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Article

SPECIAL GUEST EDITOR SECTION

Review of Analytical Methods to Detect Adulteration in Coffee

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Abstract

As one of the most consumed beverages in the world, coffee plays many major socioeconomical roles in various regions. Because of the wide coffee varieties available in the marketplaces, and the substantial price gaps between them (e.g., Arabica versus Robusta; speciality versus commodity coffees), coffees are susceptible to intentional or accidental adulteration. Therefore, there is a sustaining interest from the producers and regulatory agents to develop protocols to detect fraudulent practices. In general, strategies to authenticate coffee are based on targeted chemical profile analyses to determine specific markers of adulterants, or nontargeted analyses based on the "fingerprinting" concept. This paper reviews the literature related to chemometric approaches to discriminate coffees based on nuclear magnetic resonance spectroscopy, chromatography, infrared/Raman spectroscopy, and array sensors/indicators. In terms of chemical profiling, the paper focuses on the detection of diterpenes, homostachydrine, phenolic acids, carbohydrates, fatty acids, triacylglycerols, and deoxyribonucleic acid. Finally, the prospects of coffee authentication are discussed.

Coffee is one of the most commonly consumed beverages in the world. In general, coffees from *Coffea Arabica* (commonly known as Arabica) tend to carry more fruity notes, a buttery odor, and a roasty aroma compared to brews from *Coffea canephora* var. *Robusta* (commonly known as Robusta), which tends to be more bitter (1). Due to their finer sensory profile and other agronomical reasons (i.e., growing at elevated altitude, greater susceptibility to diseases), Arabica coffee beans command a higher price than Robusta (2), creating conditions that are prone to fraudulent practices along the value chain. In the context of "speciality coffees" [e.g., Antigua from Guatemala, Bourbon Pointu from Reunion Island, Blue Mountain from Jamaica, Kalossi from Indonesia, Tres Rios from Costa Rica, Yirgacheffe from Ethiopia, palm civet coffee (*Kopi Luwak*) from Indonesia], which are characterized by their unique sensory properties and/or limited supplies, it is susceptible to adulteration due to higher market prices (3, 4). Possible adulteration scenarios in coffee are: misrepresenting low value beans as high value counterparts, blending single-origins with other beans, mixing of two species (e.g., adding cheaper Robusta to pure Arabica), and the incorporation of undeclared plant materials (e.g., chicory, coffee husks, cereals, malt, or starch). Accidental adulteration could also occur through lack of proper bean identification techniques at processing and café levels (5). The detection of adulteration is therefore of interest to both regulatory authorities, producers, and consumers.

Through sensory cues (sight, taste, and smell), expert coffee procurers and cuppers may be capable of discerning the origins and varieties of green and roasted whole beans. However, the accuracy and repeatability can vary substantially depending on the level of experience, sensory acuteness towards specific coffee sensory traits, physiological well-being, environmental conditions, and so on. The discrimination will be more challenging for ground and brew samples, since the specific features associated with particular coffee beans may be reduced or eliminated during the grinding process. Optical and electron microscopy may be used to detect the presence of adulterants, although this approach is subjective and inefficient. Hence, other more accurate, repeatable, and reproducible analytical techniques (physical, chemical, and biological) are required (6).

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A typical workflow for the detection of adulteration first involves sample extraction by a myriad of different techniques (e.g., liquid-liquid extraction, solid-phase extraction, solid-phase microextraction), followed by the detection of chemical species of interest using analytical instruments (e.g., chromatography, spectroscopy, mass spectrometry, electronic sensors) (7). The detection of adulteration can be based on "fingerprinting" (untargeted, i.e., determination of as many metabolites as possible without specifically identifying the metabolites) or chemical "profiling" (targeted, i.e., identified and quantified related metabolites) approaches. The former is useful for rapid classification and screening of samples, while the latter yields direct functional information useful for confirming the identity of the adulterants and addressing specific objectives of the investigation. In the context of coffee authentication, both approaches exploit the use of analytical techniques based on chemical (organic marker compounds, metabolites, mineral, etc.), biomolecular (DNA, proteins), or isotopic (H, C, O, N) principles (8, 9).

The objective of this paper is to review the literature related to adulteration detection of coffee, specifically focusing on the techniques and methodologies relevant for coffee brew. Although some of the literature studies reviewed are based on the analysis of coffee beans and grounds, they are included because the experimental techniques and approaches may be adaptable for the analysis of brews, with additional sample preparatory steps.

Fingerprinting Analysis through Chemometrics

Spectral data derived from electromagnetic radiation [e.g., visible, near-infrared (NIR), mid-infrared (MIR), ultraviolet (UV)], nuclear magnetic resonance (NMR), and mass spectrometry contain molecular information that can be used as unique fingerprints of coffee species and varieties. Spectral data are highly complex; statistical procedures are often used to reduce the dimensionality of the data in order to be useful for coffee authentication, such as identification of spectrum regions that are relevant to the quality parameter, pattern recognition, and detecting the presence of outliers. For this purpose, non-supervised exploratory methods, including principle component analysis (PCA), factorial analysis, and cluster analysis are often being used (8, 10). While the chemometric approach allows the use of spectral data for sample detections, DiFoggio discussed several issues related to error propagation, such as long-term stability of the spectrometer, spectral noises, and artefacts that can result in poor prediction (11). Unlike explicit modeling where phenomenon of interest is described by a specific model (e.g., kinetic), implicit modeling methods, such as principle component regression (PCR) and partial least square (PLS), do not impose fundamental theory on the data. Moreover, fingerprint data often include variations that are not related to the phenomenon of interest, and thus must be validated and confirmed if the model includes meaningful variance (12). This section provides an overview of chemometric techniques exploited by researchers for the detection of adulteration in coffee brews.

NMR spectroscopy

NMR reveals the spin state information of carbon and hydrogen nuclei, providing specific information regarding the number of magnetically distinct atoms of the compounds being investigated. With ^{1}H-NMR, one can determine the number of distinctive hydrogen nuclei present, as well as the nature of their surrounding environment (13). This characteristic is attractive for coffee brew analysis as the technique allows analysis of brew samples without chemical derivatization and separation, which greatly simplifies the workflow.

Cagliani et al. evaluated the feasibility of using ^{1}H-NMR spectroscopy to determine Robusta composition in Arabica/Robusta blends. They prepared water extractions of Arabica and Robusta coffees, followed by analyzing the supernatant, after centrifuge treatment, with ^{1}H-NMR spectroscopy (14). They employed Orthogonal Projection to Latent Structures (OPLS) to analyze the NMR spectral data, which is a preprocessing method to remove systematic variation from the spectral data matrix, **X**, that are not correlated with the response matrix, **Y** (e.g., concentration) of interest. Examples of systematic variations that are irrelevant for the analysis could be scattering of light due to sample heterogeneity, differences in path length, limited signals in narrow spectral regions, and so on. With the removal of systematic variability in ***X*** that is orthogonal to **Y**, robust models can be achieved. Moreover, the non-correlated variations in ***X*** can be analyzed separately to understand the system better. For instance, the non-correlated variation may be due to perturbation of spectrum baseline or slope variations (15). Using the OPLS approach, Cagliani et al. were able to develop a multivariate model capable of predicting the Arabica content in the coffee blends accurately, despite the different geographical origins and roasting conditions (14). The chemical compounds that can be assigned to the ^{1}H-NMR spectra of brew samples were acetate, chlorogenic acids, caffeine, quinic acids, trigonelline, 2-furylmethanol, N-methyl pyridine, and formate. The same research group applied the similar OPLS ^{1}H-NMR technique to discriminate coffee from different geographical regions (16). Forty coffee ground samples from three continents (America, Africa, and Asia) were evaluated by extracting in deuterated water, centrifuged, and decanted for analysis. They concluded that the coffees from America were mainly characterized by fatty acids. Coffees from Africa are characterized by their chlorogenic acids and lactate. On the other hand, acetate and trigonelline were the signature species for the Asian coffee that accounted for sample differentiation (16). Using similar methods, Consonni et al. can distinguish organic coffee from conventional coffee, regardless of different geographical origins and roasting conditions applied. From OPLS discriminant analysis (DA), they determined that fatty acids, β-(1–3)-D-galactopyranose, β-(1–4)-D-mannopyranose, quinic acid and its cyclic ester are useful marker metabolites for organic coffee. On the other hand, trigonelline, chlorogenic acid (CGA) isomers, caffeine, and acetate were used as the key indicators for the conventional coffees (17). Wei et al. also successfully applied OPLS to process their ^{1}H-NMR spectra of D_2O coffee extract to achieve the taste prediction of Robusta and Arabica coffees, with light and dark roast degrees (18).

Arana et al. exploited ^{1}H-NMR spectral fingerprints to discriminate Colombian coffees from those of other regions in the world (Vietnam, Guatemala, Cameroon, Vietnam, China, Mexico, Peru, Brazil, Uganda). They prepared extracts of coffee grounds (0.2 g) in non-deuterated chromatographic grade methanol (1 mL) and then centrifuged. The supernatant was transferred to the NMR tube followed by the addition of 50 μL of deuterated methanol and then analyzed with a NMR spectrometer. The spectra were analyzed using hierarchical PLS-DA. They first identified whether the coffees were 100% Arabica (i.e., not blend, not Robusta), followed by classifying the Arabica samples into Colombian versus non-Colombian groups. Score plots of PCA exhibited clear separation between the two species.

Score plot clusters also separated into two distinctive groups; one being the Colombian coffees and the second one from other origins. The "other origins" cluster was substantially more scattered as compared to the Colombian cluster, which was attributed to the larger variances in composition of coffee samples from different parts of the world (19).

Besides being used for the discrimination of Robusta and Arabica coffees, ^{1}H-NMR spectroscopy is useful for the detection of non-coffee adulterants. Ribeiro et al. applied a simple ^{1}H-NMR method for the detection of corn, coffee husks, barley, and soybean in aqueous extract of coffee samples (20). Ground samples (150 mg) were extracted with 1000 μL of deuterated water, vortexed for 1 min and centrifuged for 10 min. The supernatant was then transferred to an NMR tube for analysis using a 600 *MHz* NMR spectrometer. To detect the presence of corn, an intense peak at 5.30 ppm was used. Signals at 5.30 and 3.15 ppm were chosen for barley detection. Peaks at 5.30 and 4.87 ppm were used for the detection of soybean and signals at 5.08 and 4.98 ppm were identified as the markers for coffee husk. Results from the NMR were in accordance with the certified pure and adulterated samples from Associação Brasileira da Indústria de Café.

These observations suggest that ^{1}H-NMR, along with multivariate analysis, can be a very powerful technique for adulteration detection in coffee (21). Further refinement of NMR methodology will expand its applicability for coffee testing, including the detection of adulterants, and elucidating how roasting conditions (e.g., time-temperature profile, degrees of roast) affect the fingerprints of coffees. Figure 1 summarizes the peak assignments of major chemical species in ^{1}H-NMR spectrum for aqueous and lipophilic extracts of Arabica and Robusta coffees.

Chromatography

Chromatography is one of the most versatile techniques for fraud detection in coffee. With optimal columns and mobile phases, mixtures of chemical species in coffee samples can be effectively separated either in gaseous [gas chromatography (GC)] or liquid [liquid chromatography (LC)] phases. In the presence of a suitable detector [e.g., flame ionization detector (FID), thermal conductivity detector (TCD), mass spectrometer (MS), diode array detector (DAD), fluorescence detector, and so on], physical or chemical properties of the eluates can be monitored as a function of time, yielding chromatograms that show unique fingerprints of the coffee specimens (23). The usefulness of the chromatographic fingerprints depends on the number of theoretical plates attained by the column for separating the mixtures, the detector's sensitivity towards the eluates, and more importantly, the reproducibility of the sample preparation step in extracting the chemical species relevant for authentication purposes. In addition, due to the inherent variation of retention time from changing of the mobile phase, stationary phase, operating pressure, and temperature and so on, pretreatment of chromatogram data is essential before chemometric analysis.

Oliveira et al. evaluated the feasibility of using GC-MS for the detection of coffee adulteration with roasted barley, focusing on looking at the headspace volatile compounds above ground samples using a solid phase micro-extraction (SPME) technique. Only chromatogram peaks with signal-to-noise ratios of higher than 50 were selected for PCA analysis. Their method was capable of detecting coffee containing roasted barley at concentrations as low as 1% (w/w). They observed that this level of detection was more feasible for dark roast coffees, for which adulteration tends to be more prevalent to evade sensory detection (24). Piccino et al. employed a solid phase extraction (SPE) method to extract the brew volatiles in order to discriminate the coffee brew of three trade classifications ("Grand cru", "Sublime", and "Authentique") of Bourbon Pointu coffee, and *Coffea arabica* var. laurina, from Reunion Island. With their GC-MS method, potent odorants specific to the trade classification were identified by their odor activity value. They established that "Grand cru" classification is characterized by aldehydes

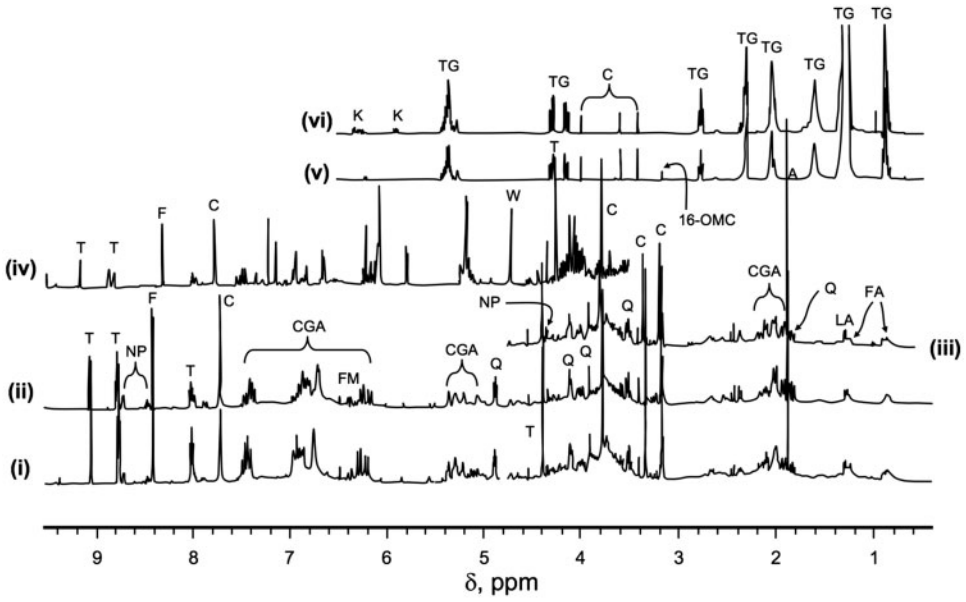

Figure 1. Peak assignments of ^{1}H-NMR spectra for (i) water extract of Robusta coffee (14); (ii) water extract of Arabica coffee (14); (iii) water extract of Arabica coffee (16); (iv) methanol extract of Arabica coffee (19); (v) lipophilic extract (in deuterated chloroform) of Robusta coffee (22); and (vi) lipophilic extract (in deuterated chloroform) of Arabica coffee (22). T: trigonelline; NP: N-methyl pyridine; F: formate; C: caffeine; CGA: chlorogenic acids; FM: 2-furyl methanol; Q: quinic acid; LA: lactic acid; FA: fatty acids; w: water; K: kahweol; TG: triglyceride; 16-OMC: 16-O-methylcafestol. Figures were re-created based on the spectral data from Cagliani et al. (14), Consonni et al. (16), Arana et al. (19), and Monakhova et al. (22).

[e.g., (E,E)-2,4-nonadienal; (E,Z)-2,4-heptadienal], "Sublime" by 2-phenylacetaldehyde, and "Authentique" by pyrazine compounds (e.g., 2-methyl-5-propylpyrazine) (3).

Carbohydrates are very useful tracers for the authenticity assessment of coffee. High performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) is the most commonly-used technique to study the carbohydrate composition of coffee and to detect coffee frauds (25). HPAEC exploits the weak acidic nature of carbohydrates to achieve selective separations at elevated pH, using an anion-exchange stationary phase without the need of derivatization. PAD of carbohydrates, with typical signal-to-noise ratios of ~10 picomol, is based on measuring the electrical current generated by the oxidation of carbohydrates at the surface of a gold electrode at high pH, by applying a positive potential. The current is proportional to the carbohydrate concentration. Between runs, the electrode needs to be regenerated by increasing the electrical potential to oxidize the gold surface, followed by a reduction in potential to reduce the electrode surface back to gold. The official method ISO 11292 is essentially based on HPAEC-PAD for the determination of free and total carbohydrates in soluble coffees (26). Domingues et al. applied a high-performance liquid chromatography (HPLC)-HPAEC-PAD method for carbohydrate analysis to detect adulteration of Brazilian coffees with triticale and acai seeds (25). They concluded that galactose was characteristic for the Arabica coffee matrix. By contrast, glucose and xylose were predominantly present in triticale, and mannose in the acai matrix. On the other hand, Domingues et al. performed the carbohydrate analysis with the post-column derivatization reaction with a HPLC-UV-Vis system. Overall, the post-column derivatization approach had lower resolution and sensitivity than the HPLC-HPAEC-PAD technique. However, in view of the faster and easier operating protocols, and the availability in most laboratories, the former can be more preferable for routine screening of adulterants during quality control, while the latter is more superior for quantification and the development of predictive modelling (25). In another study, Cai et al. applied a similar liquid chromatography technique to profile oligosaccharides in coffee. They used ultra-performance liquid chromatography (UPLC)-high resolution mass spectrometry (HRMS) to establish the oligosaccharide profiles for the detection of rice and soybean adulterants in ground coffee. Oligosaccharides were extracted, purified, and derivatized with 2,4-bis(diethylamino)-6-hydrazino-1,3,5-triazine for UPLC-HRMS analysis. From OPLS DA, they identified 17 oligosaccharide indicators that were present in rice and soybean, but not in coffee. Focusing on these oligosaccharides from the adulterants, the chemometric analysis allowed the detection of rice or soybean contents of as low as 5% in coffee (27).

Choi et al. applied an integrated metabolomic approach of combining multiple analytical data to determine the geographical origins of coffee. They fingerprinted the water:methanol (1:1) extracts of 21 coffee samples from different origins, using LC-MS and GC-FID. In addition, they conducted targeted analyses on total protein, carbohydrate, monosaccharide, and amine. Through PCA analysis of the integrated data from the non-targeted fingerprinting and targeted analyses, the researchers accurately classified the coffees into the regions of Asia, South America, and Africa (28). In an attempt to discriminate Arabica from Robusta coffees, Casal et al. investigated trigonelline, nicotinic acid, and caffeine contents in hot brews. Samples were filtered and analyzed using an HPLC equipped with a reversed-phase column and a diode array detector. A gradient mobile phase of phosphate buffer (pH 4.0; 0.1 M) and methanol was used, allowing simultaneous detection of the three analytes (29). They concluded that trigonelline and caffeine can be used in the discrimination of pure roasted Arabica and Robusta coffees, but not enough for identification of geographical origins.

Infrared (IR) and Raman Spectroscopy

The electromagnetic spectrum useful for vibrational analysis spans three regions. The mid-infrared region spans from 2.5 to 15 μm, corresponding to 4000 to 400 cm^{-1} wavenumber. The near-infrared spans from 0.77 to 2.5 μm (13 000 to 4000 cm^{-1}), while far-infrared is the region of >25 μm (<400 cm^{-1}). Because covalent bonds absorb electromagnetic radiation at the frequencies in the infrared regions, and infrared spectra is unique for specific molecule, infrared spectroscopy is a useful technique for obtaining the "fingerprints" of coffee samples. Moreover, since IR analysis is non-destructive and requires minimal sample preparation, it is a powerful tool for rapid analysis of coffee samples (13, 30).

Briandet et al. employed Fourier transform infrared (FTIR) techniques, in attenuated total reflection (ATR) and diffuse reflectance modes, to discriminate instant coffee from adulterated counterparts doped with glucose, starch, or chicory (5). They applied PCA to reduce the dimensionality of the spectral data, followed by linear discriminant analysis (LDA) of the samples. They also applied PLS regression and artificial neural network to discriminate the adulterated coffee samples. Coffee and adulterant were thoroughly mixed and then scanned directly in reflectance measurement, while for ATR analysis, samples were dissolved in water (0.33 g/mL) and then scanned. They reported that both methods were capable of 100% discriminating samples with adulterants, at a level of as low as 20 g/kg. Despite the strong absorbance from water in ATR mode, the FTIR spectral data in the fingerprint region (1000 to 1500 cm^{-1}) provided adequate signals to discriminate the adulterated from the authentic products. Similarly, Kemsley et al. employed FTIR diffuse reflectance spectroscopy and successfully discriminated Arabica (20 varieties) from Robusta (8 varieties) coffee grounds (31).

Wang et al. combined ATR-FTIR and chemometric analysis to discriminate Arabica coffees from different geographical origins (Colombia, Costa Rica, Ethiopia, Kenya) and of different degrees of roast, by analyzing the extracts obtained by exposing the coffee grounds to organic solvents (hexane, dichloromethane, ethyl acetate, and acetone; polarity indices of 0.1, 3.1, 4.4, and 5.1, respectively) in the presence of an equal volume of water (32). From PCA, the different clustering behaviors were observed for extracts prepared from different solvents, attributing to the different polarities of the solvents used. Hexane tended to extract nonpolar compounds from the coffee, while acetone extracted polar compounds. For dichloromethane and ethyl acetate, both polar and nonpolar compounds are extracted. Ethyl acetate extracts provided more distinctive PCA clusters for coffees from four countries. The main spectral regions that contribute to the differences were 1697, 1647–1643, and 1743–1741 cm^{-1}, due to isolated and conjugated C=O stretching. With this approach, coffees from different origins were all correctly classified (100%) when ethyl acetate was used as a co-solvent, by classification models based on soft independent modeling of class analogy (SIMCA) (32).

Giraudo et al. employed Fourier transform near-infrared (FT-NIR) spectroscopy and multivariate analysis to classify 191 green coffee beans from 2 continents and 9 countries (88 from central South America and 103 from Asian countries). NIR spectra of whole green beans were collected using a spectrometer

equipped with an integrating sphere in diffuse reflectance mode without pre-treatment. Their PLS-DA models were based on a hierarchical approach of considering first the continent and then the country of origin. The model allowed them to achieve more than 98% accuracy in prediction (33). Similarly, Luna et al. applied Raman spectroscopy directly on green coffee beans to classify their clonal varieties, focusing on a spectral range of 1200 and 1800 cm^{-1} (34). They compared the performance of various chemometric techniques, including LDA, mixture discriminant analysis (MDA), quadratic discriminant analysis (QDA), regularized discriminant analysis (RDA), PLS–DA, and SIMCA. Using mean centering (MC) spectral preprocessing, they reported relatively poor prediction accuracy (63, 71, 63, 63, 61, and 98% correction for LDA, MDA, RDA, QDA, PLS–DA, and SIMCA, respectively). Multiplicative scatter correction (MSC) provided more accurate results when compared to the MC technique (98% for LDA, and 100% for all other statistical methods). These findings highlight that, for whole bean analysis, due to the heterogeneity of the specimens, pre-processing treatment on the spectral data is essential.

Assis et al. combined ATR-FTIR and a novel paper spray ionization mass spectrometry (PS-MS) (35) to evaluate 120 Arabica/Robusta blends adulterated with Robusta (0.0–33.0%). PLS models based on fusion of ATR-FTIR and PS-MS data sets resulted in better predictions than the individual datasets. Furthermore, genetic algorithms (GA) and ordered predictors selection (OPS) variable selection methods allowed the removal of irrelevant information and redundant variables, reducing the number of variables to about 10% of the original spectra, facilitating the spectral interpretation. They concluded that trigonelline, chlorogenic acid, quinic acid, and sugar are the main markers that distinguished Robusta from Arabica coffees (36).

Array Sensors/Indicators

Array sensors/indicators are based on the technique of collecting a combined response simultaneously from an array of sensing elements that are responsive to the complex mixtures of compounds present in the samples, without attempting to separate them. Conventional electronic noses and tongues are based on this concept, by using multiple cross-reactive nonspecific sensors responsive to changes in physical properties or surface reaction, such as those based on metal oxide, conductive polymer, acoustic wave, quartz crystal microbalance, and so on (37, 38). This approach has been used by researchers for the discrimination of various food products, including coffees (39–43).

Suslick and colleagues adopted an alternate approach of applying colorimetric indicator array with specific chemistries for coffee aroma analyses (44). Unlike the conventional electronic nose technology that relies on nonspecific van der Waals interactions, their colorimetric array employed specific chemical reactions. They created a 6 × 6 indicator array by printing various classes of chemically responsive pigments on a poly(ethylene terephthalate) substrate, including metalloporphyrins (sensitive to Lewis bases, i.e., electron pair donation, metal ion ligation), acid indicators, base indicators, vapochromic (dyes with large permanent dipoles that respond to local polarity), and metal salts (respond to redox reactions). Saturating the colorimetric array to the aroma of commercial roasted coffee samples caused color changes of the dye elements. Subtracting the final color with the initial color of each of the elements resulted in a color difference map that represent the molecular fingerprint for each of the coffee aroma (Figure 2a). By analyzing the 108-dimensional vector [i.e., changes in red, green, and blue (RGB) values in the 36 elements) using hierarchical cluster analysis, they were able to accurately identify the coffees in all 55 cases. Their PCA also revealed extremely high levels of dimensionality for the array indicator; 18 dimensions were required to define 90% of the total variance as compared to 2–3 dimensions in traditional electronic nose analysis that are relying on physisorption onto metal oxides or into polymer films (44). Suslick et al. further demonstrated the feasibility of using the colorimetric array indicator for discriminating Columbian coffees roasted at different time-temperature conditions with 100% accuracy in 45 trials (Figure 2b). Kim and Kang adopted a similar approach to use a 3 × 4 colorimetric sensory array for the classification of coffees (45). Their approach is based on printing twelve sensing elements on a polyvinylidene fluoride hydrophobic membrane, forming an array indicator capable of interacting with coffee aroma volatiles and displaying different color patterns. The first group of sensing array elements was made up of a mixture of a pH indicator (thymol blue, cresol red, bromocresol purple, neutral red, bromoxylenol blue, and metanil yellow) with 2,4-dinitrophenylhydrazine stabilized with PEG 400. These elements react with carbonyl compounds (e.g., Strecker aldehydes and α-diketones) and change color. The second group of elements was targeting sulfur compounds (e.g., thiols and mercaptophenol, 2,5-dimethylfuran-3-thiol, methanethiol, 2-furfurylthiol, etc) (46, 47), made up of a mixture of 5,5′-dithiobis(2-nitrobenzoic acid) and a pH dye (bromothymol blue, cresol red, bromoxylenol blue, bromophenol blue, chlorophenol red, or metanil yellow) with PEG 400 as a stabilizer. The colorimetry sensory array displayed distinctive color patterns which differentiated Arabica and Robusta coffees, on the basis of their brew methods and degree of roast (Figure 2c). The researchers demonstrated that the array indicator, in conjunction with principal component and hierarchical cluster analyses, was capable of rapid discrimination of Arabica and Robusta coffees with different roast degrees and brewing procedures. The same research group applied a similar colorimetric indicator array and was able to discriminate coffee samples processed to different roast degrees (48).

Chemical Profiling through Compositional Analyses

Diterpenes

Cafestol, kahweol, and 16-O-methylcafestol (16-OMC) are the three main diterpenes found in the lipid fraction of coffees (Figure 3). Arabica coffees contain substantially higher contents of cafestol and kahweol. On the other hand, Robusta coffees contain mainly cafestol with a small amount of kahweol, along with 16-OMC. Free diterpenes are present in small quantities in coffee oils; the majority of them present as diterpene esters with fatty acids. Approximately 98% of these diterpene esters are with palmitic, linoleic, oleic, stearic, arachidic, and behenic acids, representing 9.4–21.2 g/kg and 2.2–7.6 g/kg on a dry weight basis for Arabica and Robusta coffees, respectively. Cafestol contents are 5.2–11.8 g/kg and 1.2–4.2 g/kg for Arabica and Robusta, respectively (49).

Since 16-OMC esters are present only in Robusta (~10–50 mg/kg) and not in Arabica coffees, and as it is stable during the roasting process, 16-OMC can be used as a reliable indicator of the presence of Robusta in coffee brews (49). Currently, the official method for the detection of 16-OMC is DIN 10779 (51, 52). To detect 16-OMC, coffee oil is extracted from the coffee samples using methyl tert-butyl ether and dichloromethane, followed by saponification. The diterpene is then determined from the unsaponifiable fraction by GC or reversed-phase

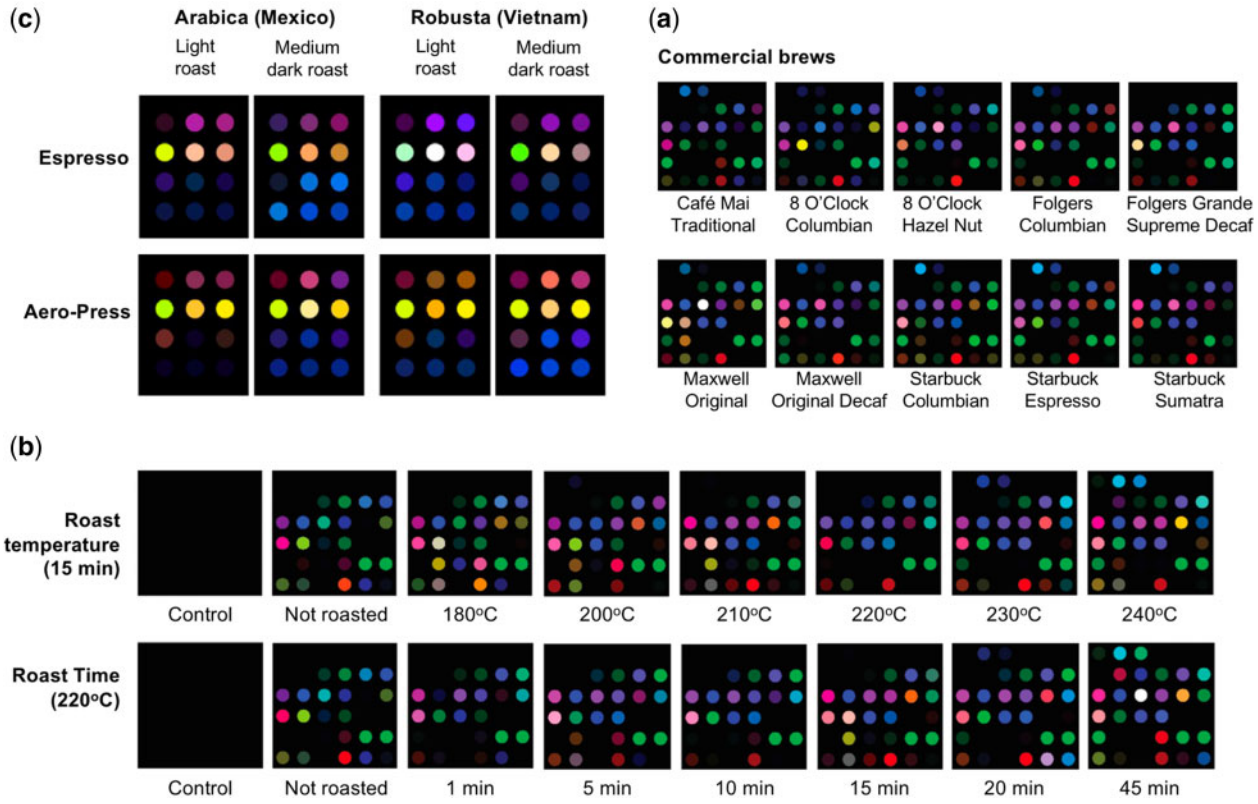

Figure 2. (a) Color changes after 2 min of exposure to commercial roasted coffees. First, second, third, fourth, and fifth rows within each of the colorimetric arrays contains different classes of chemical responsive pigments based on metalloporphyrins, acid indicators, base indicators, vapochromic, and metal salts, respectively (44). (b) Color changes of the same colorimetric array as in (a), after 2 min exposure to a Colombian coffee bean roasted at 220 °C for different durations (44). (c) Colorimetric sensor array responses to Arabica and Robusta coffees, processed to light and medium dark roast degrees, brewed using espresso and Aero-press methods (45). Within each of the 3 × 4 arrays, the top six sensor elements are sensitive to carbonyl compounds, while the bottom six elements are sensitive to sulfur compounds. Colors of the array elements shown are based on subtracting the RGB color values of the initial image from those of the final images. Adapted from Kim and Kang (45) and Suslick et al. (44), with permissions from American Chemical Society and Elsevier.

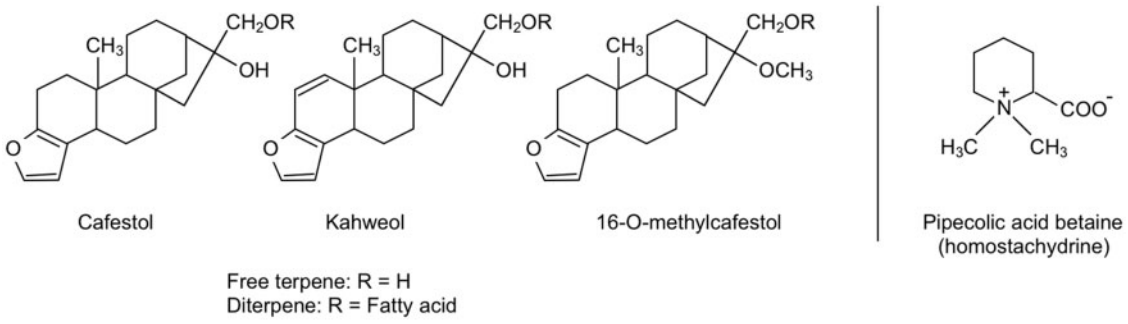

Figure 3. Chemical structures of the major diterpenes and homostachydrine in coffees. Adapted from Speer and Kölling-Speer (49) and Servillo et al. (50).

chromatography using acetonitrile/water as eluent. This process is laborious and time consuming (~5 to 6 h). Recently, a rapid method based on a high-resolution ^{1}H-NMR spectroscopy for the detection of 16-OMC was reported by Schievano et al. In their method, roasted coffee beans were grinded in liquid nitrogen, and then extracted with deuterated chloroform for 15 min. The extract was filtered through a cotton wool filter directly into the NMR tube, and ^{1}H-NMR spectrum was acquired immediately (52). This 16-OMC detection method had a limit of detection of 5 mg/kg and limit of quantitation of 20 mg/kg, which allowed the detection of the presence of Robusta at <0.9% levels. Considering the simplicity of the procedure and relatively short turnaround time, this method can be quite appealing for routine testing of large quantities of samples. Based on that, Monakhova et al. conducted ^{1}H-NMR spectroscopy analyses of lipophilic ($CDCl_3$ with 0.1% tetramethylsilane) and aqueous (warm water) extracts of 77 commercial coffee samples. NMR analysis on the aqueous extracts did not reveal any specific markers, but the analysis of the lipophilic fraction confirmed that the signal at chemical shift 3.16 ppm is related to 16-OMC and can be used to detect the presence of Robusta. Meanwhile, a signal at chemical shift 10.21 ppm, which is related to kahweol can be used as a marker for coffees from Arabica species, since this signal is negligible for Robusta coffees (22).

Recently, the peak at the chemical shift 3.16 ppm was detected in Arabica coffees by Gunning et al., using 60 and 600 MHz fields NMR with improved preparation procedure (extraction in chloroform, filtration, evaporation, followed by redissolving in deuterated chloroform) (53). Contrary to the previous belief that the peak at 3.16 ppm only arose from 16-OMC in Robusta, their findings showed that the chemical shift at 3.16 ppm could be caused by 16-OMC or 16-O-methylkahweol (16-OMK) from both Arabica and Robusta. This finding implies that the existence of the peak at 3.16 ppm cannot be used as simple indication of presence/absence test for Robusta, and the detection limit of the approach must be considered as well. Since typical Arabica coffees contain about 1–2% combined 16-OMC and 16-OMK of a typical Robusta, it is not possible to detect Robusta adulteration in Arabica below this level using 16-OMC/16-OMK as markers (53).

Other than ^{1}H-NMR method, GC has been employed by researchers to detect the presence of Robusta. For example, Pacetti et al. for the determination of Robusta content in Italian espresso coffee blends, by taking the peak ratio of kahweol (A_K) and 16-OMC (A_{OMC}), i.e., $A_K/(A_K + A_{OMC})$, without the requirement of quantifying these compounds (54). The method requires coffee oil extraction (with hexane) and saponification processes. The unsaponifiable fraction was silylated before GC analysis with flame ionization detector. They established a cubic polynomial function to correlate Robusta content with $A_K/(A_K + A_{OMC})$ ratio, yielding a strong correlation ($r^2 = 0.998$). Reportedly, the roasting conditions did not affect the accuracy of the results.

Wermelinger et al. applied the Raman spectroscopy to determine the Robusta content in Arabica/Robusta blends by analyzing the lipid fraction, obtained from Soxhlet extraction (55). They took the ratio between two Raman peaks, i.e., one for kahweol (1570 cm^{-1}) and the other for fatty acids (1460 or 1665 cm^{-1}), to determine the Robusta content in the coffee mixture. The results agreed well with the laborious HPLC-based official method DIN 10779. The Raman method had a detection limit of 4.9 to 7.5% of Robusta level. Also based on kahweol content, Keidel et al. successfully employed Fourier transform Raman spectroscopy (1064 nm excitation) to discriminate Robusta and Arabica green coffee beans from different origins (Asia, Africa, and South America). The approach resulted in decent reproducibility with an average mean standard deviation of 3.5%. Moreover, the method is singe-bean sensitive, allowing rapid detection of adulteration of low-value Robusta coffee in Arabica coffee (56). It is noteworthy that since kahweol is unstable in light, with Raman spectroscopy approach, irradiation time should be minimized and controlled.

These studies show that the determination of diterpene indicators, i.e., 16-OMC and kahweol, can be an effective strategy to detect the adulteration of Arabica with Robusta in coffees. Since diterpenes are hydrophobic, lipophilic extract fraction should be used for analysis. In the context of coffee brew, depending on the brewing method employed, the available target analytes can vary substantially due to the variation in lipid content. For example, drip coffee passing through filter paper tends to have minimal diterpene due to absorption by the cellulosic filtration medium, while Turkish coffee tends to be the highest. Light roast coffee prepared by French press or boiled preparations have the highest cafestol extraction yield, while dark roast Mocha and Turkish preparations have the lowest extraction. Also, capsule coffee tends to contain the highest cafestol and kahweol (57–61). Therefore, using diterpenes as indicators to discriminate coffee species is more optimal at coffee bean and grind levels than the final brew products.

Homostachydrine

Besides 16-OMC, one of the potential signature compounds for the detection of Robusta is homostachydrine. The compound is a positively-charged betaine (pipecolic acid betaine; Figure 3), naturally present in some plant tissues, such as those from Medicago (alfalfa), Citrus, and Achillea genera. It is believed to be an osmolyte in plants, playing important roles in plant resistance to drought and/or salinity stress (62). Homostachydrine is derived from pipecolic acid, an amino acid of higher homologue of proline (50, 63). Recently, Servillo et al. discovered that homostachydrine is present in Robusta and Arabica coffees. More importantly, they observed that the homostachydrine content was considerably higher in Robusta (31.0 ± 10.0 mg/kg) than Arabica beans (1.5 ± 0.5 mg/kg).

The homostachydrine content was detected based on a HPLC electrospray ionization (ESI)-MS technique, using a C8 column with 0.1% formic acid in water as a mobile phase. The method of extraction is relatively straightforward. Coffee grounds were extracted in 0.1% formic acid in the ratio 1:50 (w/w) under agitation for 1 h and then centrifuged at 18 000 *g* for 30 min, filtered, and then analyzed. This approach is simpler and less time-consuming than the official DIN 10779 method for 16-OMC detection. Also, since 16-OMC content spans a large range (0.8 to 2.5 g/kg) in Robusta coffees, using this indicator compound for accurate determination of Robusta in a blend is challenging. On the other hand, the relatively narrow variation of homostachydrine content in Robusta beans (31.0 ± 10.0 mg/kg) can serve as a more accurate indicator to quantify Robusta percentage in coffee blends (63).

Phenolic Acids

Coffee brew contributes significantly to the dietary intake of antioxidants for consumers, attributing to the antioxidant capacity of phenolic compounds and melanoidins. Chlorogenic acids, a family of quinic acid esterified with a trans-cinnamic acid moiety (e.g., caffeic, ferulic, and p-coumaric acid), are the main phenolic acids in coffee, ranging from 3 to 12 g/100 g of green coffee (dry weight basis) (64–66).

Górnaś et al. investigated the phenolic acid profiles of boiled-type coffee brews, both regular and decaffeinated beans from different regions and species, roasted to different degrees (67). The phenolic acid was extracted from the brew using ion exchange column and characterized using HPLC. The predominant phenolic acids in green and roasted coffee samples are 3-, 4- and 5-caffeoylquinic acids, with the content in green beans being 2- to 6- fold higher than the roasted samples. Moreover, Robusta has a higher content of phenolic acid as compared to Arabica species, which was reported by other researchers as well (64–66). However, total phenolic acid alone is not enough to accurately distinguish Arabica and Robusta roasted coffee beans, considering the sensitivity to degree of roast and relatively small differences in concentration.

Recently, Mehari et al. profiled the phenolic compounds of 100 green Arabica coffee beans using UPLC-MS, in order to determine the geographical origin of green coffee beans from Ethiopia (68). Green coffee beans were powdered and extracted in 95% aqueous methanol and then centrifuged. The supernatant was treated with 15 mL each of Carrez reagents to remove polymeric components, filtered, and then analyzed using UPLC-MS. Principal component analysis of the data identified 3-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5 dicaffeoylquinic

acid, and 4,5-dicaffeoylquinic acid as the most discriminating phenolic compounds for the authentication of the various regional and sub-regional green coffee beans in Ethiopia (68). These findings suggest the feasibility of using phenolic compounds to distinguish coffee brews from different origins.

Carbohydrates

Polysaccharides constitute about 50% of the dry weight of green coffee beans. Branched arabinogalactan, linear mannan (and/or galactomannans), and unsubstituted cellulose are the three main fractions of polysaccharides in coffee. Arabinogalactans comprise about one-third of the polysaccharides in mature green beans, with its content in green Arabica beans lower (14%) than the Robusta beans (17%). Mannan and cellulose contents in Arabica and Robusta green beans are similar, at 22 and 7%, respectively (69–71).

Blanc et al. were among the first who studied the sugar profiles of coffee (72). They looked at more than a hundred of commercial instant coffees and extracts of roasted Arabica and Robusta coffees, and concluded that pure soluble coffees, regardless of the extraction conditions used, contained maximum levels of about 0.3% total xylose and sucrose and about 2% total glucose. Elevated levels of total xylose indicate the presence of coffee husks/parchment. In this case, the type of husks/parchments added (unroasted or roasted) can also be determined by looking at the levels of free fructose and glucose. The addition of maltodextrins and caramelized sugar can be recognized by elevated levels of maltose and total glucose, and elevated levels of sucrose and total glucose, respectively (72). Based on the same concept, Daniel et al. applied capillary electrophoresis-tandem mass spectrometry to identify coffee adulteration. It was reported that fucose can be used to detect coffee adulterated with soybean, while elevated concentrations of glucose and xylose were observed in coffee adulterated with corn (73). Thus, analysis of monosaccharide profiles can be one of the techniques available for initial screening of suspected samples.

Fatty acid and triacylglycerol

Coffee oil is made up of approximately 75% triglyceride, with linoleic (*cis*18:2n-6) and palmitic (16:0) acids being the main fatty acids. The remaining fraction constitutes unsaponifiable components, including ~19% total free and esterified diterpene alcohols, ~5% total free and esterified sterols, and a small amount of tocopherols (49). The fatty acid profiles between coffee species are different, which have been exploited by researchers as a basis for the determination of Arabica and Robusta ratio in a mixture. For example, Romano et al. analyzed the fatty acid profiles of mixed coffee grounds by gas chromatography, after Soxhlet lipid extraction (AOAC Method 14.029) and derivatization to form methyl esters. They concluded that total monounsaturated fatty acids (TMUFA) concentration, linolenic acid (*cis*18:3*n*-3) concentration, the 18:0/*cis*18:1*n*-9 ratio, and the TMUFA/(total saturated fatty acid) ratio are useful indicators for the determination of Arabica-Robusta blend proportions (Figure 4) (74). Similarly, Martin et al. extracted the lipid fractions of green and roasted coffee beans, and then analyzed their fatty acids contents using gas chromatography. They selected ten fatty acids [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)] for the differentiation of Arabica and Robusta coffees. Chemometric analysis by PCA and LDA achieved a complete separation of Arabica and Robusta coffees, with oleic, linolenic, linoleic, and myristic acids identified as the most differentiating descriptors (75). Alves et al. employed a similar technique to analyze the fatty acids of 24 coffee samples from different botanical and geotechnical regions, and achieved the discrimination of Arabica and Robusta coffees both in green and roasted stages (76). They cautioned that the fatty acid profiles of coffees were affected by roasting, specifically, trans isomers increased sharply, which may be useful as an indicator of roast-processing conditions.

Instead of analyzing the fatty acids, Cossignani et al. investigated the approach of analyzing triacylglycerol (TAG) structure (three *sn*- position of TAG) to differentiate roasted Arabica and Robusta coffees. Stereospecific analysis of TAG started with the isolation of TAG fraction from total fat by thin layer chromatography (TLC) using silica gel plates, with petroleum ether/diethyl ether/formic acid (70 + 30 + 1, v/v/v) as a developing solvent. Then, the *sn*-2-monoacylglycerols (*sn*-2-MAG) was prepared by pancreatic lipase hydrolysis, and the mixture of *sn*-1,3/*sn*-1,2(2,3)-diacylglycerols (DAG) through Grignard deacylation by adding ethyl magnesium bromide in anhydrous ethyl ether. The *sn*-1,2(2,3)-DAG was isolated by TLC from the mixture of *sn*-1,3/*sn*-1,2 (2,3) DAG using hexane/diethyl ether (1 + 1, v/v) as developing solvent, then were reacted with *sn*-1,2-diacylglycerols kinase and adenosine triphosphate disodium aqueous solution to form the *sn*-1,2-phosphatidic acids (*sn*-1,2-PA). The

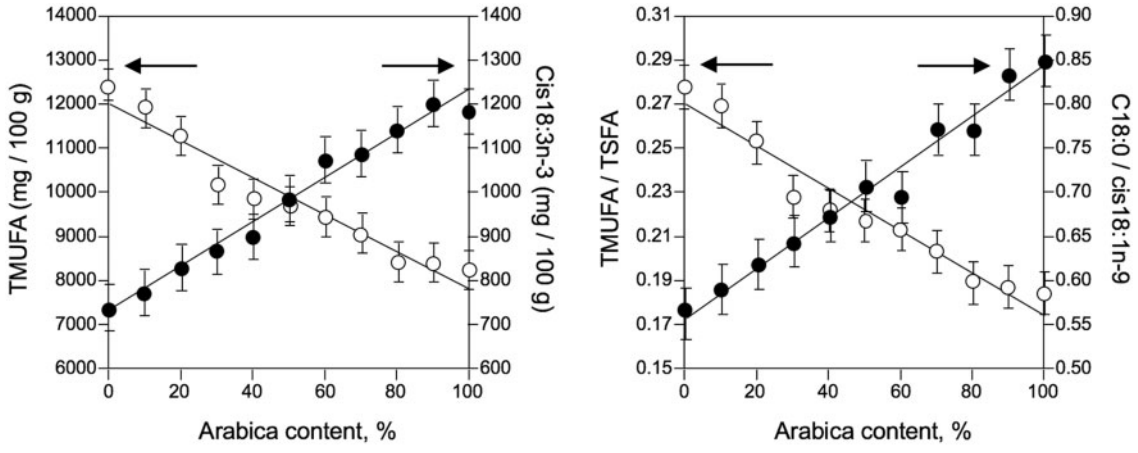

Figure 4. Correlations between TMUFA, TMUFA/TSFA, *cis*18: 3n-3, and C18: 0/*cis*18: 1n-9 with Arabica content. TMUFA: total monounsaturated fatty acid; TSFA: total saturated fatty acid; *cis*18: 3*n*-3: linolenic acid; *cis*18: 1*n*-9: oleic acid; C18: 0: stearic acid. Figures are recreated based on the data from Romano et al. (74).

sn-1,2-PA was purified by TLC using chloroform/methanol/25% ammonia (65 + 25 + 5, v/v/v) as the developing solvent. The purified TAG, *sn*-2-MAG, and *sn*-1,2-PA were transesterified for fatty acid methyl esters analysis. It was observed that Arabica coffee had a higher content of palmitic and α-linolenic acids in *sn*-1-position than Robusta, while linoleic acid tended to occupyboth *sn*-1- and *sn*-3- positions. Moreover, oleic acid was more dominant in Robusta at *sn*-1- and *sn*-3- positions. Reportedly, this procedure, in conjunction with LDA allowed the researchers to characterize roasted pure coffee samples and coffee mixtures with 10% Robusta coffee (77).

DNA

DNA detection is another powerful tool for specific detection of adulterants in coffee. This approach is capable of detecting and quantifying small amounts of specific nucleic acid sequences present in each specific coffee species, as well as other foreign biological materials. The method is based on real-time PCR, which is a technique that monitors the amplification of a targeted DNA region that exhibits genetic variations existing between coffee species.

Recently, Ferreira et al. reported the use of real-time PCR for detection and quantification of cereals as adulterants in ground roasted and soluble coffees (78). The method was sensitive and specific, with limit of quantification (LOQ) down to 0.6, 14, and 16 pg/μL, for barley, corn, and rice DNA, respectively. The marker genes that they used for barley, corn, and rice were cytochrome f, zein protein, and a hypothetical protein chromosome 1, respectively, which had no similarity to organisms of *Coffea arabica* and *C. canephora* (78, 79). Combes et al. developed a rapid and low cost DNA-based method to detect and quantify the adulterations in coffee (80). They adopted high resolution melting (HRM) analysis that allows genotyping by discriminating single nucleotide polymorphisms (SNPs), based on the melting profile of real-time PCR products. The identification of Arabica and Robusta coffee species and quantification of their blend ratios were performed by HRM analysis targeting sequences of chloroplast genome SNPs. For green beans, thresholds of admixture detection ranged from 1 to 5% of Robusta in Arabica were established, indicating the detection of adulteration and its quantification were possible for green coffee beans using this DNA method. However, they highlighted that quantification of roasted Robusta adulterants in Arabica products by HRM was not repeatable. For accurate quantification, further optimization of DNA extraction method, to dissociate inhibitors from DNA before the precipitation step, is critical (80).

A low-cost, single-use, rapid dipstick approach was developed by Trantakis et al. for the qualitative detection of Robusta in Arabica coffee mixtures. This detection method is based on visual observation of reagent dried on a nitrocellulose strip, with gold nanoparticles used as the reporters that enable visual detection (81). The principles of their method were based on PCR amplification of the DNA segment that flanks the unique species marker using a thermocycler for PCR. A 15 min DNA polymerase reaction extended an allele-specific primer occurred only if the samples contained DNA complementary to the target sequence. The products from the extension reaction were then transferred onto the gold nanoparticles conjugated nitrocellulose test strip, followed by immersing the wicking pad of the test strip into a phosphate buffer for visual detection. The development of a red-colored line, within 10 min, indicated positive response. Besides, Spaniolas et al. applied a PCR- restriction fragment length polymorphism (RFLP) and lab-on-a-chip capillary electrophoresis approach to detect and quantify the Robusta in Arabica coffees (82). Due to the simplicity, these DNA based approaches do not require highly qualified personnel and expensive equipment, which is appealing for routine testing. However, one potential concern with the molecular biology detection method is the susceptibility of thermal degradation of DNA during the roasting treatment. Detection limit of adulterant is likely affected by the different degrees of roast.

Conclusions

This review provides an overview on the analytical techniques for the authentication of coffee. The majority of the literature focused on the detection of Robusta in Arabica, while others investigated the detection of foreign components, and misrepresentation of coffees of the intended origins. The detection of coffee adulteration is technically challenging due to many possible fraudulent scenarios involved. Concerted data on coffee adulteration are practically nonexistent due to different socioeconomic situations of various producing and end-use regions, although the establishment of standard detection protocols agreeable to the industry would be beneficial to prevent fraudulent practices. Advanced analytical and instrumental techniques are available for the detection of unintended materials in coffee by identifying/quantifying their specific chemical/biological markers with sensitivity higher than ever before. However, many of these techniques require specific technical expertise for data interpretation. Moreover, the instruments are expensive and their operation tends to be costly. Moving into the future, the development of rapid, low cost, and less specific fingerprinting instruments accessible to the coffee industry will likely be the trend. The availability of accessible adulterant detection technology will enable parallel detection of adulteration on a large scale and routine quality assurance testing, which will improve the economic viability of various coffee varieties, and allow the implementation of more effective traceability programs to monitor the beans as they move though the value chain. The establishment of official protocols will be important for transferability of methods, such as using standard reference materials to normalize fingerprinting signals between equipment or labs. Sophisticated techniques will continue to find their niche in confirmation analysis of suspected adulterants and specific forensic investigation. Miniaturization and cost reduction of advanced instruments will pave the way to big data analytics (83), which capture comprehensive information from farm to cup, useful not only in the context of authentication, but also to shed light on product quality, safety, and productivity.

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