Current strategies to guarantee the authenticity of coffee

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Abstract: As they become more health conscious, consumers are paying increasing attention to food quality and safety. In coffee production, fraudulent strategies to reduce costs and maximize profits include mixing beans from two species of different economic value, the addition of other substances and/or foods, and mislabelling. Therefore, testing for coffee authenticity and detecting adulterants is required for value assessment and consumer protection. Here we provide an overview of the chromatography, spectroscopy, and single-nucleotide polymorphism-based methods used to distinguish between the major coffee species *Arabica* and *Robusta*. This review also describes the techniques applied to trace the geographical origin of coffee, based mainly on the chemical composition of the beans, an approach that can discriminate between coffee-growing regions on a continental or more local level. Finally, the analytical techniques used to detect coffee adulteration with other foods and/or coffee by-products are discussed, with a look at the practice of adding pharmacologically active compounds to coffee, and their harmful effects on health.

Keywords: *Arabica* and *Robusta* varieties, geographical origin, adulterants, chromatographic techniques, spectroscopic techniques.

Introduction

Coffee is a beverage with a distinctive taste and aroma made from ground roasted coffee beans. Due to its aromatic flavor and the beneficial effects of caffeine and other bioactive components, millions of people consume coffee every day. The world produces 6.3 million tons of coffee per year in about 60 tropical and subtropical countries (mainly, Hawaii, Jamaica, Ethiopia, Kenya, Brazil and Vietnam), some producing coffee as their main agricultural export. The coffee plant belongs to the *Coffea* genus of the Rubiaceae family, which has more than 100 species, although most of the coffee consumed is produced from *Coffea arabica* (*Arabica*) and *Coffea canephora* (*Robusta*) (Núñez et al. 2020).

The composition of green coffee beans is dominated by carbohydrates (~60% dry weight) and lipids (8-18%), with a minor amount of proteins, peptides, and free amino acids (9–16%) (Ludwig et al. 2014). The phytochemical profile of green coffee beans is complex, with over 1000 different chemical classes, including diterpenes (cafestol and kahweol), methylxanthines (e.g., caffeine, theobromine, and theophylline), nicotinic acid (vitamin B3), and trigonelline (Jeszka-Skowron, Zgoła-Grześkowiak, and Grześkowiak 2015). For years, coffee has been valued for its stimulating effect, associated mainly with caffeine (Butt and Sultan 2011; George, Ramalakshmi, and Mohan Rao 2008). However, it is now known that coffee contains many other bioactive components with valuable health-promoting properties. Coffee is rich in antioxidant substances such as phenolic compounds, the most abundant being ellagic, caffeic, and chlorogenic acids (Butt and Sultan 2011; George, Ramalakshmi, and Mohan Rao 2008). Studies have attributed many potential health benefits to coffee intake, including the prevention of several chronic and degenerative diseases, such as cancer, type 2 diabetes, cardiovascular conditions and Parkinson's disease (Esquivel and Jimenez 2012; Ludwig et al. 2014; George,

Ramalakshmi, and Mohan Rao 2008). Among the bioactive compounds responsible for these effects, polyphenols are the most important (Bułdak et al. 2018). Chlorogenic acid, the major polyphenol of coffee, is reported to have antibacterial, antifungal, antiviral, antioxidant, and chemo-protective properties (Bharath, Sowmya, and Mehta 2015; Hayakawa et al. 2020). Furthermore, caffeic acid exerts anticancer effects through the inhibition of DNA methylation and prevention of tumorigenic processes (Yu et al. 2011). Coffee polyphenols have also demonstrated potential anti-obesity effects and they can improve metabolic risk factors such as hypertension, abdominal obesity, and hyperglycaemia (Ohishi et al. 2021; Gökcen and Şanlier 2019).

The chemical profile, and therefore the antioxidant characteristics of coffee, can vary depending on the origin, variety, degree of roasting, and storage conditions, among other factors (George, Ramalakshmi, and Mohan Rao 2008; Herawati et al. 2019). The frequent and diverse adulteration practices in coffee production can involve the quality of the coffee beans (substitution by beans of other species or geographical origin, or defective beans), or the addition of external agents (for example, coffee husks and stems, soybeans, maize, barley, brown sugar), strategies that reduce production costs and increase profits from the final product (Toci et al. 2016).

For the consumer, flavor is what matters most in a high-quality coffee, which is described as having a balanced combination of body, aroma and flavor without any defects (Sunarharum, Williams, and Smyth 2014). Whereas green coffee has a mild, bean-like aroma, the desirable fragrance associated with coffee beverages is developed during roasting. The air temperatures in standard roasting are in the range of 180–250 ºC, and roasting time can vary between 25 min at the lowest temperatures to 2 min at the highest, depending on the desired degree of roasting and the technique employed (Parliment, Ho, and Schieberle 2000). The flavor and aroma of brewed coffee is intrinsically linked to

this roasting process, during which the chemical composition changes profoundly due to Maillard and Strecker reactions (Flament 2001; Ishwarya S and Nisha 2021). The substances produced in these reactions are responsible for the characteristic aroma of coffee and its pleasant bitterness. The characteristic flavor and aroma that these components provide make possible to classify coffee according to its quality based on sensory analysis. This approach relies on the evaluation of coffee quality from an olfactory and sensory perspective by trained panelists in a score scale developed by the Speciality Coffee Association of America (SCAA) (Batali et al. 2020) .

This review takes a look at the current strategies employed to assess the quality of coffee, including methods that can distinguish between the two main species used in its production, trace the geographical origin of coffee, and detect the addition of adulterants.

Discrimination between *Arabica* and *Robusta* coffee species

C. arabica (*Arabica*) and *C. canephora* (*Robusta*) differ in several aspects, for example, morphology, bean size and color, chemical components, and sensorial properties (Davis et al. 2006; Keidel et al. 2010; Feria-Morales 2002). Coffee is generally marketed as a mixture of the two species blended in different amounts to achieve the desired sensory characteristics (Martín, Pablos, and González 1998). *Arabica* is employed to enhance aroma, whereas *Robusta* is usually added to improve the body and foam of some coffee beverages (e.g., espresso coffee) and in instant coffee production (Wongsa et al. 2019; Clarke 2012).

Due to differences in price and organoleptic properties, *Robusta* can be considered as an adulterant of *Arabica*, and its illegal addition constitutes fraud. The more expensive *Arabica* coffee (reaching 20-25% higher market prices) has a more pronounced and refined flavor. On the other hand, *Robusta* crops are more resistant to disease, but the coffee they produce is considered to have an inferior flavor. It is therefore important to develop analytical methods that allow the reliable identification of both species and the estimation of their content in coffee products. Several approaches to coffee varietal identification have been applied with relative success, but many require techniques that are expensive and/or time-consuming (Esteban-Díez et al. 2007).

Chromatographic techniques

Chromatography is one of the most versatile methods for detecting fraud in coffee (Wang, Lim, and Fu 2020). The triglyceride and tocopherol contents of green and roasted coffee beans of the *Arabica* and *Robusta* were determined by reversed phase and normal high performance-liquid chromatography (HPLC), respectively, after Soxhlet extraction with hexane (González et al. 2001). Applying principal component analysis (PCA) and linear discriminant analysis (LDA), species discrimination was achieved with both

parameters, but only tocopherols allowed differentiation between green and roasted coffees. Similarly, the tocopherol profile in the two coffee species was analyzed by normal-phase HPLC/diode-array/fluorescence detection (Alves et al. 2009), and the higher content of β-tocopherol in *Arabica* after roasting permitted a clear separation; in *Robusta*, the mean degradation of this antioxidant was approximately 25% when expressed as dry weight. The ratio between α:β:γ tocopherol homologues determined by reversed phase-ultra HPLC electrospray ionization/mass spectrometry (RP-UHPLC-ESI/MS^n) was reported as a marker of authentication able to distinguish between coffee varieties even in roasted samples (Górnaś et al. 2014). In this study, an alkaline saponification procedure followed by extraction with a mixture of organic solvents was necessary to improve the recovery of tocopherols from coffee beans.

HPLC was also employed to evaluate the content of hydrosoluble compounds (caffeine, trigonelline, 5-caffeoylquinic acid, and nicotinic acid) as a method to discriminate between *Arabica* and *Robusta* in coffee blends (Dias and Benassi 2015). The most efficient discriminator was caffeine, which was unaffected by the degree of roasting, unlike the other tested compounds, whose application as markers required an additional step to characterize the roasting. To circumvent these difficulties, in the HPLC-diode-array-based method developed by Casal et al. (2000), all samples were roasted to the same degree. Multivariate and nonparametric analysis of the chromatographic results revealed that trigonelline and caffeine effectively discriminated between *Arabica* and *Robusta*, but not nicotinic acid (Casal et al. 2000).

Other potential biomarkers for *Arabica* and *Robusta* coffee are biogenic amines (putrescine, cadaverine, serotonin, tyramine, spermidine, and spermine). Using a method based on reversed-phase HPLC after derivatization with dansyl chloride and multivariate analysis, it was determined that putrescine, the predominant biogenic amine in green

beans, could be used for species discrimination, even after different post-harvest processes, but the statistical significance decreased considerably after roasting (Casal et al. 2004). Recently, non-targeted approaches relying on HPLC-UV chromatographic fingerprints together with partial least squares regression-discriminant analysis (PLS-DA) have also been applied for the evaluation of varietal classification and authentication (Núñez et al. 2020; De Luca et al. 2018).

Excellent results for *Arabica* and *Robusta* discrimination have been achieved with fatty acids (FA) (Bertrand et al. 2008). Gas chromatography (GC) analysis of lipid extracts from ground green and roasted coffee beans has been performed by various research groups to discriminate between *Arabica* and *Robusta* coffees using pattern recognition methods (Martín et al. 2001; Rui Alves et al. 2003; Romano et al. 2014). Total lipids were extracted from coffee beans using a Soxhlet apparatus, and the FA content was determined by their corresponding methyl esters. According to Martin et al, (2001) ten FA could serve as descriptors to differentiate between the coffee species: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3). arachidic (C20:0), eicosenoic (C20:1) and behenic acid (C22:0) (Martín et al. 2001). In another study, the levels of C18:1 and C20:1 (higher in *Robusta*) and C18:3 and C18:2 (higher in *Arabica*) were the most efficient markers (Rui Alves et al. 2003). Similarly, the total monounsaturated fatty acids (∑MUFA), the concentration of linolenic acid (*cis* 18:3 n–3), the 18:0/*cis* 18:1 n–9 ratio, and the ∑MUFA/ ∑SFA ratio was used to determine the relative amounts of *Arabica* and *Robusta* in coffee blends (Romano et al. 2014). GC also allowed the species classification according to the content of D- and L-amino acid enantiomers (Casal et al. 2003).

The GC analyses of the free amino acids, as well as the amino acids obtained after acid hydrolysis, were performed after derivatization. Multivariate analyses applied to the

179 results showed that the free amino acids can serve as a tool to discriminate between
180 *Arabica* and *Robusta*, especially L-glutamic acid, L-tryptophan, and pipecolic acid.
181 Although they have less discriminatory capacity, the amino acid levels after acid
182 hydrolysis can also be used.

183 In summary, the advantages of the chromatographic techniques allow the
184 identification of a large number of biomarkers (triglycerides, tocopherols, hydrosoluble
185 compounds, biogenic amines, aminoacids and FA) to discriminate between *Arabica* and
186 *Robusta* coffee species. Another advantage is that little amount of sample is required
187 compared to spectroscopy techniques.

188 ***Spectroscopic techniques***

189 Spectroscopic techniques have emerged as an attractive and useful tool for varietal
190 identification purposes: methods based on nuclear magnetic resonance (NMR)
191 spectroscopy and Raman spectroscopy, also combined with near infrared (NIR)
192 spectroscopy, have been developed. Table 2 provides a general description of the
193 spectroscopic methods used to distinguish between *Arabica* and *Robusta* coffee species,
194 highlighting the strengths and weaknesses of each. These methodologies have proved to
195 be easily implemented in routine analysis. In most of these studies, multivariate methods
196 such as PCA, LDA, or partial least squares regression (PLS) were employed to evaluate
197 the complex spectral information and to identify the compounds responsible for
198 differentiation.

199 An ultraviolet–visible (UV-Vis) spectroscopy-based determination of caffeine
200 and chlorogenic acid contents to discriminate between green coffee beans of *Arabica* and
201 *Robusta* was reported recently (Adnan et al. 2020). Seventy-four green coffee bean
202 samples from Indonesia were analyzed in this study, and the data related to both
203 compounds were processed using LDA, achieving an accuracy of 97%.

The original NIR spectra of roasted coffee samples can be used directly to develop a classification model with a moderate to high discrimination ability for pure varieties. However, after applying the orthogonal signal correction methods to remove information, Esteban-Diez et al obtained a notably less complex model with excellent classification power (Esteban-Díez et al. 2007). The same research group applied NIR spectroscopy combined with multivariate calibration methods to quantify the content of *Robusta* in roasted coffee samples as a means of controlling coffee adulteration (Pizarro, Esteban-Díez, and González-Sáiz 2007). PLS regression and a wavelet-based pre-processing method (called OWAVEC) were applied in this case to simultaneously operate two crucial pre-processing steps in multivariate calibration: signal correction and data compression. Another study also showed NIR spectroscopy to be a very consistent and useful tool to classify coffee samples (Buratti et al. 2015). The practicability of the approach was demonstrated by LDA, and an external test set validation showed the samples were 100% correctly classified. More recently, this technique has been applied to intact beans, achieving high classification accuracy (95%) when wavelength was selected by multivariate analysis (Adnan et al. 2020).

Fourier transform (FT) Raman spectroscopy is a dispersion process that allows discrimination between coffee beans of different species, both green and roasted, through their lipid fraction, which is extracted by diethyl ether in a Soxhlet system (Rubayiza and Meurens 2005). Taking advantage of two specific scattering bands at 1567 and 1478 cm^{-1} in the Raman spectra of the diterpene kahweol (present in 0.1-0.3% of dry matter in *Arabica* beans and only in traces in *Robusta*), a set of 86 green and 82 roasted coffees were grouped by species with a high degree of accuracy after PCA.

NMR spectroscopy is a powerful tool for the qualitative and quantitative analysis of complex mixtures of small molecules in solution and has been used with great success

to analyze foods and beverages. This approach is especially suitable for the quantification of minor components in complex matrices (Olmo-Cunillera et al. 2020). Using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, kahweol and 16-*O*-methylcafestol (16-OMC) were established as markers of *Arabica* and *Robusta*, respectively, in the lipophilic extracts of authentic roasted and green coffees (Monakhova et al. 2015). The integration of the 16-OMC signal (δ 3.165 ppm) was used to estimate the amount of *Robusta* in coffee blends with an approximate limit of detection of 1–3%. The method was successfully applied for the analysis of 77 commercial coffee samples (coffee pods, coffee capsules, and coffee beans). Another study revealed that the two species can be quickly discriminated by quantitatively evaluating the major metabolites of green coffee beans using carbon-13 nuclear magnetic resonance (^{13}C NMR)-based metabolite profiling coupled with chemometric analysis (PCA or orthogonal partial least squares discriminated analysis (OPLS-DA)) and by applying signal assignment information. Additionally, ^{1}H NMR and multivariate statistical analysis was used to develop an OPLS model based on multiple chemical components, which successfully determined the composition of coffee blends of unknown *Arabica* and *Robusta* content, regardless of the geographical origin of the analyzed samples (Cagliani et al. 2013).

A method based on direct-infusion electrospray ionization−mass spectrometry (ESI−MS) data calibrated by a PLS multivariate technique allowed the rapid detection and quantification of adulterations of *Arabica* coffee with *Robusta* (Garrett et al. 2012). A total of 16 PLS models were built using ESI(±) quadrupole time-of-flight (QToF) and ESI(±) Fourier transform ion cyclotron resonance (FT-ICR) MS data from hot aqueous extracts of certified coffee samples. The 30 most abundant ions accurately predicted the composition of commercial *Robusta* and *Arabica* coffee blends. In addition, ESI(±) FT-

ICR MS analysis identified 22 compounds in *Arabica* and 20 compounds in *Robusta*, mostly phenolics, which were responsible for the distinction between the coffee varieties.

The proton transfer reaction–time of flight–mass spectrometry (PTR-ToF-MS) technique for the analysis of volatile organic compounds (VOCs) can be used for a rapid and correct classification of *Arabica* and *Robusta* coffee at different stages of processing, from the roasted beans to the brewed coffee, but not for green beans (Colzi et al. 2017). After multivariate statistical analysis, the identified VOCs (16 for roasted beans, 12 for ground coffee and 12 for brewed coffee) were able to characterize the different aromatic profiles of the two species and discriminate between them. The best results were obtained with roasted beans, which may therefore be the most suitable coffee matrix for authentication screening.

In brief, spectroscopic methods have been widely used to distinguish between *Arabica* and *Robusta* coffee species. Within the strengths of these techniques, we would like to emphasize: i) simplified measurement procedures, ii) high throughput, iii) fast and low cost and iv) (lipid fraction, caffeine and chlorogenic acid, 16-OMC and VOCs). In addition, these methods can be affected by environmental conditions and that the success depends on signal pre-processing methods applied to minimize the spectral variation, due to the alteration in sample preparation and conditions.

Single-nucleotide polymorphism-based methods

Single-nucleotide polymorphisms (SNPs) are single-base changes in DNA that discriminate between closely related species and/or varieties. SNP-based methods are therefore useful for authenticity testing of coffee beans by enabling the differentiation between *Arabica* and *Robusta* varieties. The method developed by Trantakis et al. (2012) (Trantakis, Christopoulos, et al. 2012), based on the detection of an SNP in the chloroplastic trnL(UAA)-trnF(GAA) intergenic spacer, accurately determined the

percentage of *Arabica* and *Robusta* beans in a mix. After polymerase chain reaction (PCR) amplification of this genomic region, the resulting DNA fragments were subjected to extension reactions by DNA polymerase using *Robusta*-specific and *Arabica*-specific primers. In the reaction, the extended strands were labelled with oligo(dA) tags and biotin. The products were immobilized in streptavidin-coated microtiter wells and hybridized with the oligo(dT)-conjugated photoprotein aequorin. The fragments were then quantified by measuring the presence of aequorin via its characteristic bioluminescent reaction following the addition of Ca^{2+}.

In subsequent work (Trantakis, Spaniolas, et al. 2012; Trantakis, Christopoulos, et al. 2012), this SNP-based authentication assay was further developed into a low-cost, disposable, dipstick-type test that allows DNA-based coffee bean authenticity testing by the naked eye. After the described PCR amplification of the chloroplastic intraspacer region and fragment extension using species-specific primers, the fragments are applied to the dipstick, followed by a carrier buffer. While being transferred through a membrane, DNA fragments take up gold nanoparticles. Species-specific fragments are held back by immobilized streptavidin due to their biotin labelling, while unspecific fragments bind to a final zone on the membrane and serve as a control. The presence and quantity of labelled fragments can be easily assessed by the intensity of the nanoparticle staining.

To date, very few studies have used SNPs to discriminate between closely related species and/or varieties. However, SNP-based methods are useful for authenticity testing of coffee beans by enabling the differentiation between *Arabica* and *Robusta* varieties.

Geographical origin authenticity

The worldwide growth of the coffee market has increased the importance of the geographical origin of coffee, and this information is increasingly included on product

labels. As the quality of this globally appreciated beverage is associated with specific growing areas, mislabeling has become another area of fraud.

Tracing the geographical origin of coffee is challenging, mainly because the chemical composition of beans is influenced not only by agronomic practices and the climate of the growing area, but also by the post-harvest processing methods, storage conditions, distribution, and roasting procedures (Alves et al. 2009). The choice of a discrimination technique depends not only on its performance, but also the time required for analysis, the cost of the analytical equipment, and the possibility of automation (Anderson and Smith 2002; Perez, Lopez-Yerena, and Vallverdú-Queralt 2020). Table 3 provides an overview of the methods commonly used to distinguish the geographical origin of coffee.

Discrimination between major coffee-growing regions

NMR has emerged as a promising technique for the traceability of coffee from the largest growing areas. In this context, the metabolite content of *Arabica* roasted coffee samples from America, Africa, and Asia was investigated by NMR spectroscopy by Consonni et al. (Consonni, Cagliani, and Cogliati 2012). The samples were clearly separated according to origin when OPLS-DA models were applied to ^{1}H NMR data. The main compounds characterizing the American samples were FA, whereas chlorogenic acids and lactate were the key compounds for African coffee, and acetate and trigonelline for the Asian samples. On the other hand, the geographical origin of green coffee beans can be rapidly discriminated by quantitative ^{13}C NMR-based metabolomics (Wei et al. 2012). The content of caffeine was found to be higher in *Robusta* green coffee beans from Vietnam compared to Indonesia, or in those from Central America compared to South America and Africa, therefore serving as an indicator of origin. Other reported indicators are chlorogenic acids, acetic acid and amino acid levels.

Coffee bean samples from three major coffee-growing regions (Indonesia, East Africa, and Central/South America) were analyzed by elemental analysis using inductively coupled plasma atomic emission spectroscopy (ICPAES) (Anderson and Smith 2002). A computational evaluation of the data sets from 11 elements was carried out using statistical pattern recognition methods, including PCA, discriminant function analysis, and neural network modeling, resulting in 70–86% of successful classification. Similarly, the trace element composition of coffee beans from six different regions (Brazil, Colombia, Vietnam, Indonesia, Tanzania, and Guatemala) was analyzed using a high sensitivity X-ray fluorescence spectrometer with three-dimensional polarization optics (Akamine et al. 2010). After optimization of the experimental conditions and the construction of the linear calibration curves, the analytical results of six trace elements were used in the PCA to classify both roasted and green beans according to their growing area.

Regarding stable isotope ratios of elements, it was found that the ratio of carbon, nitrogen, and boron of green coffee beans produced in three continents (Africa, Asia and America) were good indicators of geographical origin (Serra et al. 2005). The combination of the isotopic fingerprints of these three elements and the subsequent PCA successfully identified the continental origin of 88% of the analyzed samples. Although this approach has produced promising results, it fails to distinguish between adjacent countries with similar climatic environments. Moreover, samples from large countries with a variety of climatic areas may also result in an extensive range of isotope ratio values, and therefore a wide dispersion. Multi-element stable isotope analysis of caffeine isolated from green coffee beans of different geographical origins (Central and South America, Africa, Indonesia, Jamaica and Hawaii) was carried out using isotope ratio mass spectrometry (IRMS) and elemental analysis (EA) (Weckerle et al. 2002). Data evaluation

by LDA and classification and regression tree (CART) analysis showed that the $\delta^{18}O_{VSMOW}$ values were highly significant for origin assessment.

Based on the volatile and semi-volatile profiles in coffee, ToF-MS has also been applied to trace the origin of coffee bean samples. In this regard, a rapid analytical method to distinguish the geographical origin of coffee samples from America, Africa and Asia was developed (Risticevic, Carasek, and Pawliszyn 2008) using headspace solid-phase microextraction (HSSPME)–GC–ToF-MS and submitting the semi-quantitative results to statistical evaluation by means of PCA. Similarly, the aroma profiles of different roasted coffees from Brazil, Ethiopia and Guatemala were analyzed by PTR-ToF-MS (Yener et al. 2014), with the aid of a multipurpose autosampler. After the application of unsupervised and supervised multivariate data analysis techniques, significant differences were found in the volatile profiles of the coffee according to origin, as visualized by PCA, and classification prediction accuracy was established by further partial least square regression-discriminant analysis.

The geographical origin of green coffees from the major growing regions of America, Africa, Asia, and Oceania was also analyzed by HPLC coupled with UV spectrophotometry (Alonso-Salces et al. 2009). Phenolic and methylxanthine profiles provided classification models that correctly identified all authentic *Robusta* green coffee beans from Cameroon and Vietnam and 94% of those from Indonesia after multivariate data analysis, LDA and PLS-DA. Moreover, PLS-DA afforded independent models for *Robusta* samples from these three countries with classification sensitivities and specificities close to 100% and for *Arabica* samples from America and Africa with sensitivities of 86 and 70% and specificities of 90 and 97%, respectively. The content of chlorogenic acids, caffeine and total polyphenols were analyzed by means of UHPLC coupled to an exactive Orbitrap MS for the geographical assessment of coffee samples

377 from China, India and Mexico (Mullen et al. 2013). *Arabica* and *Robusta* coffee from
378 India and Mexico showed similar contents of chlorogenic acids and polyphenols, whereas
379 significantly lower contents were found in samples from China.

380 To date, few published studies have compared the different analytical techniques
381 applied to trace geographical origin. However, quite recently, Medina et al. published a
382 collective comparative analysis of ^{1}H NMR, attenuated total reflectance – mid infrared
383 (ATR-MIR), and NIR applied to detect fraud in Colombian coffee (Medina et al. 2017).
384 For each technique, classification models were constructed for discrimination by origin
385 and ATR-MIR emerged as the best candidate, as it showed the same ability as ^{1}H NMR
386 to determine the Colombian origin, but more rapidly and at a lower cost; NIR fell short
387 in comparison with the other methods.

388 In summary, NMR is the most powerful technique for the traceability of coffee
389 from the largest growing areas, although IRMS and EA seem to have gained interest in
390 the last few years. MS and UV coupled to GC and HPLC have also been used to determine
391 the volatile and semivolatile profile of coffees, but further research is necessary to
392 improve the applicability of these techniques.

393 ***Discrimination between local/regional growing areas***

394 The effectiveness of chlorogenic acids, FA, and elements analysed by HPLC, GC, and
395 ICPAES, respectively, for the discrimination of five (one traditional and four
396 introgressed) *Arabica* varieties from three Colombian locations was compared by
397 Bertrand using PCA and discriminant analysis (Bertrand et al. 2008). Although elements
398 provided an excellent classification of the three locations studied, this chemical class was
399 ineffective for *Arabica* discrimination. Chlorogenic acids gave satisfactory results, but
400 FA were clearly the most effective in distinguishing between varieties (*Arabica* versus
401 *Robusta*) and regions, with very high percentages of correct classification (79 and 90%,

respectively). On the other hand, green coffee samples proceeding from four different cities in the south of Brazil were successfully distinguished by NIR spectroscopy (Marquetti et al. 2016) after the complexity and quantity of information within the spectra was simplified by PLS-DA.

Recently, the phenolic profile obtained by UPLC-MS was applied to determine the geographical origin of green coffee beans produced in four Ethiopian regions (Mehari et al. 2021). PCA of the data identified 3-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinicacid, and 4,5-dicaffeoylquinic acid as the most discriminating phenolic compounds for authentication, with a moderate classification efficiency (74% prediction success rate). On the other hand, the metabolite variability in coffee grown in Indonesia, a top exporter of *Arabica* coffee, was analyzed by means of non-targeted GC/MS according to species and geographical origin (Putri, Irifune, and Fukusaki 2019).

In summary, in an effort to confirm the validity of the information on the product label regarding origin, numerous technologies have been applied to discriminate major coffee-growing regions and between local/regional growing areas. While some biomarkers show high classification efficiency (e.g. chlorogenic acids, FA, lactate, acetate and trigonelline, caffeine, carbon, nitrogen, and boron) others biomarkers (phenolic profile) are characterized to have moderate classification efficiency.

Other adulteration practices in roasted coffee

Fraudulent or accidental adulteration is the most serious problem affecting the coffee trade (Nogueira and do Lago 2009). To lower the production costs, beans from two species of different economic value may be mixed and other substances added. The major adulterants of coffee include roasted and unroasted coffee husks or parchments, coffee stems, maize, barley, chicory, cereals, wheat middlings, brown sugar, soybean, rye, triticale, acai (Toci et al. 2016), malt, starch, maltodextrins, glucose syrups, and

427 caramelized sugar (Nogueira and do Lago 2009).

428 As well as devaluing the coffee product, the addition of substances could also
429 affect consumer health, which has prompted the development of several analytical
430 techniques to detect the presence of adulterants in coffee. Microscopy analysis and visual
431 inspection have been traditionally used to examine roasted and ground coffee, but they
432 are not suitable to identify impurities in processed coffee (Cai, Ting, and Jin-Lan 2016;
433 Nogueira and do Lago 2009). Therefore, other methods have been developed that provide
434 more reliable and reproducible results, including chromatographic, spectroscopic,
435 voltammetric and biological techniques (Figure 1).

436 ***Chromatographic techniques***

437 Adulteration in commercial coffee can be indicated by carbohydrate levels. Thus, by
438 determining the concentration of free and total carbohydrates, it was possible to detect
439 the deliberate contamination of coffee with coffee husk and ligneous material (sticks), as
440 this resulted in a higher content of mannitol, xylose, glucose, and fructose; pure and
441 adulterated products were also distinguished on this basis (Nogueira and do Lago 2009).

442 Carbohydrates are usually analyzed by HPLC. Accordingly, roasted soybean and
443 wheat adulterations were revealed by a method combining HPLC - high performance
444 anion exchange chromatography with pulsed amperometric detection (HPLC-HPAEC-
445 PAD) with chemometric tools. After characterizing pure roasted coffee beans and
446 adulterants by their carbohydrate profile and monosaccharide content (Cai, Ting, and Jin-
447 Lan 2016), glucose and fructose were established as markers for adulteration with wheat
448 and soybean, respectively. In another study, the standardized ISO 11292:1995
449 methodology (HPLC-HPAEC-PAD) for the determination of free and total carbohydrate
450 content in soluble coffee was compared with HPLC coupled to UV-Vis to characterize
451 the carbohydrate profile of the adulterants triticale and acai (Domingues et al. 2014).

Although both chromatographic methods effectively detected the coffee adulterants, pulsed amperometry was superior for quantification. Nevertheless, the HPLC-UV-Vis system was faster, cheaper and easier to operate. Another study also demonstrated that HPLC–HPAEC–PAD associated with chemometrics has potential as a routine system for adulteration and authenticity tests in ground roasted coffee (Pauli et al. 2014). It was found that pure roasted coffee has higher levels of galactose and mannose, and that glucose and fructose can indicate adulteration with wheat and soybean, respectively. A novel method developed by Cai et al. (2016) used ultra performance liquid chromatography – high resolution mass spectrometry (UPLC-HRMS) technology to determine the oligosaccharide composition of coffee and common adulterants. This approach identified up to 17 oligosaccharide markers and detected the presence of soybeans and rice in ground coffee when these adulterants were present in amounts of 5% (Cai, Ting, and Jin-Lan 2016). Based on chemometric analysis (PCA), HPLC was also used in a non-targeted analysis of coffee adulteration with soybeans and green mung beans. Unlike targeted analysis, this method allowed the identification of unknown additive compounds without sample preparation. Compared to FTIR, HPLC provides more detailed information because the peaks in the chromatogram represent different compounds, whereas Fourier transform infrared spectroscopy (FTIR) spectra only indicate functional groups. However, the detection limit of adulterants was 5%, whereas in many chemometric analyses with IR it is below 1% (Cheah and Fang 2020).

Tocopherol fingerprinting is another potential approach to detect coffee adulteration. In a study of tocopherol levels based on HPLC-PDA/UV and mean tests, regression analysis, PCA, LDA and SIMCA (Tavares et al. 2016), tocopherol ratios indicated the presence of maize, husks and cleaned husks, γ–tocopherol being the main descriptor for adulterations with both maize and coffee by-products. Another study

477 analyzing α, β, γ and δ-tocopherol by HPLC-UV also found that γ- tocopherol was the
478 best indicator of coffee adulterations with corn (Jham et al. 2007).

479 In 2009, Oliveira et al. used solid-phase microextraction (SPME) -GC-MS and
480 chemometrics to study coffee adulteration with roasted barley, carrying out a comparative
481 analysis of the volatile profiles of both coffee and barley, pure and mixed, and at several
482 roasting degrees (Oliveira et al. 2009). The results demonstrated that the higher the degree
483 of roasting, the easier it was to distinguish the adulterated samples, allowing the detection
484 of roasted barley in quantities as low as 1% (w/w) in dark roasted coffee samples.

485 ***Voltammetry***

486 A new approach to detect coffee adulterations involves voltammetry coupled with
487 chemometrics. This simple low-cost technique avoids the common disadvantages of
488 physical, chemical, and biological methods, such as high costs, long analysis times and
489 the need for skilled manpower. The voltammetric analysis is performed by an electronic
490 tongue, an electronic system that generates complex data requiring chemometric tools to
491 extract the information. This system was first used in coffee samples by Arrieta et al. in
492 2019 for the detection of adulterations with roasted soybean and corn. They achieved
493 sample discrimination using an electronic tongue equipped with a polypyrrole sensor
494 array, followed by either PCA or cluster analysis. The method was also successfully
495 applied for quantitative analysis by partial least squares regression (Arrieta, Arrieta, and
496 Mendoza 2019; de Morais et al. 2019).

497 ***Capillary electrophoresis***

498 Capillary electrophoresis is a powerful tool that can detect and quantify a wide range of
499 food-related molecules with different chemical properties (Papetti and Colombo 2019).
500 In processed coffee, this technique has been applied to detect adulterations with cereals

and coffee husks (Nogueira and do Lago 2009), soybeans and corn (Daniel et al. 2018) by evaluating the monosaccharide profile. Even though capillary electrophoresis has proven to be a useful technique for the analysis of carbohydrates, it has the disadvantage that monosaccharides need to undergo acid hydrolysis and a neutralization step, which is time-consuming. However, Daniel et al. developed an optimized procedure by using $Ba(OH)_2$ to neutralize the medium, as this reduces the amount of salt and the ionic conductivity of the sample (Daniel et al. 2018). In another study, a strong-base anion resin was used, because it exchanges chloride for hydroxide, which simultaneously neutralizes the medium and reduces the ionic strength (Nogueira and do Lago 2009).

Spectroscopic techniques

FT-MIR has been employed to determine the quality of different food products, including coffee (Karoui, Downey, and Blecker 2010). The spectral variability between pure and adulterated coffee samples are fundamental in building chemometric models (Flores-Valdez et al. 2020). Thus, the characteristic spectral regions of pure coffee (assigned to chlorogenic acid, lipids, lignin, quinic acid, amides, caffeine, among others) (Flores-Valdez et al. 2020; Reis, Franca, and Oliveira 2013a; Reis, Franca, and Oliveira 2013b; Craig, Franca, and Oliveira 2012), tocopherols (Winkler-Moser et al. 2015) and/or coffee adulterants such as sibutramine have been used (Cebi, Yilmaz, and Sagdic 2017). Both FT-MIR and FT-NIR are rapid, direct, and simple techniques, but the NIR bands are more difficult to interpret and less reproducible and specific. Moreover, the mid-infrared region is more sensitive to the chemical composition of the samples (de Oliveira et al. 2014). Flores-Valdez et al. (2020) developed a method based on FT-MIR spectroscopy coupled with chemometrics that allowed the identification and quantification of coffee adulterants (coffee husks, barley, corn, soy, oat and rice) at concentrations ranging from 1 to 30% (Flores-Valdez et al. 2020). The amount of barley added to coffee samples using a method

526 based on FT-NIR spectral information also have been study (Ebrahimi-Najafabadi et al.
527 2012). In this study, the excellent predictive ability obtained by multivariate calibration,
528 which was confirmed by the low values of root mean square errors (RMSE), indicated
529 that non-destructive NIR measurements can successfully detect and quantify the
530 fraudulent addition of roasted barley (up to 2% w/w) to roasted coffee. In addition,
531 variable selection using genetic algorithms helped to determine which spectral regions
532 would be most useful to identify the adulteration. ATR-FTIR combined with PCA was
533 also employed to detect sibutramine, an oral anorexiant that may be illicitly included in
534 green coffee. This method was based on the presence of an absorption band at 2698 cm^{-1}
535 , which is specific to sibutramine hydrochloride monohydrate (Cebi, Yilmaz, and Sagdic
536 2017).

537 A different FTIR procedure, known as diffuse reflectance Fourier transform
538 infrared spectroscopy (DRIFTS), was used to determine roasted corn and coffee husks in
539 roasted and ground coffee (Reis, Franca, and Oliveira 2013a). The same research group
540 developed a method using DRIFTS and PLS that allowed the detection and quantification
541 of roasted coffee husks, barley and corn (Reis, Franca, and Oliveira 2013b). To date, no
542 published studies have compared ATR-FTIR and DRIFTS for the analysis of coffee
543 adulteration. Comparisons of other coffee-related applications, such as discrimination by
544 quality or maturity, have shown that DRIFTS provides a more effective differentiation
545 and higher intensity spectra than ATR-FTIR(Craig, Franca, and Oliveira 2012).

546 Winkler-Moser et al. (2015) performed a comparative analysis of HPLC and NIR
547 to detect adulteration with corn. HPLC analysis was based on the determination of
548 tocopherol in coffee, as corn and coffee differ in their tocopherol profile. The sensitivity
549 of both HPLC and NIR was about 5%, but NIR has the advantage of being a simple and
550 faster technique that does not require sample treatment (Winkler-Moser et al. 2015).

NMR has been successfully employed to discriminate between coffee species and geographical origins, as already described, and in the authentication of other foods (Hong et al. 2017), but it has been underused for the identification of coffee adulterants. A methodology based on ^{1}H NMR combined with PCA and soft independent modelling of class analogies (SIMCA) for the identification and quantification of coffee contamination was recently developed (Milani et al. 2020). The technique was able to quantify six adulterants (coffee husks, soybean, corn, barley, rice, and wheat) in coffee with two different degrees of roasting.

A novel technique, laser-induced breakdown spectroscopy (LIBS), combined with PLS and PSA, has proven to be a reliable method to detect and quantify the coffee adulterants chickpeas, corn, and wheat. Based on a laser that detects atomic and molecular emission signals of elements, LIBS is a rapid technique that does not need any sample preparation and determines adulterations in coffee below 0.6% (Sezer et al. 2018).

Biological methods

DNA-based techniques have emerged in the last years as useful methods to guarantee food authenticity and safety (Laube et al. 2010; Fuchs, Cichna-Markl, and Hochegger 2012). PCR is a fast, specific and sensitive method that can be used to obtain DNA from roasted beans and instant coffee (Martellossi et al. 2005). This approach was adopted by Ferreira et al., who developed a real-time PCR-based method to detect and quantify barley, maize, and rice in roasted and soluble coffee. Marker genes for coffee and the targeted adulterants were tested using the Basic Local Alignment Search Tool (BLAST). Primer sensitivity and efficiency revealed that this method was suitable for authenticity control in the coffee industry (Ferreira et al. 2016).

In summary, a large number of methods (chromatographic, voltammetry, capillary

electrophoresis, spectroscopic and biological methods) have been used by the scientific community and the coffee industry as a strategy to identify other added substances with lower value. However, more efforts are needed to curb adulteration in the coffee sector, towards high-quality production.

Coffee adulteration and its effect on human health

Adulterants have been studied for their effect on the bioactive constituents of coffee, and it was found that levels of caffeine, chlorogenic acid and other phenolic compounds decreased with increasing adulterant concentration (de Pádua Gandra et al. 2017), as did the antioxidant capacity. The results therefore show that adding coffee hulls, coffee straw, and corn affects the health benefits of coffee beverages, reducing protection against oxidative stress.

Sibutramine is an oral anorexiant that may be illicitly included in herbal slimming foods and supplements marketed as "100% natural" to enhance weight loss. However, sibutramine consumption has been associated with increased blood pressure and heart rate (Bertholee et al. 2013), and heart attacks and strokes (Cebi, Yilmaz, and Sagdic 2017). Numerous efforts have therefore been invested in developing an effective and rapid method for its detection in weight-loss products such as green coffee (Cebi, Yilmaz, and Sagdic 2017), coffee (Suryoprabowo et al. 2020), and Brazil Potent Slimming Coffee (Bertholee et al. 2013) to guarantee the quality of functional foods and protect consumer health.

Phosphodiesterase-5 inhibitors (PDE-5i) are another family of drugs that have been used as adulterants in coffee. PDE-5i are employed for the medical treatment of erectile dysfunction, but they are known to have side effects, such as headaches, nausea, skin flushes, muscle pain or prolonged erection (Suryoprabowo et al. 2020). In recent years, the detection of illegal PDE-5i and analogues in herbal supplements has been

reported in many regions, including Asia, Europe and North America (Dong et al. 2020). In order to protect public health, Suryoprabowo et al. (2020) developed a fluorescence-based method that allowed rapid and sensitive determination of tadalafil in coffee (Suryoprabowo et al. 2020).

Coffee seeds are liable to become contaminated with mold, including ochratoxin A, especially if they are not dried properly or become rehydrated during any stage of drying, storage and transportation (Blanc 2004). As coffee is one of the most consumed beverages worldwide, this nephrotoxic and nephrocarcinogenic mycotoxin is a potential risk factor for human health. Notably, the levels of ochratoxin A were highest in soluble coffees that had been adulterated with coffee husks and/or coffee parchments (Pittet et al. 1996).

Conclusions

In this review, we have provided an extensive overview of analytical techniques and multivariate data analyses successfully applied to detect adulteration or authenticity in coffee, focusing on the most common species, *Robusta* and *Arabica*. Advances in technology have allowed the detection of fraudulent practices in coffee through the identification/quantification of specific chemical or biological markers with a higher sensitivity than ever before, although each method has its limitations. Additionally, we have comprehensively compared the capacity of the different analytical techniques to discriminate between *Arabica* and *Robusta* and trace geographical origin, pointing out their respective drawbacks. We have also looked at the advancements in methods to detect fraudulent or accidental adulteration with other foods and/or substances. It can be concluded that more efforts are necessary to protect coffee producers from the huge economic losses and consumers from the health risks these practices entail.

624 **Abbreviations**

ATR	Attenuated total reflectance
BLAST	Basic Local Alignment Search Tool
^{13}C NMR	Carbon-13 nuclear magnetic resonance
CART	Classification and regression tree
DRIFTS	Diffuse reflectance Fourier transform infrared spectroscopy
DA	Discriminated analysis
EA	Elemental analysis
FA	Fatty acids
FT	Fourier transform
FTIR	Fourier transform infrared spectroscopy
FT-ICR	Fourier transform ion cyclotron resonance
GC	Gas chromatography
HSSPME	Headspace solid-phase microextraction
HPAEC-PAD	High performance anion exchange chromatography with pulsed amperometric detection
HPLC	High performance liquid chromatography
^{1}H NMR	Proton nuclear magnetic resonance
ICPAES	Inductively coupled plasma atomic emission spectroscopy
IRMS	Isotope ratio mass spectrometry
LIBS	Laser-induced breakdown spectroscopy
LDA	Linear discriminant analysis
MIR	Mid infrared
NIR	Near infrared
NMR	Nuclear magnetic resonance
16-OMC	16-*O*-methylcafestol
OPLS	Orthogonal partial least squares
PLS	Partial least squares regression
PDE-5i	Phosphodiesterase-5 inhibitors
PCR	Polymerase chain reaction
PCA	Principal component analysis
PTR-ToF-MS	Proton transfer reaction–time of flight–mass spectrometry
QToF	Quadrupole time-of-flight

RMSE	Root mean square errors
RP-UHPLC-ESI/MSn	Reverse phase-ultra high performance liquid chromatography-Electrospray ionization/mass spectrometry.
SNP	Single-nucleotide polymorphism
SIMCA	Soft independent modelling of class analogies
SPME	Solid-phase microextraction
∑MUFA	Total monounsaturated fatty acids
UPLC-HRMS	Ultra-performance liquid chromatography – high resolution mass spectrometry
UV-Vis	Ultraviolet-visible
VOC	Volatile organic compound

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980

981 Table 1. Overview of chromatographic methods to distinguish between *Arabica* and *Robusta* coffee species.

Object of the analysis	Samples analyzed	Strong points	Weak points	Ref
HPLC				
Triglycerides from Soxhlet extraction with hexane	Green and roasted coffee beans -24 samples belong to *Arabica.* -8 to *Robusta*	-Complete discrimination.	-Extraction of the coffee oil is required. -No differentiation between green and roasted coffees.	González et al. (2001)
Tocopherols from Soxhlet extraction with hexane		-Complete discrimination. -Differentiation between green and roasted coffees.	-Extraction of the coffee oil is required.	
Tocopherols from extracts (solid-liquid micro-extraction)	Green and roasted coffee beans -16 samples belong to *Arabica* -13 to *Robusta*	-Either green or roasted coffee can be used.	-Extraction of the coffee tocopherols is required. -There is also no evidence that these compounds can be used for discrimination between coffees subjected to different post-harvest procedures.	Alves et al. (2009)
Hydrosoluble compounds: Caffeine, trigonelline, 5-caffeoylquinic acid, nicotinic acid	Blends (*Robusta/Arabica*: 0:100, 20:80, 30:70, 50:50 and 100:0) at the three roasting degrees.	-Species and degree of roasting were predicted by multiple linear regression with high coefficient of determination values.	-Characterization of roasting degree of the sample is required.	Dias and Benassi (2015)
Hydrosoluble compounds: Caffeine and trigonelline	Roasted coffee beans -9 samples belong to *Arabica* -20 to *Robusta*	-Suitable for routine analysis in the coffee industry.	-There was no association with the geographical origin of the samples.	Casal et al. (2000)
Fingerprint from solid-liquid extracted using water/methanol mixture.	Green coffee beans -12 samples belong to *Arabica* -12 to *Robusta*	-PLS-DA achieved 100% correct classification.	-Concentration of individual analytes, such as caffeine and chlorogenic acid, proved to be insufficient. -Extraction of the phenolic fraction from ground coffee is required.	De Luca et al. (2018)
Fingerprint from the coffee brewing using an espresso machine	-160 samples belong to *Arabica* -20 to *Robusta* -60 to *Arabica*/*Robusta* mixtures	-Classification rates higher than 89.3%.	-Detects and quantifies coffee frauds only to 15% adulterant level.	Núñez et al. (2020)
Biogenic amines	-19 samples belong to *Robusta* -11 to *Arabica*	-Putrescine shows a high potential as a coffee species discriminator.	-Biogenic amine extraction and their derivatization are required. -Biogenic amine approach cannot be used in roasted beans.	Casal et al. (2004)
UPLC				
Tocopherols from saponification and organic solvent extraction.	Green and roasted coffee beans -15 samples belong to *Arabica* -6 to *Robusta*	-Species discrimination even in roasted samples.	-Saponification and organic solvent extraction are required.	Górnaś et al. (2014)
GC				

FA from Soxhlet extraction with hexane or petroleum ether	Green and roasted coffee beans -27 samples belong to *Arabica* -13 to *Robusta*	-Complete separation of *Arabica* and *Robusta* coffees	-FA extraction followed by derivatization to form the corresponding methyl esters is required.	Martín et al. (2001)
	Green and roasted coffee beans -8 samples belong to *Arabica* -16 to *Robusta*	-FA profile can be used as a coffee variety marker.	-FA extraction followed by derivatization to form the corresponding methyl esters is required. -No geographical relationships could be found.	
	Roasted coffee beans -6 samples belong to *Arabica* -5 to *Robusta* 8 laboratory and 13 commercial blends	-Useful and suitable tool to assess the amounts of *Arabica* and *Robusta* in a coffee blend.	-The variability of FA composition in *Robusta* reduces applicability in blends containing a high percentage of *Robusta*.	
Amino acids	Green and roasted coffee beans (30:30) -22 samples belong to *Arabica* -38 to *Robusta*	-High potential for use as coffee species discriminators.	-Amino acid extraction followed by derivatization is required.	Casal et al. (2003)
FA composition from Soxhlet extraction with petroleum ether.	Green and roasted coffee beans -8 samples belong to *Arabica* -16 to *Robusta*		-FA extraction followed by derivatization to form the corresponding methyl esters is required. -No geographical relationships could be found.	Rui Alves et al. (2003)

982 FA: Fatty acids

983 Table 2. Overview of spectroscopic methods to distinguish between *Arabica* and *Robusta* coffee species.

Spectroscopic techniques	Object of the analysis	Samples analyzed	Strong points	Weak points	Ref
UV-Vis	Caffeine and chlorogenic acid	Green coffee beans -32 samples belong to *Arabica.* -42 to *Robusta*	-Simplified measurement procedures. -Accuracy of 97%.	-Limited by the environmental conditions	Adnan et al. (2020)
NIR	Selected wavelengths		-High throughput -Fast and low cost -Accuracy of 95%.	-Limited by the environmental. conditions -The study did not clarify the chemical composition of the beans.	Adnan et al. (2020)
	Selected wavenumbers	Green beans + ground roasted beans -99 samples belong to *Robusta.* -54 to washed *Arabica* -41 to natural *Arabica*	-100% samples correctly classified. -Fast, clean, and inexpensive compared to classical analysis.	-Slight misclassification of *Arabica* and washed *Arabica* samples.	Buratti et al. (2015)
	Spectra with orthogonal signal correction to remove information not related to the caffeine content.	Roasted beans -36 samples belong to *Arabica.* -47 to *Robusta* 108 blends of *Arabica* and *Robusta* coffee varieties	-Spectra directly acquired on untreated samples (all NIR analysis). -Excellent results obtained.	-Pilot study	Esteban-Díez et al. (2007)
	Spectra with signal correction and data compression	Roasted beans -36 samples belong to *Arabica.* -47 to *Robusta* 108 blends of *Arabica* and *Robusta* coffee varieties	-Allows the quantification of the *Robusta* content in roasted coffee samples. -Spectra directly acquired on untreated samples (all NIR analysis).	-The success depends on signal pre-processing methods applied to minimize the spectral variation, not due to the parameter of interest but due to variation in experimental or sample conditions.	Pizarro, Esteban-Díez, and González-Sáiz (2007)
FT-Raman	Kahweol from the lipid fraction extract	Green beans + ground roasted beans -124 samples belong to *Arabica.* -42 to *Robusta.* -2 to *Liberica.*	-Handheld Raman spectrometers are available and easy to use.	-Kahweol content may be affected by temperature, coffee cultivars, soil, and post-harvest processing.	Rubayiza and Meurens (2005)
^{1}H NMR	16-OMC and kahweol from $CDCl_3$ lipophilic extracts	Green beans + ground roasted beans -12 samples belong to *Arabica.* -8 to *Robusta* 77 commercial coffee samples (coffee beans or ground coffee)	-Quick, non-destructive, structure elucidation capabilities. -Chemometric discrimination.	-When using 16-OMC as a marker, it is not possible to detect less than 2% of *Robusta* in *Arabica* coffee.	Monakhova et al. (2015)
	Water-soluble chemical compounds from room temperature extracts	Roasted and ground coffee blends	-Chemical derivatization or separation techniques are not required.		Cagliani et al. (2013)

			- High accuracy in prediction of *Arabica* content in roasted coffee blends.		
^{13}C NMR	14 metabolites from green coffee bean extracts	Green coffee beans - 40 samples belong to *Arabica*. - 20 to *Robusta*	- Quick, non-destructive, and non-targeted. - Fewer overlap-problems than ^{1}H NMR spectroscopy.	- The quantification of metabolites can be problematic.	Wei et al. (2012)
ESI-MS	From hot-aqueous extracts: • 22 compounds for *Arabica* • 20 compounds for *Robusta*	Six blends (10, 20, 30, 40, 50, and 70% of *Robusta* coffee in *Arabica*) made by mixing five *Robusta* with six *Arabica* coffees.	- Fast method to quantify blends of *Robusta* and *Arabica* coffee.		Rubayiza and Meurens (2005)
PTR-ToF-MS	VOCs: 16 for roasted coffee 12 for ground coffee 12 for brewed coffee	13 samples	- Enables real-time analysis of VOCs without a sample pretreatment.		Colzi et al. (2017)

16-OMC: 16-*O*-methylcafestol; VOCs: volatile organic compounds.

985 Table 3. Overview of methods to distinguish geographical origin.

Techniques	Object of the analysis	Samples analyzed	Strong points	Weak points	Ref
^{1}H NMR	Deuterated water extracts of ground roasted coffee	40 roasted coffee samples (America: 20, Africa: 11, Asia: 9)	Null sample derivatization. Detection of several water-soluble compounds in a single experiment. High reproducibility and short experimental time.	A supervised discriminant analysis with OPLS-DA was required for a better interpretation of data. Extraction of ground roasted coffee is required.	(Consonni, Cagliani, and Cogliati 2012)
^{13}C NMR	14 metabolites from green coffee bean extracts	60 coffee bean samples *Arabica*: (Brazil, Colombia, Guatemala, and Tanzania) *Robusta* (Indonesia and Vietnam).	-Quick, non-destructive, and non-targeted. -Fewer overlap problems than ^{1}H NMR spectroscopy.	The quantification of metabolites can be problematic. An extraction process is required.	(Wei et al. 2012)
ICPAES	11 elements	160 coffee samples from Indonesia, East Africa, and Central/South America	70-85% successful classification. Single analysis on a commonly available automated instrument.	Simple inspection of elemental concentrations cannot be used to differentiate between growing areas.	(Anderson and Smith 2002)
Elemental analyzer	Stable isotope ratio of carbon, nitrogen, and boron	Green coffee beans from Africa, Asia, and America	88% of successful classification.	Mismatch between national borders and climatic borders.	(Serra et al. 2005)
Elemental analyzer-IRMS	Stable isotope analysis of carbon, hydrogen, and oxygen in caffeine	45 coffee samples (Central and South America: 20, Africa: 16, Indonesia: 6, Jamaica and Hawaii: 3)	The $\delta^{18}O_{VSMOW}$ values are highly significant.	Caffeine has to be isolated. Limited number of samples.	(Weckerle et al. 2002)
GC–ToF-MS	Volatile and semi-volatile profile in coffee	33 samples (Brazil: 11, Colombia: 8, Costa Rica: 3, Guatemala:4, Ethiopia: 3, Indonesia: 4)	Automated HSSPME–GC–TOFMS methodology. Rapid analytical methodology.		(Risticevic, Carasek, and Pawliszyn 2008)
PTR-ToF-MS		Roasted *Arabica* coffees Brazil, Ethiopia, and Guatemala	Rapid, direct, and non-invasive technique.		(Yener et al. 2014)
HPLC + UV	Phenolic and methylxanthine profiles	107 green coffee bean samples (America: 36, Africa: 27, Asia: 44)	CART correctly classified all Vietnamese samples and recognized 88% of Indonesian samples.	Between 30 and 60% of the Cameroonian samples were misclassified.	(Alonso-Salces et al. 2009)
HPLC-Orbitrap	Chlorogenic acid content and profile	China, India, and Mexico		No details regarding soil or climatic conditions were available.	(Mullen et al. 2013)
mIR	Fingerprinting	97 samples of roasted coffee beans *Arabica*: (Colombia: 34, Guatemala: 15, Peru: 11, Brazil: 9, Costa Rica: 5, Panama: 1)	Simple implementation, and short time of analysis. Spectra acquired directly on finely powdered samples.		(Medina et al. 2017)

		Robusta (Vietnam: 8, India: 4, Uganda: 3, Indonesia: 3, Togo: 1, Tanzania: 1, Ivory Coast: 1, Cameroon: 1)	High-quality results.	
NIR	Fingerprinting		Low cost. Spectra were acquired directly on finely powdered samples.	Misclassification of Colombian samples.
NMR	Methanol extract of finely powdered samples		High-quality results.	Sample extraction is required.

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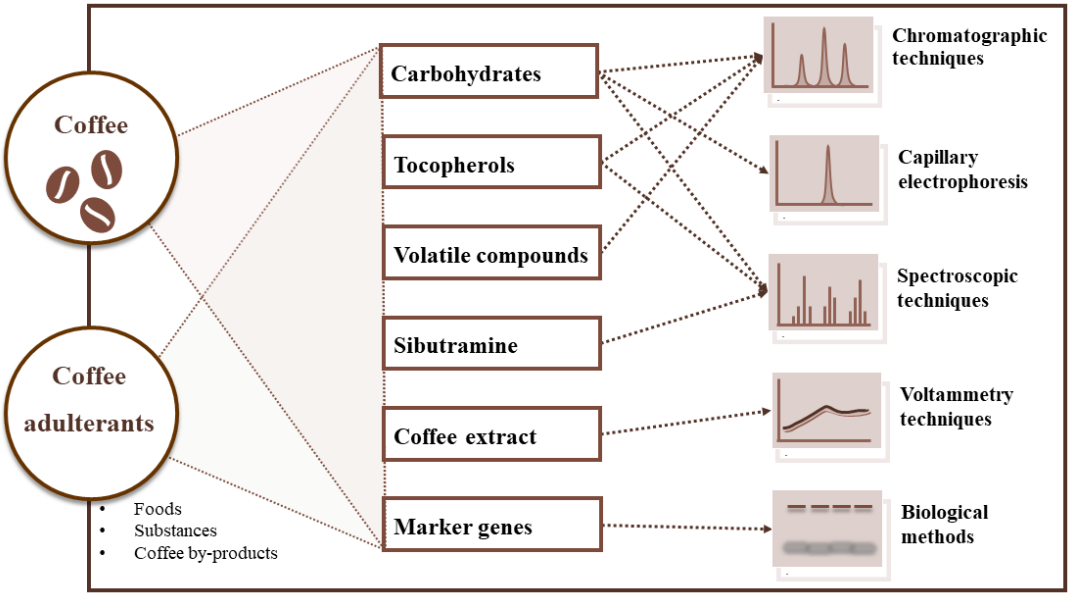

Figure 1. Methods applied to detect coffee adulteration.