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TRACING THE TRUE COFFEE BEANS: A CLOSER LOOK AT ADULTERATION AND AUTHENTICATION

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ABSTRACT

Coffee is among the most widely consumed beverages worldwide and represents a high-value commodity with a complex global supply chain, making it particularly vulnerable to adulteration and other forms of food fraud. This review summarizes the historical background of coffee adulteration and critically discusses the major authenticity challenges associated with species substitution, geographical origin mislabeling, and the addition of low-cost adulterants such as cereals, chicory, husks, silverskin, spent coffee grounds, and legumes. Emphasis is placed on the evolution of authentication strategies, from traditional chemical and microscopic approaches to modern analytical techniques, including chromatography, spectroscopy, DNA-based tools, and emerging omics platforms. Attention is given to metabolomics, volatomics, proteomics, and fingerprinting approaches coupled with chemometric and machine learning tools, which have significantly improved the detection of adulteration and the verification of coffee origin and species identity. Despite substantial progress, important limitations remain, including matrix complexity, the effects of roasting and processing, limited availability of representative reference materials, and insufficient harmonization of food fraud reporting. Overall, the review highlights the need for integrated, rapid, reliable, and cost-effective authentication systems to strengthen regulatory control, protect consumers, and preserve trust and transparency in the global coffee market.

Keywords: coffee, adulteration, authentication, food fraud

Literature Search Strategy and Review Methodology

To provide a comprehensive overview of current approaches used for coffee adulteration detection and authentication, a structured literature search was conducted by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis). Scientific publications were retrieved from major databases including Web of Science, PubMed, Scopus, and Google Scholar. The search strategy was based on combinations of keywords such as coffee adulteration, coffee authentication, coffee fraud, coffee spectroscopy, coffee metabolomics, coffee DNA authentication, and coffee origin authentication. The literature search focused primarily on studies published between 2000 and 2024, while earlier publications were included where relevant to provide historical context. Only peer-reviewed research articles and review papers written in English were considered. Studies were selected based on their relevance to analytical methodologies for the detection of coffee adulteration, including spectroscopic, chromatographic, genetic, and omics-based techniques. Publications focusing solely on agronomic aspects of coffee cultivation without analytical relevance were excluded.
The collected literature was critically evaluated and grouped according to the type of analytical approach applied for coffee authentication. The aim of this review is therefore to provide a structured and critical overview of current analytical strategies used to detect coffee adulteration and verify coffee authenticity.

INTRODUCTION

Plant History

The coffee tree is classified within the Angiospermae kingdom, a group of plants that reproduce via seeds enclosed in an ovary at the base of the flower. It is part of the Rubiaceae family, which includes around 500 genera and over 6,000 species, such as gardenias **(Ekblaw & Ukers, 1925).** Coffee falls under the genus Coffea, the most economically significant member of the Rubiaceae family. Within Coffea, coffee belongs to the subgenus Eucoffea. Another genus in the Rubiaceae family, Psilanthus, has often been mistaken for Coffea species **(Farah & Santos, 2014).** The *Coffea* plant is a tropical, perennial woody shrub that can grow to heights of 3–12 meters if not pruned **(Mekete *et al.*, 2008).** Coffee cultivation spans about 85 tropical and subtropical countries in Coffee Belt area (Figure 1). As a short-day plant, Coffea requires a specific length of nighttime darkness to trigger flowering. Its root system is extensive, primarily concentrated within the top 60 cm of soil but capable of reaching depths of up to 3 meters **(Davis *et al.*, 2006; Masarirambi *et al.*, 2009).**
Most coffee species have persistent leaves that remain on the plant after maturity or flowering. Typically, Coffea develops a single main trunk, though it can be grown with multiple trunks. Vertical (orthotropic) branches grow from the trunk, from which near-horizontal (plagiotropic) branches emerge. Inflorescences appear in clusters along the axils of plagiotropic branches, with each axil bearing 2 to 20 flowers **(Nagai *et al.*, 2008; Ngo *et al.*, 2011**). The coffee flower structure is well-documented. Flowers are borne on short pedicels, with a five-petal calyx, white corolla, and relatively short anthers. Coffee fruits, known as drupes, usually develop with two ovules **(De Castro & Marraccini, 2006).** When only one ovule matures and the other aborts, the result is a single-seeded fruit called a peaberry **(Ngo *et al.*, 2011).** Although peaberries are often considered undesirable due to their deformity, a niche market exists for peaberry coffee **(Ricketts *et al.*, 2004).** Coffee shrubs typically require 3 to 5 years after germination to produce flowers and fruit **(Wintgens, 2018).** Since the sixteenth century, when coffee trees were first documented, coffee has captivated explorers and botanists worldwide. This interest surged in the late nineteenth century, leading to the discovery of many new species. Due to the significant diversity among coffee plants and seeds, botanists have struggled to establish a unified classification system or confirm certain plants as true members of the *Coffea* genus. While hundreds of species have reportedly been described, scientists have identified over 90 species within the genus, with 25 receiving more detailed study **(Davis *et al.*, 2006; Davis *et al.*, 2007; Farah & Santos, 2014).** Of these, only two species hold substantial commercial importance: *Coffea arabica* and *Coffea canephora*. *C. arabica* (tetraploid, 2n = 4x = 44) is believed to have originated from natural hybridization between *C. canephora* and *Coffea eugenioides* or related diploid ecotypes (2n = 2x = 22). Triploid hybrids resulting from crosses between C. arabica and diploid species are vigorous but nearly sterile. *C. arabica* trees grow up to 6 meters tall, thrive in high-elevation, mild climates, and produce seeds that contribute to over 60% of global coffee production **(Lashermes *et al.*, 1999; Anthony *et al.*, 2002).** *C. canephora* trees, on the other hand, grow up to 10 meters tall, prefer low-elevation, warmer climates, and are more disease-resistant but produce beans with inferior flavor and lower market value, accounting for less than 40% of global production. *Coffea arabica* is self-compatible, meaning it can self-fertilize, a trait observed in only two other coffee species: *C. heterocalyx* and *C. anthonyi*. In contrast, *C. canephora* is self-incompatible and relies on cross-pollination, which helps maintain genetic diversity despite its lower cup quality **(Maurin *et al.*, 2007)**. The third notable species, *Coffea liberica* (diploid, up to 18 meters tall), grows in hot, low-elevation climates but is characterized by poor flavor quality, disease susceptibility, and minimal market contribution (under 1%) **(Hendre *et al.*, 2008)**. *Coffea liberica*, a diploid species, is cultivated on a limited scale due to its high susceptibility to

diseases, particularly Fusarium xylarioides. Its seeds generally produce a cup quality superior to *C. canephora* but inferior to C. arabica. Although closely related phylogenetically, *C. liberica* and *C. canephora* exhibit significant morphological differences (Figure 2), making *C. liberica* a potential candidate for interspecific breeding programs **(N'Diaye *et al*., 2005; Farah, 2019).**

Coffee Market

Coffee has become an integral part of modern daily life, serving as both a source and a vehicle for caffeine, the most widely consumed psychoactive substance globally. Despite its widespread use, the health effects of coffee and caffeine remained largely unexplored until recent years. While coffee was introduced to Europe in the late 16th century and caffeine was isolated in the early 19th century, it is only in recent decades that significant attention has been given to the diverse array of chemical compounds found in this complex beverage **(Dye, 2001; Farah *et al*., 2019).** In recent decades, coffee has transitioned from being a simple commodity to a specialty product, a shift often described through the framework of the "three waves of coffee consumption" **(Manzo, 2014).** The "first wave", beginning in the 1960s, was defined by mass-market appeal, rapid growth in consumption, and the widespread availability of coffee. In the early 1990s, annual coffee consumption was on a steady decline, dropping from about 35 gallons per person in 1970 to 25 gallons per person by 1990. During this time, the coffee industry underwent significant consolidation, with most consumers relying on drip and instant coffee. This era (the "first wave"), was characterized by stagnant market shares and declining consumption despite substantial investments in advertising **(Samoggia & Riedel, 2018).**

By the early 1990s, however, a premium coffee market began to emerge. Dominated by major players like Kraft General Foods (Maxwell House) and Procter & Gamble (Folgers), which together controlled 65% of the market, the industry largely overlooked the rise of smaller, specialized coffee brands entering the market **(Dolbec *et al*., 2022).** Starbucks and other coffee houses rose within this upscale segment and drove the market throughout the 1990s. This period is colloquially referred to as the "second wave". These establishments introduced specialty coffee, catering to a growing consumer interest in coffee quality. During this period, coffee transitioned from a commodity to a luxury product **(Carvalho *et al*., 2015)** and "transformed gourmet coffee from a yuppie status symbol into a mainstream consumer good" (Sood & Tellis, 2005). The "third wave" of coffee originated with small roasters emphasizing specific regions and innovative brewing techniques. Coffee has since evolved into a high-quality artisanal product, often likened to wine. Drinking coffee has transcended mere consumption, becoming associated with pleasure, experience, lifestyle, and social status. This shift in consumer behavior is driven by three key aspects that now define coffee as a consumer product: pleasure, health, and sustainability **(Samoggia & Riedel, 2018).**

ICO reported that in 2015–2016, global coffee consumption reached approximately 151.3 million 60-kg bags. The United States led as the largest single-country consumer, with 25 million bags, followed by Brazil at 20 million bags, which also remains the world's largest coffee producer, contributing 55 million bags. The European Union collectively had the highest consumption at 42 million 60-kg bags. The fastest growth in coffee consumption was observed in Asia and Oceania **(Samoggia & Riedel, 2018).** Scandinavians had the highest per capita coffee consumption, with Finland leading at 12.2 kg per person. Italy, renowned for its strong coffee culture, recorded a per capita consumption of 5.6 kg. The United States, home to Starbucks, the world's leading specialty coffeehouse chain, had a per capita consumption of 4.5 kg. In comparison, the UK and Japan, countries with a rich history of tea consumption, reported lower per capita coffee consumption of 3.6 kg **(Samoggia & Riedel, 2018).** The coffee industry has evolved into a highly lucrative sector, achieving a retail market value of approximately USD 102 billion in 2020. This market is projected to grow at a compound annual growth rate (CAGR) of at least 4.28% between 2021 and 2026. Globally, the coffee value chain provides direct employment to an estimated 125 million people **(Bermudez *et al*., 2005).** In the United States alone, the coffee industry employed nearly 1.7 million people in 2015. Coffee cultivation occurs on 12.5 million farms worldwide, with the majority being smallholder operations. Notably, 95% of coffee farms are 5 hectares or smaller, and 84% are less than 2 hectares in size **(Jones *et al*., 2024).**

Global coffee production has experienced fluctuations over the past five years, influenced by factors such as weather conditions, biennial bearing cycles, and market dynamics. In recent years, the coffee market has gained considerable global significance due to its growth and extensive production. South America, particularly Brazil, stands out as a leading region for coffee production and export. According to the International Coffee Association (ICO), in 2021 worldwide coffee production was estimated at 168.5 million 60-kg bags, while global consumption reached 175.6 million bags. Brazil led the market with an output of approximately 69 million 60-kg bags, followed by other major contributors such as Vietnam, Colombia, and Indonesia **(Freitas *et al*., 2024).** Furthermore, coffee bean prices reached their highest levels in a decade, providing a significant boost to the economies of Brazil and other coffee-producing nations. Coffee consumption has also risen, with an estimated 166.34 million 60-kg bags consumed globally in 2022 **(Ridder, 2025; Desk, 2023).** As a traditional and widely consumed beverage, coffee plays a vital role in both the global and Brazilian economies. To meet the growing demand for high-quality products, the coffee market has diversified, offering a variety of options such as freshly ground coffee, instant coffee, instant coffee blends, fresh ground coffee pods, and standard decaffeinated instant coffee **(Gosalvitr *et al*., 2023).** The two most commercially significant coffee varieties are *Coffea arabica* (arabica) and *Coffea canephora* var. robusta (robusta). These species differ substantially in agronomic traits, geographical distribution, chemical composition, and sensory characteristics. Roasted arabica coffee is renowned for its refined and distinctive flavor, attributed to its higher concentration of odorants—excluding sulfur compounds—making it highly sought after. In contrast, roasted robusta beans have elevated levels of caffeine and chlorogenic acids. Local traditions also influence consumer preferences, with arabica favored for its aroma in blends, while robusta contributes to taste and body **(Sanz *et al*., 2002; Speer & Kölling-Speer, 2006).**

Price is a key differentiator, as arabica commands nearly double the market value of robusta. This economic disparity has led to a temptation to unlawfully substitute arabica with robusta. Consequently, authenticating coffee products has become a pressing challenge, driven by the widening price gap between the two species. Ensuring authenticity is crucial for the coffee industry, regulatory bodies, and consumers alike to maintain quality standards and trust in the market **(Frega, 2014).** With the expansion of the coffee market, there has been a corresponding increase in research on coffee adulteration, highlighting the need for effective regulation and quality control measures. Adulteration of roasted coffee is both common and varied, involving issues such as bean quality (including species, geographical origin, and defective beans) and the addition of cheaper substances like coffee husks, stems, maize, barley, chicory, wheat middling, brown sugar, soybean, rye, triticale, and açai to coffee blends to reduce costs **(Toci *et al*., 2015). Spink and Moyer (2011)** describe food fraud as a broad term that includes deliberate actions such as substitution, addition, tampering, or misrepresentation of food, its ingredients, or packaging, as well as false or misleading claims about a product, all carried out for economic gain. While cases of coffee adulteration typically do not pose health risks and may occasionally enhance nutritional value, such as with the addition of barley or corn, it remains essential for consumers to be informed about the contents of the products they purchase and consume. This is particularly important when higher-value food items are replaced with lower-cost alternatives, as is often the case with adulteration. Furthermore, the potential for food allergies arising from these substitutes must also be considered **(Ferreira *et al*., 2021).** Ensuring the authenticity of coffee origin is a vital component of the industry, as it guarantees that consumers receive a product that aligns with its labeling and that producers are fairly compensated for their efforts. Traditional authentication techniques, such as sensory analysis, have been widely used in the coffee industry for many years. However, these methods can be subjective, time-intensive, and susceptible to inaccuracies **(Aurum *et al*., 2023).**

Coffee Adulteration: Types, Drivers, and Economic Impact

In the early 19th century, Hassall's seminal work, *Food and Its Adulterations* (1855), provided some of the earliest documented methods for coffee authentication. This underscores the fact that coffee adulteration—like food fraud in general—is not merely a modern issue but a long-standing challenge that has persisted for centuries. Based on coffee world-market status it needs to be emphasized that due to its high commercial value, coffee is frequently subjected to fraudulent practices. Coffee adulteration has been a widespread practice since the 18th century, when coffee became popular in the West. The most common adulterants used before the 20th century were chicory, followed by corn and other cereals. Additionally, various other food items such as cereals (wheat, rye, oats, buckwheat, rice), legumes (soybeans, peas, lupin seeds), roots (chicory, beet, dandelion), vegetables (sweet potatoes, potatoes), nuts (almonds, peanuts), fruits (figs, prunes), and even non-food substances like stones and sawdust were used **Stevenson (1830)**; **Simmonds (1864)**; **Draper (1869)**; **Walsh (1894)**; **Ukers (1922)**; **Dahlgren (1938)**. Based on information published in database Knowledge Centre for Food Fraud, the EU Commission's platform, **Poláková *et al*. (2024)** points out that that coffee adulteration cases were registered in Central America countries in 2023. Based on the information in the database Knowledge Centre for Food Fraud and Quality we did research into reported cases of coffee adulteration. The figure indicates that, within the specified time frame, the highest incidence of cases was observed in the categories of product tampering and grey market activities. Nevertheless, it is important to acknowledge the potential underreporting and undetected occurrences, suggesting that the actual extent of such incidents may be significantly greater than documented. Various studies were focused on describing the potential coffee adulterants. **De Carvalhko Counto *et al*. (2022)** reported that the addition of non-coffee adulterants in roasted, and ground coffee powder compromises the quality of the beverage. In Brazil, common adulterants found in roasted and ground coffee include coffee husks, sticks, corn, barley, brown sugar, and soy. More recently, new plant-based adulterants such as açaí and triticale have also been introduced **(Pauli *et al*., 2014)**. The following table (Table 1) summarizes the most frequently reported adulterants in coffee, based on a review of current scientific literature, the nature of each adulterant, the motivation for its use, and commonly applied detection methods.

Table 1 List of most used coffee adulterants

Adulterant	Description	Detection Methods
Coffee	Addition of lower quality coffees to specialty coffee, or mixing of coffee from various geographical areas	Chromatography, omics approaches, fingerprinting
Chicory	Roasted root of Cichorium intybus; used to enhance flavor and reduce cost.	FTIR, NIR, Chromatography, Microscopy
Caramel	Caramelized sugar to mimic roast color and flavor.	HPLC, UV-Vis spectroscopy
Roasted barley	Ground roasted Hordeum vulgare; used for cost reduction and flavor mimicry.	FTIR, NMR, GC-MS, Microscopy
Maize (corn)	Roasted and ground Zea mays as a bulking agent.	DNA-based methods (PCR), NIR, FTIR
Tamarind seed powder	Ground Tamarindus indica seeds; used as bulking agent.	HPLC, FTIR, GC-MS
Date seed powder	Ground Phoenix dactylifera seeds; similar appearance to coffee.	Chromatography, FTIR, Sensory analysis
Coffee husks/silverskin	By-products reused in ground coffee for cost saving.	TGA, FTIR, Microscopy
Spent coffee grounds	Previously brewed coffee reused fraudulently.	Thermal analysis, Moisture content, Caffeine quantification
Soybeans	Ground soybeans used for protein enrichment and bulking.	ELISA, DNA barcoding, GC-MS

Note: The list was prepared based on references **Coqueiro *et al*. (2024); Ferreira *et al*. (2021); De Carvalho Martins *et al*. (2018); Winkler-Moser *et al*. (2015); Yulia and Suhandy (2021); Pauli *et al*. (2014)**.

Analytical Approaches for Coffee authentication

To detect these adulterants, early methods in the 19th century relied on chemical analysis, such as measuring caffeine, fat, and ash content. Microscopy was also employed to identify specific cellular structures of the adulterants, alongside physical examination methods like assessing color, density, and solubility. In the 20th and 21st centuries, more advanced techniques were developed to address coffee adulteration. These included spectroscopic methods, such as FTIR, NIR, and UV-VIS, often combined with chemometrics. Chromatographic methods, including HPLC and GC, were utilized to analyze specific chemical markers, while DNA-based methods like qPCR were employed to detect the presence of specific adulterants **(Mendonça *et al*., 2009; Ciabotti *et al*., 2016; Song *et al*., 2018; Cheah & Fang, 2020)**. Despite these advancements, modern methods still face challenges, including matrix effects, lack of control over reference samples, and decreased sensitivity with increased roasting of the coffee. Consequently, an ideal assessment of coffee authenticity often requires the use of multiple techniques.

OMICS APPROACHES

Strategies for coffee authentication are grounded either in targeted chemical profiling, which focuses on identifying specific markers indicative of adulterants, or in nontargeted analytical approaches that rely on comprehensive "fingerprinting" techniques to capture the holistic chemical signature of the sample **(Wang *et al*., 2020).** "Omics" approaches in food authentication refer to advanced analytical techniques that provide comprehensive datasets about the biological or chemical composition of food products. These techniques are powerful tools for detecting food fraud, identifying adulterants (Figure 1), and verifying geographical origin, species, or production methods **(Zhang *et al*., 2024)**. Due to the complexity of omics datasets, multivariate statistical methods or machine learning models are applied to classify samples, detect outliers, and to build predictive authentication models **(González-Domínguez *et al*., 2022)**.

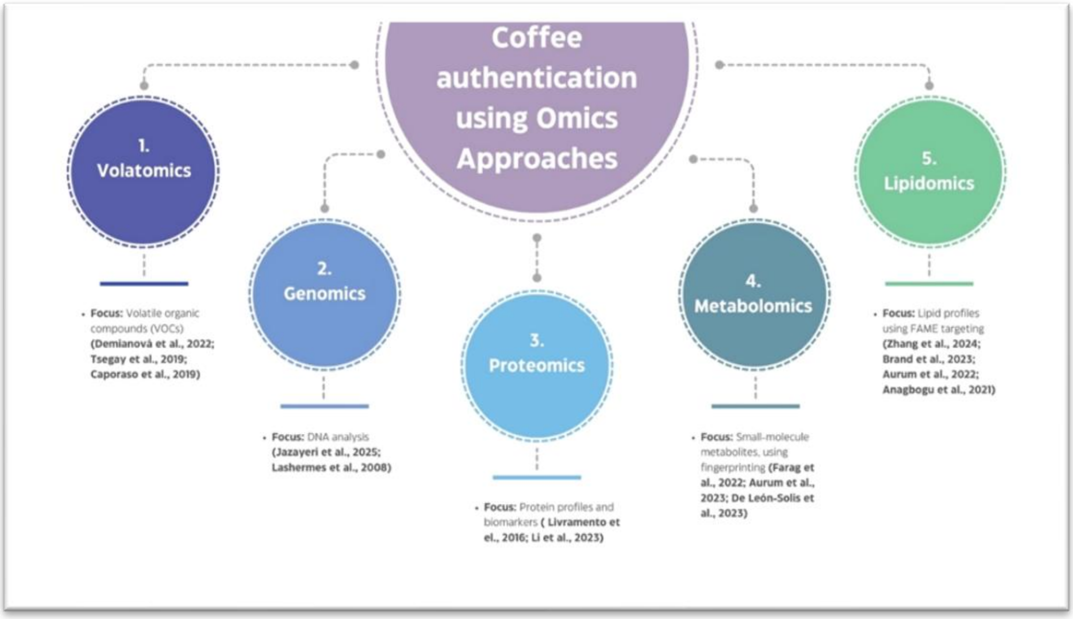

Figure 1 Classification of Omics-based strategies for the authentication of coffee

Volatomics

Metabolomics Application of GC/MS and GC/FID-based volatomics were used for the determination of markers for the authentication of Asian pal civer coffee Kopi Luwak **(Putri *et al*., 2015).** Volatomics combined with advanced statistics was used for the purpose of authentication of geographical origin of green and roasted coffee **(Caporaso *et al*., 2018; Demianová *et al*., 2022; De Vivo *et al*., 2023)**. However, such an approach can be suitable for the identification of other quality aspects, e.g., post-harvest processing. The traceability of geographical origin of coffee is challenging matter from producers and consumers perspective. Given being biological material, the concentration of VOCs can vary due to various environmental and processing reasons (Figure 2). One of the most captivating and defining attributes of coffee is its complex aroma, which is largely attributed to the presence of volatile organic compounds (VOCs). These compounds, primarily secondary metabolites, originate from various biosynthetic pathways and are structurally derived from fatty acids, isoprenoids, phenylpropanoids, and amino acids **(Bertrand *et al*., 2012; Caporaso *et al*., 2018; Yeretzian *et al*., 2019)**. The volatile profile of green coffee has been extensively investigated, revealing the presence of over 300 distinct compounds. However, only a limited number of these volatiles are considered key contributors to the characteristic aroma of unroasted coffee **(Agresti *et al*., 2007; Bertrand *et al*., 2012).**

The formation and accumulation of VOCs in green coffee are influenced by the chemical stability of their precursors and their spatial distribution within the coffee bean matrix. These biochemical precursors are, in turn, modulated by a range of environmental and agronomic conditions. Critical factors such as soil composition, microclimate, altitude, and cultivation practices play a pivotal role in shaping the volatile metabolome of coffee. Collectively, these variables are inherently linked to the geographical origin of the beans, underscoring the terroir effect in coffee aroma development **(Bhumiratana *et al*., 2011; Sunarharum *et al*., 2014; Poyraz *et al*., 2016).**

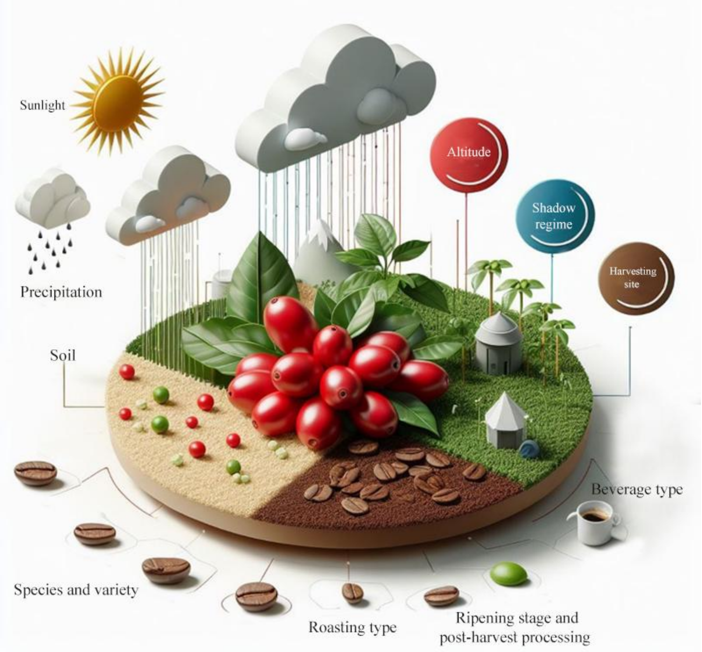

Figure 2 Influence of Agroecological Conditions and Processing Parameters on the Variability of Volatile Organic Compounds

From the perspective of advanced analytical method, Solid-Phase-Micro-Extraction (SPME) is widely used **(Caporaso *et al*., 2018; Demianova *et al*., 2022; Sádecká *et al*., 2013).** Various authors reported that a 1 cm 50/30 µm DVB/Carboxen/PDMS StableFlex fibre (Supelco, Bellefonte, USA). This type of fibre has been previously reported to be the most efficient one for coffee analysis **(Caporaso *et al*., 2018; Risticevic *et al*., 2008)**. Available literature describes that composition and concentration of volatiles vary based on chosen processing and is significantly different for green and roasted coffee. **Demianova *et al*. (2022)** identify 349 different VOCs in samples of specialty green coffee samples (n=23) from Africa, Central America, and South America. The premise of the study was that based on VOCs chemical structure and the functional group, VOCs were divided into several categories: furan derivates, aldehydes, ketones, alcohols, organic acids, hydrocarbons (alkanes, alkenes, alkynes), terpenoids, heterocyclic compounds, aromatic hydrocarbons, nitriles, amines, and others. These were used in the linear discriminant analysis with leave-one-out cross-validation for possible determination of the geographical origin of samples. The authors noticed that Wilks' Lambda test showed significant differences between geographical groups ($p < 0.0001$). The confusion matrix showed that 100% of the 23 samples under study were correctly identified. Moreover, predictions represented by prior and posterior classification and membership probabilities reached almost 87%. Variables representation pointed out that two factors can explain 100% of the variability between geographical groups. As was shown, 91.17% of variability was explained based on ketones, organic acids and esters, aldehydes, alkenes, alcohols, and nitriles. Based on vectors' length and orientation, the LDA model suggested that ketones are the strongest parameter of the green specialty coffee identification.

Proteomics

Zaman and Shan (2024) stated that a search conducted on PubMed using the keywords "Proteomics" and "Coffee" with the publication year range 2014–2024 discovered only a very limited number (n=10) of scientific articles, which emphasizes a significant knowledge gap. The availability of the coffee genome-wide sequence has enabled researchers to identify the genes and proteins present in coffee cherries through transcriptomic and proteomic analyses **(Alkimim *et al*., 2020)**. By integrating metabolomics and proteomics, both low-molecular-weight metabolites and high-molecular-weight proteins, along with the dynamic cellular state, can be examined in terms of both quality and quantity **(Berni *et al*., 2021).** Studies have reported that protein content in *Coffea arabica* and *Coffea canephora* beans varies between 10% and 15%, a variation largely attributed to the diverse methods used for protein assessment. It is estimated that around 33% of the total protein in coffee beans is bound to arabinogalactans within the cell wall. Nonetheless, detailed information regarding the specific properties of these proteins remains limited **(Anthony *et al*., 1993; Mazzafera *et al*., 2019; Zaman and Shan, 2024).** A large proportion of coffee proteins are thought to be enzymes, such as polyphenol oxidase and peroxidases, predominantly found in the cytoplasm Compared to proteins, free amino acids are present at much lower concentrations in *Coffea arabica* and *Coffea canephora*, typically ranging from 0.15% to 2.5%. Nevertheless, these amino acids play a crucial role in shaping coffee's flavor profile. In general, *C. canephora* varieties contain higher levels of all free amino acids than *C. arabica*, except for glutamate. Although the significance of various biochemical reactions in determining food quality, including coffee quality, is well understood, proteins have largely been overlooked in genetic studies focused on breeding coffee for improved quality traits **(Mazzafera *et al*., 2019; Zaman and Shan, 2024).**

A recent study published in *Eureka* leveraged the latest developments in tandem mass spectrometry (MS/MS), computational power, and proteomic database resources to perform an in-depth comparative analysis of the proteomes of *Coffea arabica* beans from two distinct cultivars. The Rwanda Shyira (RS) beans, grown at high altitudes in Rwanda's Nyabihu district, were compared with Brazil Flor de Ipe (BFDI) beans cultivated at lower elevations in the Minas Gerais region of southeastern Brazil. The study revealed, for the first time, notable differences in the proteomic profiles between the two cultivars differences that may be linked to the superior flavor attributes typically found in high-altitude *C. arabica* coffee **(Ma *et al*., 2020; Li *et al*., 2023).**

Metabolomics

Metabolomics, one of the principal "omics" technologies, is a comprehensive and systematic approach focused on analyzing metabolite concentrations and their interactions within biological systems. This methodology aims to simultaneously capture global metabolic changes across various organisms, including microorganisms, plants, animals, and humans, thereby providing direct insights into the biochemical processes occurring at cellular, tissue, organ, and organismal levels **(Selamat *et al*., 2021).** Metabolomics has been widely applied across diverse sectors such as pharmaceuticals, agriculture, and food sciences. In the context of food, metabolomics is employed to investigate food sources, production methods, processing techniques, and dietary impacts. Its application in the food sector has expanded rapidly in recent years, contributing to notable advancements alongside innovations in the food industry **(Kim *et al*., 2015; Yan & Xu, 2018).** Liquid chromatography (LC) serves as an effective alternative for the analysis of non-volatile metabolites, particularly those susceptible to degradation under gas chromatography (GC) conditions. Its separation capabilities, influenced by variations in the stationary phase, allow for broader and more detailed metabolite profiling **(Forcisi *et al*., 2013). De León-Solis *et al*. (2023)** used a total of thirty-two compounds and statistical analysis including PCA and OPLS-DA to successfully determine the geographical origin of Arabica and Robusta coffee samples from Mexico and Brazil. **El-Hawary *et al*. (2022)** revealed differences between authenticated Brazilian samples and commercially available samples obtained in the Middle East were identified using UHPLC–HRMS and UV spectroscopy in combination with multivariate data analysis.

Using metabolomic principles is not limited to authenticating the geographic origin of coffee species. Kopi Luwak is one of the most valuable coffees on the market and therefore is an interesting target for counterfeiters. **Jumhawan *et al*. (2013)** prepared counterfeited mixture of Kopi Luwak Golden and Kopi Luwak Wahana, with a commercial regular coffee (Wahana regular) in a 50:50 (wt%) ratio. Authors were able to detect and differentiate between mixed and single species samples using markers such as citric acid, malic acid, and the inositol-pyroglutamic acid ratio. Those positive results using reported markers were confirmed by **Jumhawan *et al*. (2016).** Although omics-based approaches such as metabolomics, proteomics, and volatolomics offer powerful tools for coffee authentication, several challenges limit their routine implementation in regulatory laboratories. First, omics methods generate complex multidimensional datasets requiring advanced chemometric and machine-learning approaches. Standardization of data processing pipelines remains a major challenge. Second, analytical instrumentation such as high-resolution mass spectrometry or NMR spectroscopy requires significant financial investment, limiting accessibility for routine food control laboratories. Third, regulatory validation of omics-based methods is still evolving. For practical implementation, authentication models must demonstrate reproducibility across laboratories and robustness across harvest years and geographical variations. Despite these challenges, omics technologies combined with machine learning represent one of the most promising directions for future coffee authentication systems **(Cubero-Leon *et al*., 2014; Danezis *et al*., 2016)**

Multi-omics and advanced statistics in coffee authentication

Recent advances in analytical chemistry and data science are transforming coffee authentication through the integration of multi-omics datasets with advanced statistical and machine-learning frameworks. While individual omics platforms—such as metabolomics, lipidomics, proteomics, and volatomics—provide valuable insights into the molecular composition of coffee, the intrinsic complexity of the coffee matrix and the influence of factors such as roasting, processing, and geographical variability often limit the discriminatory power of single-technique approaches. Consequently, recent studies increasingly advocate multi-omics integration, which enables the simultaneous interrogation of multiple molecular layers and provides a more comprehensive fingerprint of coffee samples. For example, metabolomic profiling using LC-MS, GC-MS, or NMR combined with multivariate analysis has demonstrated strong potential for discriminating coffee species, geographical origin, and production practices, revealing distinct metabolite signatures associated with specific cultivars and growing regions **(Belej *et al*., 2025; Gottstein *et al*., 2023).** Recent targeted metabolomics workflows coupled with machine-learning models have achieved highly accurate classification of coffee origins and varieties, highlighting the value of integrating chemical fingerprints with predictive computational algorithms **(Aurum *et al*., 2023**). In parallel, emerging lipidomic and metabolomic investigations have identified hundreds of metabolites—including chlorogenic acids, alkaloids, amino acids, and complex lipids—that serve as potential biomarkers for coffee authenticity, quality, and processing conditions **(Wang *et al*., 2024).** The interpretation of these large and complex datasets increasingly relies on multivariate chemometrics and machine learning, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), random forests, and support vector machines, which enable robust classification and predictive modeling. Moreover, data fusion strategies—ranging from low-level feature integration to high-level decision fusion—allow the combination of complementary information from spectroscopic, chromatographic, and omics-based platforms, significantly improving model robustness and detection sensitivity. Such integrative analytical frameworks represent a promising direction for the development of next-generation coffee authentication systems, enabling more reliable detection of adulteration, improved traceability of geographical origin, and enhanced protection of high-value specialty coffees within increasingly complex global supply chains **(Danezis *et al*., 2016; Demianová *et al*., 2022; Belej *et al*., 2025).**

SPECTROSCOPIC TECHNIQUES

In recent years, spectroscopic techniques have attracted increasing interest in the food industry due to their effectiveness in evaluating the chemical composition and quality of food products. These methods rely on the interaction between electromagnetic radiation and matter, generating valuable data that enable both qualitative and quantitative analyses. Compared to traditional analytical techniques, spectroscopy offers several advantages, including rapid assessment, real-time monitoring, minimal or no sample preparation, and a non-destructive approach **(Zettel *et al*., 2016).**

Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is an advanced analytical technique with significant potential for detecting adulteration in various food and beverage products **(Hachem *et al*., 2016; Hatzakis, 2018).** Although not yet widely employed for this purpose, it has been successfully utilized to authenticate oils, cereals, grains, alcoholic beverages, and fruit juices. NMR provides detailed structural and compositional information about a sample's major constituents **(Hong *et al*., 2017**). In coffee analysis, it has been applied to determine geographical origin **(Consonni *et al*., 2011**), distinguish between *Coffea canephora* and *Coffea arabica* **(Schievano *et al*., 2014; Gunning *et al*., 2017**), and establish unique chemical fingerprints for authentication **(Toci *et al*., 2017).** Key advantages of NMR include its minimal sample requirement, straightforward pretreatment, non-destructive nature, and absence of toxic waste production. Among available techniques, ^{1}H NMR is particularly valued for its simplicity and rapid analysis **(Milani *et al*., 2020)**. **Milani *et al*. (2020)** tried NMR to authenticate ground Brazilian coffee with various adulterants, such as barley, coffee husks or corn and limit of detection for all adulterants were below the 1% and the signal-to-noise ratio (SNR) below 10%. These findings confirm the reproducibility of the NMR technique, as the obtained values remained below 10%, a threshold deemed satisfactory for a complex food matrix like coffee **(Parambi *et al*., 2011).**

Lin *et al*. (2023) proved that 16-OMC (16-O-Methylcafestol) is an efficient marker for distinguishing between Robusta and Arabica coffee. The 500 MHz 1H NMR spectra were collected using a JEOL ECZ500R/S1 spectrometer running the Delta V5.3.1 software which was equipped with a 5 mm FG/RO DIGITAL AUTO TUNE probe S (NM-03822R05SS).32 mg/kg and absent in single Arabica beans. The absolute concentration of 16-OMC was calculated by comparing area value of the methyl protons at position 21 of 16-OMC 3.17 ppm (sum of all 16-OMC signals in this area) and that of the protons of pyrene at 8.09 ppc. Study indicates that 17 out of 47 blend products claiming to contain 100% Arabica had 16-OMC quantifications in the range of 155.74–784.60 mg/kg. Furthermore, all 17 products were produced by the same coffee chain. Authors also the decomposition products of 16-OMC were observed obviously in green Robusta, but the reaction mechanism is still not fully understood. **Burton *et al*. (2020)** quantitative 1H NMR methodology was developed to authenticate pure coffee (100% caffeine, trigonelline, 3- and 5-caffeoylquinic acid, lipids, cafestol, nicotinic acid, N-methylpyridinium, formic acid, acetic acid, kahweol, and 16-O-methylcafestol. Quantitative analysis was performed using a combination of traditional integration and spectral deconvolution methods and linear regression model for identification of known and unknown roasted pure coffees and blends. Authors described that the difference in distribution between kahweol and 16-OMC in arabica and robusta coffees is the basis for regression model development. Natural variations, roasting and processing might be other factors responsible for the large distribution of kahweol observed in their dataset. Although the distributions of 16-OMC and kahweol exhibit considerable variability across different geographical origins, the inclusion of a large and diverse sample set (n=292), as employed in the study, enables the regression trend line to yield consistently reliable outcomes. The findings underscore that the variability in the concentrations of 16-OMC in pure robusta and kahweol in pure arabica represents a fundamental limitation in the advancement of analytical approaches based solely on these individual markers for blend prediction. Nevertheless, establishing a correlation between the levels of 16-OMC and kahweol could significantly enhance the robustness and reliability of the proposed methodology, surpassing the predictive capacity of models that rely exclusively on 16-OMC content.

Milani *et al*. (2020) focused on using NMR to authenticate addition of barley, corn, coffee husks, soybean, rice, and wheat to medium and dark roast coffee (Figure 3).

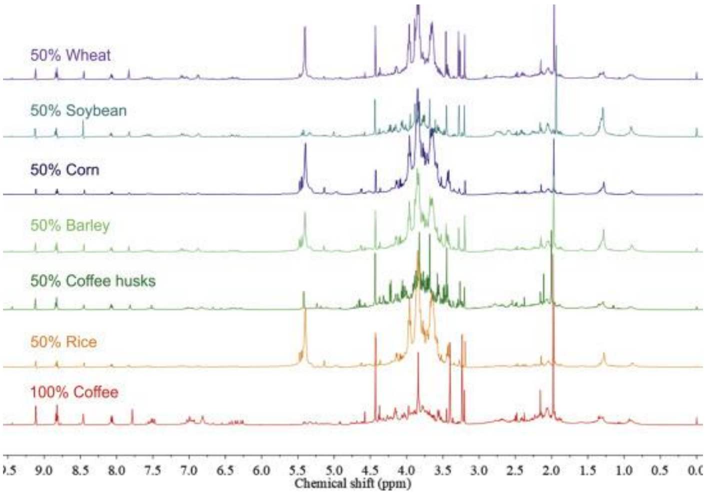

Figure 3 1H NMR pre-sat spectra for a pure medium roast coffee and coffee samples adulterated with each adulterant in the laboratory (50:50 w/w) published by **Milani *et al*. (2020).**

Their results proved that 10 of the commercial samples (n=39) were classified as adulterated given the chemical shifts found in the NMR, curves of used adulterants and the SIMCA model. Their results showed the addition mainly coffee husks and corn in medium and roasted coffees from regions: Minas Gerais, São Paulo, Argentina, and Santa Catarina.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique that examines the interaction of electromagnetic radiation within the mid-infrared range (4000–400 cm^{-1}) with various sample matrices. These interactions induce molecular vibrations, rotations, and stretching of chemical bonds, primarily involving carbon, nitrogen, hydrogen, and oxygen atoms, thus generating distinct chemical fingerprints. FTIR offers several advantages, including being non-destructive, rapid, reliable, and relatively cost-effective. Recent studies have demonstrated the successful integration of FTIR spectral data with machine learning algorithms for the authentication of coffee samples based on specific identity attributes **(Subramanian & Rodriguez-Saona, 2009; Reis *et al*., 2012; Barbin *et al*., 2014).** Consequently, developing classification models that leverage FTIR as a straightforward analytical tool targeting the organic composition of samples presents a promising alternative for verifying the global commercial quality of coffee **(Silva *et al*., 2021)** Fourier Transform Infrared spectrometry exhibits high sensitivity for caffeine quantification, utilizing its characteristic absorbance in the mid-infrared region. However, its application in coffee analysis has been relatively limited, with most research focusing on the differentiation between Arabica and Robusta coffee varieties or detecting adulterants such as sugars, starch, and chicory in instant coffee formulations **(Garrigues *et al*., 2000).** **Silva *et al*. (2021)** suggested that combination of FTIR focused on determination of carbohydrates, esters and lipids, chlorogenic acids with PCA can be used for testing the commercial coffees according to their quality degrees. **Florez-Valdez *et al*. (2020)** developed six chemometrics models' coffee–coffee husks, coffee–corn, coffee–barley, coffee–soy, coffee–oat, coffee–rice and evaluated thirty FT-MIR spectra of each adulteration system with adulteration percentage from 1 to

30%. FTIR coupled with chemometrics could correctly predict the percentage of adulterants such as coffee husks, corn, barley, soy, oat, and rice. However, **Sachairungrueng *et al.* (2022)** used FTIR to assess the levels of Robusta in Arabica coffee (Figure 4). In this case authors suggested that NIR-HIS gave higher predictive accuracy for indicating the differences between pure Arabica and Arabica blended with Robusta.

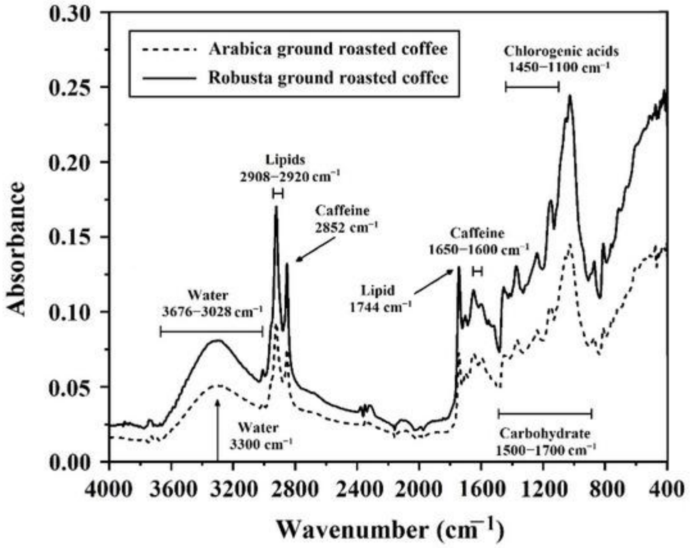

Figure 4 Average original spectra for pure Arabica and pure Robusta using FTIRs published by Sachairungrueng *et al*. (2022).

DNA METHODS

Cultivated coffee, *Coffea arabica* L. ($2n = 4x = 44$), is an allotetraploid species native to Africa, comprising two diploid genomes derived from distinct wild ancestors, *C. canephora* and *C. eugenioides* ($2n = 2x = 22$) **(Lashermes *et al.*, 1999).** C. arabica exhibits remarkably low genetic diversity, primarily due to its allotetraploid origin, reproductive biology, and evolutionary history. In contrast, diploid coffee species demonstrate substantial genetic variation **(Dereeper *et al.*, 2014).**

In recent years, DNA barcoding has become a widely adopted method for the rapid identification and classification of plants at both the genus and species levels **(Girma *et al.*, 2015; Ismail *et al.*, 2020).** This technique relies on analyzing distinct sequence variations in short genomic regions that are universally present within target lineages, exhibiting varying levels of divergence. The matK and rbcL loci have been recognized as standard plant DNA barcoding markers by the CBOL Plant Working Group of the Consortium for the Barcoding of Life, owing to their cost-effectiveness, broad applicability, high sequence quality, and strong discriminatory power, particularly at the family level **(Hollingsworth *et al.*, 2009)**. While rbcL offers higher PCR amplification success, it has lower discriminatory power due to its higher conservation across plant lineages compared to matK. In addition to coding chloroplast genes, noncoding regions have also been effectively utilized as barcode markers to establish databases for phylogenetic studies and conservation efforts **(Taberlet *et al.*, 1991)**. Several loci, including matK, rbcL, and trnL-trnF, have been extensively employed as DNA barcodes across various plant species, including certain *Coffea* species **(Maurin *et al.*, 2007; Anthony *et al.*, 2010; Kaya *et al.*, 2017; Ünsal *et al.*, 2019)**. **Mishra *et al.* (2022)** conducted study in which they tried to separate various wild coffee plant samples from India and Africa and commercially cultivated samples of *C. canephora* and *C. arabica* on their genetic level. The aligned sequences of three chloroplast loci (matK, rbcL, and trnL-trnF) were analyzed and compared with the NCBI database to identify unique sequences that could serve as potential DNA barcodes for distinguishing Indian wild *Coffea* species from other *Coffea* species. Species-specific sequences were identified in *C. jenkinsii*, *C. khasiana*, *C. travancorensis*, *C. wightiana*, and C. bengalensis. All three barcoding loci efficiently discriminate the Indian wild coffee species due to the presence of unique variable sites. The maximum likelihood tree-based phylogenetic analysis using both the individual and combined barcode loci DNA sequences yielded nearly identical dendrograms and separated the Indian and African wild coffee species effectively. Similarly, **Pokharel *et al.* (2023)** confirmed that tree chloroplast loci (matK, rbcL, and trnL-trnF) had the highest potential to separate commercially cultivated coffee varieties in Nepal.

Another DNA method used in authentication of various plants is DNA fingerprinting. **Pruvot-Woehl *et al.* (2020)** conducted study of over 2500 coffee plant samples using 8 simple sequence repeats (SSR) to create robust DNA model for authentication of *Coffea* arabica varieties. Their model was successful in tracing the history of C. arabica breeding and movement of its varieties. The genetic conformity of two commercially important varieties. Marseillesa and Gesha, proved to be 91% and 39% respectively, which is a huge difference. The high conformity of Marseillesa could be explained by their relative novelty, therefore its genetic drift is so far very limited. However, models also show that highly sought after varieties whose seeds have not moved through formal pathways have much less genetic conformity (39% for Gesha). Genetic drift, most likely related to the contamination of pollen from other varieties, is significant.

Combes *et al.* (2018) suggested usage of high-resolution melt (HRM) DNA analysis as inexpensive and quick method to distinguish between commercially most important varieties – *C. canephora* and *C. arabica*. However, this method of working properly needs a very high quality of extracted DNA. This suggests that the HRM method could be more of an additional technique of authentication and not as a primary tool.

COMPARATIVE EVALUATION OF ANALYTICAL TECHNIQUES FOR COFFEE AUTHENTICATION

The detection of coffee adulteration requires analytical methods capable of identifying subtle compositional differences between authentic coffee and potential adulterants. Numerous analytical approaches have been developed in recent years, ranging from rapid spectroscopic techniques to highly sensitive mass spectrometry-based methods and DNA-based identification strategies. Each analytical approach presents specific advantages and limitations related to sensitivity, cost, sample preparation requirements, and applicability for routine quality control.

Spectroscopic techniques, including Fourier-transform infrared spectroscopy (FTIR), near-infrared spectroscopy (NIR), and Raman spectroscopy, are widely used as rapid and non-destructive screening tools. These methods provide molecular fingerprint information that can be combined with chemometric models for the detection of adulteration or differentiation between coffee species. Spectroscopic approaches are particularly attractive for routine screening due to minimal sample preparation and rapid analysis times. However, their main limitation lies in the requirement for robust chemometric models and large calibration datasets to achieve reliable discrimination between authentic and adulterated samples **(Black, 2016; Esteki *et al.*, 2018).** Chromatographic techniques coupled with mass spectrometry, such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), allow for detailed profiling of volatile and non-volatile compounds. These techniques enable the identification of chemical markers associated with coffee species, geographical origin, and roasting conditions. GC–MS is particularly suitable for volatile compound profiling, while LC–MS is frequently used to analyze polyphenols, alkaloids, and other secondary metabolites that may serve as authenticity markers **(Pauli *et al.*, 2014; Gunning *et al.*, 2019).** Despite their high analytical sensitivity and selectivity, chromatographic approaches often require more complex sample preparation and longer analysis times compared to spectroscopic techniques.

DNA-based methods represent another powerful tool for coffee authentication, particularly for species identification. Techniques such as DNA barcoding and polymerase chain reaction (PCR) enable the detection of species-specific genetic markers even in complex mixtures. These approaches are particularly useful for distinguishing *Coffea arabica* from *Coffea canephora*, which is a common issue in coffee adulteration. However, DNA degradation during roasting and processing may limit the applicability of genetic techniques in roasted coffee products **(Martellosi *et al.*, 2005; Liu *et al.*, 2018).** More recently, omics-based approaches, including metabolomics, proteomics, and volatolomics, have emerged as powerful strategies for coffee authentication. These techniques allow comprehensive profiling of metabolites or proteins, generating large datasets that can be analyzed using multivariate statistical methods and machine learning algorithms. Omics approaches provide highly detailed chemical fingerprints that can be used for both adulteration detection and geographical origin authentication. Nevertheless, their implementation in routine quality control remains challenging due to high instrumentation costs, complex data processing, and the need for standardized databases **(Caporaso *et al.*, 2018; Ballesteros-Vivas *et al.*, 2020, Belej *et al.*, 2025).** Complex comparison of discussed techniques is shown in the Table 2.

Given the diversity of available analytical techniques, the selection of the most appropriate method depends on the specific authentication objective, available instrumentation, and required sensitivity. In practice, combined analytical strategies integrating multiple techniques and chemometric analysis often provide the most reliable approach for detecting coffee adulteration and verifying coffee authenticity.

Table 2 Comparison of Analytical Techniques Used in Coffee Authentication

Analytical method	Target analytes	Advantages	Limitations	Typical applications
FTIR / NIR spectroscopy	Molecular fingerprints	Rapid, non-destructive, minimal sample preparation	Requires chemometric models and calibration datasets	Screening coffee adulteration
Raman spectroscopy	Molecular vibrations	Rapid analysis, minimal sample preparation	Fluorescence interference possible	Species differentiation
GC–MS	Volatile compounds	High sensitivity and selectivity	Sample preparation required	Aroma profiling, origin authentication
LC–MS	Non-volatile metabolites (polyphenols, alkaloids)	High analytical resolution	Expensive instrumentation	Marker compound identification
DNA barcoding / PCR	Species-specific DNA sequences	High specificity for species identification	DNA degradation in roasted samples	Detection of *C. canephora* adulteration
Metabolomics / Proteomics	Global metabolite or protein profiles	Comprehensive chemical fingerprint	Complex data processing	Origin authentication, fraud detection

CONCLUSION

Coffee represents a globally important commodity with a complex supply chain that is particularly vulnerable to adulteration and fraud. This review summarized major adulteration practices and critically evaluated analytical strategies used for coffee authentication. Recent advances in analytical chemistry, including chromatographic, spectroscopic, DNA-based, and omics approaches, have significantly improved the detection of coffee adulteration and the verification of species identity and geographical origin. Among these, high-resolution mass spectrometry-based metabolomics and volatolomics combined with chemometric and machine learning tools appear to be particularly promising for developing robust authentication models. However, several limitations still hinder the practical implementation of these methods. Current authentication models are often constrained by limited reference datasets, compositional variability related to geographical origin and processing conditions, and the lack of standardized analytical protocols. These factors may affect the reproducibility and regulatory acceptance of proposed authentication strategies. Future research should therefore focus on the development of larger reference databases, the standardization of analytical workflows, and the integration of multi-omics approaches with advanced statistical modelling. Such developments will be essential for establishing reliable and scalable authentication systems capable of supporting regulatory control and ensuring transparency within the global coffee market.

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