processes slightly differing from each other are in use in the industry. Basically a solvent is circulated around the water soaked beans and this causes the caffeine to be released. The most widely used and less costly is extraction with an organic solvent such as methylene chloride (also known as dichloromethane) or ethyl acetate, an ester that is found naturally in fruits and vegetables. The second method is water processing: water is used as a solvent to extract the caffeine. In the third approach, carbon dioxide in supercritical state under a pressure of 250 to 300 bar circulates through a bed of green beans. At the end of the process, caffeine content is usually reduced from 1–2 g% to 0.02–0.3 g% [5].

The ICO was formed in 1962 under the auspices of the United Nations. It is a inter-government body comprising 51 coffee importing and exporting countries which aims through international co-operation on trade in coffee to achieve economic diversification and development of coffee-producing countries, increased coffee consumption, price stabilisation and improved economic relations between coffee exporting and importing countries. The ICO is well regarded for its statistical services and its role as the international forum for discussing all issues affecting the world coffee market. It also co-ordinates a number of projects (most of which deal with marketing, pest/disease/quality problems or sustainability) and holds seminars on issues such as the environmental aspects of coffee production and the use of the futures market.

The International Coffee Agreement 2007 is the legal agreement which sets out how these objectives will be met [6]. In this document, the different coffee products are defined for harmonising data collection, statistics and trade among producing and importing countries. On the other side, the International Standard Organisation has issued a standard "Coffee and coffee products – Vocabulary" (ISO 3509:2005) [7] also for setting the definitions of coffee products. The same terms, such as "Roasted coffee" or "Decaffeinated coffee" can be found in both documents, but definitions are largely consistent. Roughly ICO definitions are more statistically oriented whereas ISO focuses more on quality and process.

Table 1: Comparison of the definition of coffee and coffee products between ICO and ISO

Product	ICO Definition	ISO 3509:2005 definition
Coffee	General term for the fruits (cherries) and seeds (beans) of plants of the genus *Coffea*, as well as products from these fruits and seeds in different stages of processing, such as dry cherry, parchment, green, roasted, ground, decaffeinated, liquid and soluble coffee	–
Green coffee	All coffee in the naked bean before roasting	Commercial term designating the dried seed of the coffee plant
Roasted coffee	Green coffee roasted to any degree and includes ground coffee	Coffee obtained by roasting green coffee
Ground coffee	–	Product obtained by grinding roasted coffee
Coffee extract	–	Product obtained exclusively from roasted coffee by physical methods using water as the only carrying agent which is not derived from coffee
Soluble coffee	Dried water-soluble solids derived from roasted coffee	–
Instant coffee **Dried coffee extract**	–	Dried, water-soluble product, obtained exclusively from roasted coffee by physical methods using water as the only carrying agent which is not derived from coffee
Spray-dried instant coffee	–	Instant coffee obtained by a process in which the coffee extract in the liquid state is sprayed into a hot atmosphere and formed into dried particles by evaporation of the water
Agglomerated instant coffee	–	Instant coffee obtained by a process in which the dried particles of instant coffee are fused together to form larger particles
Freeze-dried coffee **Freeze-dried coffee extract** **Freeze-dried instant coffee** **Freeze-dried soluble coffee**	–	Instant coffee obtained by a process in which the product in the liquid state is frozen and the ice removed by sublimation
Decaffeinated coffee	Green, roasted or soluble coffee from which caffeine has been extracted	Coffee from which caffeine has been extracted

1.2. Current standards of identity or related legislation

1.2.1. Standards from ISO and the German organisation DIN

In 1980 the International Standard Organisation (ISO) created a sub-committee on coffee within its Technical Committee on Food products (TC 34 / SC 15). The scope of its work is standardisation in the field of coffee and coffee products, covering the coffee chain from green coffee to consumption. Standardisation includes terminology, sampling, test methods and analysis, product specifications and requirements for packaging, storage and transportation. About 30 standards have been written and are available on the ISO website www.iso.org.

Among these standards, two of them have a special application to instant coffee authenticity. The standard "Instant coffee - Criteria for authenticity" (ISO 24114:2011) [8] specifies criteria for authenticity of soluble (instant) coffee. Its purpose is to identify adulterated soluble coffee, defined as a "product prepared by the co-extraction or the separate extraction of roasted coffee beans and of raw or roasted materials other than coffee beans, where the product is sold as pure soluble coffee and the addition of the non-coffee bean material is not declared on the label". The aim is to avoid incorrect declarations that adulterated products with cheaper coffee substitutes are 100 % pure soluble coffee. The standard focuses on two different parameters: total glucose and total xylose, the values of which must not exceed certain limits (respectively 2.46 % and 0.45 %) for the instant coffee sample to be declared authentic.

The standard is based on a standardised method looking at the carbohydrate content of the instant coffee, under the reference "Instant coffee - Determination of free and total carbohydrate contents - Method using high-performance anion-exchange chromatography" (ISO 11292:1995) [9]. The free and total carbohydrate profiles in soluble coffee are determined by anion exchange chromatography with pulsed amperometric detection (AE-PAD).

For roasted coffee, the German standard method "Analysis of coffee and coffee products - Determination of 16-O-methyl cafestol content of roasted coffee - HPLC-method" (DIN 10779:2011) [10] can also be used for authentication purposes. It is used to quantify the amount of 16-O-methylcafestol (16-OMC) in roasted beans originally, even if applications to green coffee beans and coffee brews have also been described in the literature [11]. It is based on the observation that 16-OMC is present exclusively in Robusta.

1.2.2. EU legislation

Beyond general regulations on food products, such as the General Food Law (Regulation EC 178/2002), the European Union has set up several regulations dealing with coffee products.

The general **EU Regulation 1169/2011** [12] on the provision of food information to consumers, combines two Directives into one legislation: 2000/13/EC - Labelling, presentation and advertising of foodstuffs, and 90/496/EEC - Nutrition labelling for foodstuffs. Among other themes, it deals with the labelling of origin. No specific rules have been set up for coffee, the general principle that "information shall not be misleading" applies. Voluntary provenance labels (i.e. indication where the green coffee was grown) can be made in relation to product claims such as '100 % Brazilian coffee'.

This regulation also stipulates a list of foods, including the following coffee products, which are exempted from the requirement of the mandatory nutrition declaration:

- Products covered by Directive 1999/4/EC of the European Parliament and of the Council of 22 February 1999 relating to coffee extracts and chicory extracts,
- Whole or milled coffee beans and whole or milled decaffeinated coffee beans.

Directive 1999/4/EC [13] relating to coffee extracts and chicory extracts determines which substances may be added during manufacturing of these products, lays down common rules concerning the packaging and labelling of such extracts and specifies the conditions under which particular designations may be used for some of these products. It simplifies the legislation previously regulated by Directive 77/436/EEC.

It defines coffee extracts as "the concentrated products obtained by extraction from roasted coffee beans using only water as the medium of extraction and excluding any process of hydrolysis involving the addition of an acid or a base".

In particular it stipulates that "coffee extract must contain only the soluble and aromatic constituents of coffee", apart from those insoluble substances which it is technically impossible to remove, and insoluble oils derived from coffee.

It controls the composition of three types of coffee extracts which differ in terms of their coffee-based dry matter content:

- Dried coffee extract: not less than 95 % by weight,
- Coffee extract paste: from 70 % to 85 % by weight,
- Liquid coffee extract: from 15 % to 55 % by weight.

Liquid coffee extract is specifically allowed to contain edible sugar provided the sugar content in the final product does not exceed 12 % by weight. The Directive does not permit coffee extract in solid or paste to contain any substance other than those derived from its extraction.

This Directive also states that the term *'decaffeinated'* can only be applied to coffee extracts which have an anhydrous caffeine content of not more than 0.3 % by weight of its coffee-based dry matter content.

The Directive does not cover roast and ground coffee.

According to **Directive 2009/32/EC** [14], solvents can be used for decaffeination of coffee in the European Union. There are maximum residue limits restrictions for the extraction solvents such as methyl acetate (20 mg/kg in the coffee), dichloromethane (2 mg/kg in the roasted coffee) and ethylmethylketone (20 mg/kg in the coffee). In the United States, according to the FDA, methylene chloride may be present in coffee as a residue from its use as a solvent at a level not to exceed 10 parts per million in decaffeinated roasted coffee and in decaffeinated soluble coffee extract (instant coffee) [15].

Directive 2002/67/EC [16] on the labelling of foodstuffs containing quinine,and caffeine sets up specific rules for protecting consumers and providing them with clear information on the presence of these compounds.

Where a beverage which is intended for consumption without modification, or after reconstitution of the concentrated or dried product, contains caffeine, from whatever source, in a proportion in excess of 150 mg/l, the following message must appear on the label in the same field of vision as the name under which the product is sold: "High caffeine content". This message shall be followed by the caffeine content expressed in mg/100 ml.

However, this obligation does not apply to beverages based on coffee, tea or coffee or tea extract where the name under which the product is sold includes the term "coffee" or "tea".

One Protected designation of origin (PDO) and one protected geographical indication (PGI) have been granted by the European Union:

- **Café de Colombia** (PGI) in Regulation (EC) 1050/2007 of 12 September 2007 [17];
- **Café de Valdesia** (PDO) in Regulation (EU) 2016/1043 of 15 June 2016 [18].

1.2.3. Further legislation and standards regulating quality

In 2004, the International Coffee Organisation (ICO) has introduced voluntary targets for minimum quality export standards for Arabica and Robusta under resolution 420 [19]. Thresholds for defects (not more than 86 defects per 300 g sample for Arabica, not more than 150 defects per 300 g for Robusta) and moisture (between 8 % and 12.5 %) are defined. The resolution aims to reduce the export of inferior beans. Coffee exporters from ICO exporting Members are advised to closely follow this resolution, except for the exports of specialty coffees which can be exempt from some of the targets as long as this is clearly mentioned in the Certificate of Origin.

Different producing countries have differing quality control systems and attach differing values to certain aspects of quality. Information is also available from coffee authorities in producing countries. Some specific coffee products are also produced in some countries with specific regulations, such as "Café torrefacto" in Spain and Portugal, which is a particular process to roast coffee beans with sugar addition.

The Coffee Quality Institute, an independent organisation founded originally in the scope of the Specialty Coffee Association of America (SCAA), has developed the Q Coffee System for Quality Control. It is an initiative to introduce international standards for coffee quality. It is based on trained and certified people in the supply chain (Q graders) who test coffee samples mainly from an olfactory and sensory point of view according to SCAA protocols. Each sample is tested by three local Q graders. Coffees that meet the standards for green, roasted, and cup quality are issued a Q Certificate. Companies who wish to promote and sell Q coffee may use the Q certification marks on their product packaging.

2. Authenticity issues

2.1. Identification of current authenticity issues

As mentioned previously the two coffee species of commercial importance are Arabica and Robusta. Producer countries and coffee traders are mainly interested in being able to recognise the country of origin of coffees, whereas food processors and regulatory authorities are interested in checking on compliance of the declared composition in commercial blends and in the detection of adulteration by addition of substitutes or other ingredients

2.1.1. Adulteration by addition of substitutes

Coffee substitutes may be added to the roasted and ground coffee if they are permitted and declared on the label. However if these substitutes are not correctly labelled or not declared at all then the consumer is being misled. In the case of roasted and ground coffee, inspection with a microscope may help to determine the presence of non-coffee material. Possible ingredients that may be found in ground coffee or coffee extracts include chicory, malt, figs, cereals such as corn and barley, caramel, starch, maltodextrins or glucose syrups as well as roasted or even unroasted coffee husks/parchment [20].

This problem is more important in soluble coffee extracts due to industrial processes which merge Arabica and Robusta beans before several steps like lyophilisation. Consequently, the detection of adulteration is no longer feasible by visual inspection, microscopy or other physical means traditionally used to identify impurities or "defects" which can be present in green or roasted bean or ground coffee.

2.1.2. Geographical origin

The taste and aroma of the coffee beverage is influenced by the country of origin, and even within a certain geographical region or "terroir" some differences are to be expected as a function of specific agro-climatic conditions. Some mild Arabicas of certain countries or regions attract high prices on the world market raising the possibility of substitution with coffee of cheaper origin or mislabelling. Geographic origin claims for middle range roasted coffees have begun to appear on supermarket shelves. Geographic origin authentication is becoming increasingly of interest.

In the majority of coffee-producing countries as well as among coffee traders there are tasters who can recognise the country of origin of the coffees they deal with, however none of them can identify reliably a large number of coffee origins. Moreover the opinions of these tasters are subjective, and in cases of arbitration, disagreements frequently occur between the tasters appointed by the parties involved. Analytical techniques for checking geographical origin allow for less subjective assessment.

2.1.3. Variety substitution and falsified proportion of inter-specific blends

Arabica are more expensive than Robusta coffees. Arabica is generally viewed as superior in cup quality to Robusta and is often sought exclusively by consumers. In these circumstances, addition of Robusta coffee to Arabica offers the possibility of commercial gain to an unscrupulous dealer and represents a fraud. Green and roasted beans normally may be recognised as Arabica or Robusta by visual inspection and specifically because of their specific organoleptic characteristics, however some washed Robusta coffees approach the taste quality of Arabica. As a result there is still room for non-declared substitution. On the other hand it is important to confirm if the proportions of Arabica and Robusta in the blend correspond to the price the consumer is paying.

Coffee breeding is still largely restricted to the two species, *Coffea arabica* and *C. canephora*, that dominate world coffee production. Efforts have been greatly intensified through breeding programmes in order to develop disease-resistant varieties, in anticipation of possible coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) epidemics that earlier in the century had devastated the *C. arabica* plantations in Asia and Africa. Serious threats of coffee berry disease (*Colletotrichum coffeanum* Noack sensu Hindorf) to Arabica coffee in the highlands of Eastern and Central Africa prompted a number of entirely new breeding programmes in the early 1980s particularly in Kenya and in Ethiopia [21].

Beyond the fight against diseases, coffee production is now threatened by climate change. Arabica coffee is highly sensitive to elevated temperatures, drought, pest, and disease. The forecasted consequences of climate change include changes in rainfall patterns, more frequent drought periods, and elevated temperatures, as well as a shift in geographical coffee growing regions, leading to environmental, economic and social threats in the coming years [22]. Since the second half of the 20th century, most breeding programmes implemented throughout the world (Brazil, Colombia, Kenya, Ethiopia, Costa Rica, Honduras, Tanzania, India, etc..) have transferred resistance to the main diseases by introgression of *C. canephora* chromosomal fragments carrying resistance genes. Today Arabica cultivars derived from *C. canephora* via the interspecific 'Timor Hybrid' (a spontaneous cross between *C. canephora* x *C. arabica*) represent more than 30-40 % of the Arabica trees cultivated around the world [23]. On the other hand, introgression via the Timor Hybrid may carry not only resistance genes but also other undesirable genes involved in a substantial drop in cup quality [24]. Consequently, complex and long term genetic selections have been performed to eliminate these undesirable organoleptic properties, while keeping the plant resistance to diseases.

Coffee buyers or roasters are paying more and more attention to the cultivar of the products they buy. If some introgressed cultivars are preferred because of specific properties, coffee buyers want to check if purchased coffee batches actually originate from the expected species. Secondly it has been shown that introgression can have a negative impact on the cup quality of cultivars derived from the Timor Hybrid. Consequently, coffee buyers or roasters may wish to assess whether the coffee they are purchasing comes from introgressed varieties [25]. Finally it has been demonstrated that the variety characteristics are not stable from one harvest to the next making it necessary to use at least two harvest dates for each variety [26]. Therefore there might be a concern about procurement quality and stability in time.

2.1.4. Counterfeiting of well-known brands of coffees

Some coffees have achieved a special reputation and notoriety based upon their rarity and overall flavour. Jamaican Blue Mountain and Tanzanian Peaberry are notable examples. As such they command a premium price [27]. Other examples are civet coffees, especially the Indonesian Kopi Luwak coffee. Kopi Luwak coffee is produced from beans processed in the digestive tract of the indigenous palm civet (Luwak) and then harvested. The action of microorganisms and enzymes gives this coffee a specific taste which is highly valued by consumers. Annual production of Kopi Luwak is estimated to be lower than 250 kg in 2004 [28] and the price is about USD 200 / lb (approximately more than EUR 500 per kg) [29]. An important concern related to the price gap between civet and regular coffees is the growing attempt of fraud involving illegal mixture of cheaper coffee into premium civet coffee. This may be even considered as counterfeit in this case.

2.2. Potential threat to public health

Coffee has been known to have both beneficial and harmful effects upon health. Coffee adulteration may therefore have harmful health consequences. In case of substitution of decaffeinated coffee by genuine coffee, people suffering from caffeine dependency (caffeinism) and who want to avoid caffeine may be misled. This is also the case of pregnant and breastfeeding women who are recommended to limit coffee consumption during pregnancy, because excessive caffeine consumption has been linked to stunted foetus development [30]. Caffeine intakes from all sources up to 200 mg per day consumed throughout the day is considered to raise no safety concern.

Another safety concern has arisen about the solvents used for decaffeination of coffee. In the early 20th century, benzene, known to cause severe illnesses when inhaled, even in small amounts, has been widely used for this application. Today, coffee manufacturers have switched to safer decaffeination methods, though many still use synthetic chemicals such as ethyl acetate (even if naturally found in some fruits) and methylene chloride (commonly used in industrial applications) to strip away caffeine. Even if authorities like the FDA or the European Commission have promulgated regulations that require solvent levels, especially methylene chloride, to be below specified thresholds in decaffeinated coffee [14,15], this question remains controversial. In organic coffee, chemical solvents (e.g. methylene chloride) are not permitted for decaffeination, but the water method or the supercritical carbon dioxide method may be used [2].

3. Analytical methods used to test for authenticity

3.1. Officially recognised methods

A few standardised methods have been developed to check the authenticity of coffee.

3.1.1. Detection of adulteration with carbohydrates

In the standard ISO 11292:1995 "Instant coffee - Determination of free and total carbohydrate contents - Method using high-performance anion-exchange chromatography" [9], the free and total carbohydrate profiles in soluble coffee can be determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The HPAEC-PAD procedure enables the determination of all major carbohydrates present in soluble coffee in a single run.

Using this analytical method, fraudulent addition of cheaper coffee substitutes in commercial soluble coffee can be detected. High levels of total glucose and total xylose are good indicators of adulteration. High levels of total xylose indicate the presence of coffee husks or parchments, whereas the presence of cereals or caramelized sugar is detected by the very large amounts of total glucose. This authenticity checking procedure has been officially approved for publication as an ISO international standard ISO 24114:2011 "Instant coffee - Criteria for authenticity" [8]. Total xylose and total glucose levels of 2.46 % and 0.45 % respectively are proposed by ISO as upper limits, above which a soluble coffee should be considered as adulterated. The developed method has been tested on more than 1.000 samples [31]. The procedure has also been officially adopted as first action (Method 995.13) by the Association of Official Analytical Chemists (AOAC).

According to Thorburn Burns et al. [28], this method can also be applied to roasted ground coffee. If a specific adulterant is sought, comparison has to be made between the coffee sample and ground roasted coffee spiked with the sought adulterants.

3.1.2. Determination of substitutions with the caffeine content

The principle of the method ISO "Coffee and coffee products - Determination of the caffeine content using high performance liquid chromatography (HPLC) - Reference method" [32] is a water extraction of caffeine followed by detection and quantification by HPLC with UV detection. It can be applied to green coffee; roasted coffee; soluble coffee, regular and decaffeinated; and mixed instant coffee products (e. g. coffee/chicory mix or cappuccino-type coffee drink). The level of caffeine, usually between 1 and 2 g% and also a little bit higher in Robusta coffees than in Arabica can indicate substitution of coffee by other ingredients like husks or parchment.

3.1.3. Species determination with 16-O-methylcafestol content of roasted coffee

Roasted coffee is subject to commercial fraud, because the high-quality *C. arabica* species, described as 100 % Arabica, is often mixed with the less expensive *C. canephora* var. Robusta. A German standard DIN 10779:2011 "Analysis of coffee and coffee products – Determination of 16-O-methylcafestol content of roasted coffee – HPLC method" [10], being based on HPLC measurements for the specific determination of 16-O-methylcafestol (16-OMC), has been accepted. It is quite time expensive in sample preparation phases, even if at the end the instrument required (HPLC-UV) can be considered cheap with respect to other analytical systems. This method is tested for a mass fraction of 50 mg to 300 mg 16-OMC content per kg of roasted

coffee. It is based on the observation that 16-OMC is present exclusively in Robusta, whereas other, more abundant diterpenes, such as cafestol and kahweol, cannot be used for this discrimination.

3.2.   Other commonly used methods

3.2.1.   Detection of adulterations

Beyond the analysis of the carbohydrate profile (cf. § 3.1.1), other analytical techniques are commonly used for detection of adulteration with cheaper ingredients.

Real-time PCR has been shown to be an alternative to chemical methods for identification of diluents. DNA sequences corresponding to the endogenous genes for coffee, barley, corn and rice have been selected for amplification. To verify the applicability of the method, 30 commercial samples obtained in different countries were evaluated. Barley, corn and rice have been actually detected in different samples [33].

Chromatographic or spectrometric techniques followed by statistical models have been described in the literature for this application. UV-vis spectroscopy and the Successive Projections Algorithm for variable selection in association with Linear Discriminant Analysis (SPA-LDA) showed complete classification in both training and test sets (102 samples) [34]. Near Infra-Red (NIR) spectroscopy has also been widely studied in this purpose for roasted ground coffee. A study based on 165 aqueous extracts of ground roasted coffee samples employed Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). A Linear Discriminant Analysis classification model provided complete discrimination between roasted coffee, pure adulterants (corn and coffee husks) and adulterated coffee samples [35]. However these proofs of concept are not yet available in routine testing.

In the case of soluble coffee, analytical methods by NMR exist for detection of substitutions with ingredients such as chicory, or mislabelling of coffee / chicory proportions in commercial blends [36,37]. The presence of chicory in soluble coffee and conversely can be quantified at 10 % in aqueous solution by NMR.

3.2.2.   Authentication of the geographic origin

Several techniques have been explored by researchers in their attempt to discover a method, or combination of methods, to authenticate the origin of any green or roasted coffee samples with the highest degree of confidence possible.

3.2.2.1.   *Metabolomic profile with spectroscopic methods*

One potential approach to the problem of geographical origin involves the use of spectroscopic methods such as nuclear magnetic resonance (NMR) and near and mid-infrared techniques. They have been used to measure spectroscopic "fingerprints" of known samples to which spectra of unknowns are compared using a variety of statistical measurements for classification purposes. These techniques generally have the advantage of speed, relatively simple sample preparation requirements and are often non-destructive.

Multivariate data analysis of the phenolic and methylxanthine profiles obtained by liquid chromatography coupled with UV spectrophotometry provided preliminary results that showed their potential for the determination of the geographical origin of green coffees. Classification

models correctly identified all authentic Robusta green coffee beans from Cameroon and Vietnam and 94 % of those from Indonesia. Moreover, PLS-DA afforded independent models for Robusta samples from these three countries with sensitivities and specificities of classifications close to 100 % and for Arabica samples from America and Africa with sensitivities of 86 and 70 % and specificities to the other class of 90 and 97 %, respectively [38].

Using both ^{1}H-NMR and ^{13}C-NMR spectroscopy, it has been shown that metabolite levels in coffee were significantly different between the Arabica and Robusta species, and secondarily influenced by geographical origins [39]. OPLS-DA models performed on ^{1}H-NMR data led to a clear separation of samples according to their origin: fatty acids, chlorogenic acids and lactate and finally acetate and trigonelline were shown to be the main compounds characterising the American, African and Asian samples respectively. The analytical approach presented here confirmed the potential of joint NMR analysis and statistical treatment in coffee authentication [40]. Classification models were built on aqueous NMR profiles allowing the distinction of 192 coffees on countries or continents of origin [41]. More precisely, 50 samples of Colombian have been differentiated from 22 Asian, 12 African and 108 other American origins. Although the discrimination was based on the global fingerprint, fatty acids, acetate and caffeine were identified to having a particular part in the differentiation. However, some impacts of roasting processes were observed on spectral profile as well as the post-harvest processes, the ripening periods and the year of harvest.

NIR spectroscopy has also demonstrated its potential in geographical origin authentication. Fourier transform infrared spectroscopy (FTIR) following solvent extraction permits examination of molecular variation to distinguish degrees of roast and country of origin, as between Columbia, Costa Rica, Ethiopia and Kenya [42]. Near-infrared spectroscopy (NIR) has been used to distinguish geographic origin and genotype of samples grown in Brazil [43].

3.2.2.1. *Isotopic ratios*

The possibility of using the isotopic ratio of caffeine to distinguish between geographical origins was investigated a few decades ago. Isotope ratio mass spectrometry (IRMS) was used to determine the ^{13}C/^{12}C and ^{15}N/^{14}N isotope ratios and Site-Specific Isotopic Fractionation - Nuclear Magnetic Resonance (SNIF-NMR) for the ^{2}H/^{1}H ratio [44]. However it was not possible to discriminate within the African or the American group. Another study also reported the use of ^{13}C/^{12}C, ^{2}H/^{1}H and ^{18}O/^{16}O ratios of the caffeine to check origins [45]. In addition using the carbon and nitrogen isotopic ratios of caffeine it is possible to fully discriminate plant origins from synthetic ones.

Some studies were performed directly on green coffee beans using multi-isotope analysis by IRMS associated with elemental analysis (EA). A study applied on 68 green coffee beans has demonstrated the potential of the combination of δ^{13}C (VPDB), δ^{15}N (VAIR), δ^{18}O (VSMOW) and percentages of carbon and nitrogen in the discrimination of 20 different geographic origins distributed over Central America, Pacific, South America, Africa, Asia and Oceania [46]. Another study was applied to 54 samples of roasted coffee beans of 20 different countries of origin [47]. This second work combined stable isotope analysis by IRMS, Elemental Analysis by ICP-MS and an analysis of δ^{13}C of extracted caffeine. It has demonstrated to some extent the potential of δ^{13}C and δ^{15}N in the discrimination of coffees from Africa, Asia and Central / South America. Moreover δ^{13}C, δ^2H and δ^{18}O combined with 5 elements (Ca, Ti, Fe, Ni, Zn) could discriminate all the considered origins at 77 %.

As a conclusion, the direct multi-isotope analysis of green or roasted coffees (after grinding) is also possible for a routine control of declared origins, provided that suitable databases are available.

3.2.2.2.   *Elemental analysis*

Element-specific techniques, especially inductively coupled plasma emission spectroscopy (ICP-OES), have been used to examine the trace element composition of coffee samples and have shown interesting results.

In a survey including the major growing areas worldwide (Brazil, Ethiopia, Colombia, India, Mexico, Honduras, Guatemala, Papua New Guinea, Kenya, Cuba, Timor, Mussulo and China), the variation in trace element composition has been characterised and compared [48]. These mineral profiles have also been used to differentiate coffee origins. Intercontinental and inter-country discrimination between the major world coffee producers were achieved by applying canonical discriminant analysis. Manganese and calcium were found to be the best chemical descriptors for origin. This conclusion is consistent with the results obtained on green coffee by Krivan et al., who analysed green Arabica coffees from eight different countries for twenty elements and found manganese to be the best suited element for origin discrimination [49].

Although much attention has been given to patterns of amounts of trace elements, this technique is not considered as robust enough by some authors due to the possibility of perturbations, for example from the use of fertilizers [28].

3.2.2.3.   *Volatile compounds*

Chemical profiles of volatile compounds have been studied to determine the geographical origin of coffee. For instance changes in volatile components analysed by direct injection headspace analysis by proton transfer reaction-time of flight mass spectrometry (PTR-TFMS) enable the distinction between beans from Ethiopia, Columbia, Brazil and India [50].

An HS-SPME–GC–TOFMS methodology was developed by an academic laboratory for the purposes of verifying its capability in terms of tracing back the coffee samples to their production area. Acquired data related to naturally volatile and semi-volatile analytes from 47 samples was submitted to principal component analysis and the corresponding geographical origin discrimination of coffee from South and Central America, Africa and Asia was successfully established [51].

However, many factors, such as the origin and the type of the coffee beans, roasting time and temperature, and the degree and method of roasting, affect the resulting volatile profile. Environmental factors like temperature during seed development also influence the sensory profile, and consequently the volatile profile [52]. The variability of volatile constituents in coffee caused by the different parameters do not appear to favour the volatile approach for the identification of origin in roasted coffee samples.

3.2.3.   Determination of Arabica and Robusta blends

3.2.3.1.   *NMR profiling*

The need related to coffee species determination is first to discriminate between Arabica and Robusta species and secondly to determine the proportion of Robusta and Arabica in commercial blends.

The verification of species authenticity was well established in NMR spectroscopy on the lipid fraction using the combination of two markers roughly specific to one species: 16-OMC for Robusta and kahweol for Arabica [23]. Up until recently, it was believed that 16-OMC is exclusively present in Robusta. This compound was therefore considered as an adequate marker in the

differentiation of Arabica and Robusta coffees. Indeed, the reference method DIN 10779:2011 [10] has proven the existing correlation between the 16-OMC concentration and the Robusta rate in Arabica roasted coffee. In parallel, kahweol was shown to be a key compound in species differentiation and was considered as a marker of Arabica species, although this compound is structurally very close to cafestol, compound present in both species.

Blend compositions were determined by ^{1}H-NMR spectral fingerprints with a high accuracy for 56 mixtures in aqueous solution using Orthogonal - Partial Least Square (OPLS) regression models [53]. This NMR method was proven to be a substitute for the official method because it requires only limited preparation, thus avoiding the loss of analytes. It was also shown that this technique could reach low limits of detection and quantification (5 and 20 mg/kg, respectively). This performance is adequate to detect the presence of Robusta at percentages lower than 0.9 % and down to 0.2 %, thus lower than the official method by HPLC (about 2 %) [11]. Furthermore, a recent paper has proven the presence of 16-OMC, a marker of Robusta, in ground roasted Arabica coffee in the order of 1-2 % [54]. Consequently the limit of quantification for Robusta content must be defined at 5 % and 10 % respectively in roasted and green Arabica coffee, in order to avoid false negative results. Moreover, this recent paper detected 2 doubtful market samples of Arabica coffee with adulterations at levels up to 30 % (w/w) in a panel of 60 retail purchased coffees using a limit of detection at 1 % and of quantification at 4 % [54].

3.2.3.2. NIR spectrometry

A near infrared spectroscopy signature, acquired over a set of harvests by keeping the most heritable zones of the spectrum, can therefore effectively characterize a coffee variety [26]. In a set of 191 roasted coffees from both pure Arabica and Robusta varieties and blends varying the final Robusta content from 0 to 60 % (w/w), classification models were built using NIR spectroscopy with Direct Orthogonal Signal Correction (DOSC) pre-processing method. It has been demonstrated that classification between pure Arabica, Arabica-Robusta blends and pure Robusta could be achieved.

3.2.3.3. Chemical compounds

The lipid content of Arabica coffee beans averages some 15 %, whilst Robusta coffees contain much less, namely around 10 % lipid. By Principal Component Analysis, oleic, linolenic, linoleic, and myristic acids used as chemical markers obtained by capillary gas chromatography were demonstrated as useful for differentiating varieties [55]. Six fatty acids were also analysed by Linear Discriminant Analysis (LDA) for a clear discrimination between Arabica and Robusta, green and roasted, coffee samples. Total monounsaturated (MUFA) and saturated fatty acids (SFA) could be used to determine amounts of Arabica and Robusta in a coffee blend [56].

Bertrand et al. compared the effectiveness of three chemical families, namely, chlorogenic acids, fatty acids, and minerals, for the discrimination of Arabica varieties (traditional *versus* modern introgressed lines) and potential *terroir* within a given coffee-growing area [57]. Although minerals provided an excellent classification of three locations under study, they were useless for Arabica variety discrimination. Chlorogenic acids gave satisfactory results, but fatty acids clearly offered the best results for the determination of both varieties and environments, with very high percentages of correct classification (79 and 90 %, respectively).

Roasted Arabica and Robusta coffees differ in their aroma as a consequence of their different chemical composition. Robustas show (due to their high content of free amino acids and chlorogenic acids) significantly higher concentrations of pyrazines, phenols and phenol ethers than Arabicas. Direct correlations were established between individual amino acids of green coffee and

aroma compounds which are formed during roasting. Arabicas contain (due to their high sucrose content) considerably higher amounts of steam-volatile furans, hydroxymethylfurfural and some aliphatic sugar degradation products than Robustas [58]. In a recent paper, a comparison between GC-C-IRMS, GC-MS, and ^{1}H-NMR was carried out to discriminate coffees from Colombia versus nearby countries (Brazil and Peru). According to the authors, results show that the quality of the classifiers depends mainly on the number of variables included in the analysis, which does not favour GC approaches [59].

3.2.3.4. DNA-based methods

Identification of Arabica and Robusta coffee species, as well as the quantification of their relative proportion in blends were performed by High Resolution Melting (HRM) analysis on green and roasted coffee products [60]. For a more sensitive detection method, chloroplastic rather than nuclear genetic variations were targeted, leading to the selection of 24 SNPs.

3.2.4. Detection of introgressed varieties

Introduction of new hydride varieties mostly induces an increase in the variability of the Arabica species, making differentiation between the Arabica and Robusta species more and more difficult for the analyst. For this purpose, chemometric approaches based on spectral profiles obtained by NMR or IR screening are being increasingly developed. They enable the extraction and combination of several species-characteristic signals from substantial datasets of coffee spectra. Consequently, coffee buyers or roasters could assess whether the coffee they are purchasing comes from traditional or introgressed Arabica varieties.

A chemometric method Independent Components - Discriminant Analysis (IC-DA) was applied to the ^{1}H-NMR fingerprints of lipophilic extracts from 272 authentic green coffees. Some signals of terminal methyl group of the fatty acid chains were identified as possible markers for the distinction between introgressed and native Arabica green coffee [61].

The NIR spectroscopy has also demonstrated its potential to be used to detect introgression in *C. arabica* cultivars on a dataset composed of 62 samples from Nicaragua and 61 from Costa Rica [25]. Moreover, particular metabolites were also identified such as fatty acids and caffeine, but also chlorogenic acids.

3.2.5. Authentication of coffee cultivars

Visual inspection can authenticate green coffee species and varieties, but after roasting, and particularly after grinding, this distinction becomes very difficult due to the morphological changes of beans induced by the high temperatures. Therefore, more sophisticated techniques have been developed, mainly based on genomics approaches, to check cultivars of roasted coffee, especially after grinding.

PCR amplification techniques are generally sensitive, reproducible and routinely available in testing laboratories. They can provide results even when testing very small amounts of degraded DNA as in the case of roasted coffee [62]. They have been effectively employed in the identification of roasted coffee species. An approach based on amplified fragment length polymorphism (AFLP) and simple-sequence repeats (SSRs) has shown to be useful for calculating genetic distance among 15 Arabica varieties from Yemen [63]. Applied to coffee species authentication, the potential of 33 SSR markers was assessed in 24 accessions of the Coffea genus [64]. The analysis included six Arabica (*C. arabica*) accessions, five Robusta accessions (*C. canephora*), three Híbrido de Timor (*C. arabica* x *C. canephora*), three Triploids (*C. arabica* x *C.*

racemosa) and one Racemosa (*C. racemosa*) accession. Six leaf rust resistant Arabica were also included. Authors concluded that that it is possible to use these SSRs for coffee variety identification. Single Nucleotide Polymorphism (SNP) has also been studied for identification of coffee germplasm with good results. A panel of 180 SNPs has been validated on 25 *C. arabica* and *C. canephora* accessions from Puerto Rico [65]. All the Robusta accessions were differentiated, as well as 10 out the 12 Arabica accessions (the 2 remaining ones were considered as synonymous).

All these tools are available for coffee players to assist in coffee germplasm management, quality control of planting material propagation, coffee cultivar authentication and protection of varietal rights in the international coffee community.

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
NMR profiling	^{1}H NMR spectrum	Arabica-Robusta proportion in blends (green & roasted coffee) Geographical origin Adulteration with cheap ingredients Chicory content confirmation Identification of introgressed varieties
NIR spectroscopy and profiling		Substitution with cheaper ingredients (coffee husks, parchments) Geographical origin Arabica-Robusta proportion in blends Identification of introgressed varieties
UV-vis spectrometry	Whole spectrum	Substitution with cheaper ingredients (coffee husks, parchments) Geographical origin of green coffee
HPAEC-PAD	Carbohydrates	Substitution with cheaper ingredients (coffee husks, parchments, cereals, sugar)
HPLC	Caffeine	Substitution with cheaper ingredients (coffee husks, parchments)
HPLC	16-O-Methylcafestol	Dilution of Arabica with Robusta
HPLC	Chrologenic acids	Identification of varieties
SNIF-NMR and IRMS	^{13}C/^{12}C and ^{15}N/^{14}N, ^{2}H/^{1}H, ^{18}O/^{16}O, isotope ratios	Geographical origin Naturality of caffeine
ICP-OES, ICP-AES	Trace elements (minerals) notably Mn and Ca	Geographical origin Arabica-Robusta proportion in blends Identification of varieties
Capillary GC	Lipid content	Arabica-Robusta proportion in blends Identification of varieties
GC-MS	Volatile compounds	Geographical origin Arabica-Robusta proportion in blends Specialty coffee authentication
Real-time PCR	Endogenous genes	Dilution with barleycorn and rice
SSR fingerprinting	Genome	Varietal identification Arabica / Robusta proportion in blends
SNP fingerprinting	Genome EST transcriptome	Varietal identification

5. Conclusion

Coffee authentication is a major concern for the coffee sector. The product itself, once roasted, ground or processed as instant coffee, can be easily adulterated. Furthermore coffee is one of the most appreciated and valued food commodities. Extensive research has been carried out on coffee authentication over the last few decades with the results that robust authentications methods are now available for the industry throughout the supply chain in order to ensure that genuine products are delivered to consumers.

The problem of determining the proportion in blends or the adulteration of Arabica with Robusta has been addressed and there are techniques that provide a good estimation of mixtures. However, under pressure of changing climate conditions, new varieties are being created by breeding Arabica and Robusta cultivars, for instance. Current differentiation between these two species is becoming more and more complex. New knowledge is needed in the future to ensure accurate results and to avoid false positives.

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