

## Review

# Fraud investigation in commercial coffee by chromatography

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## Abstract

Coffee is currently the second largest commodity on the world market today, and there is great concern about the quality of the beans exported from producer countries to Europe and USA. Practices such as using blends of different species and adding low-cost raw materials, such as chicory, corn, and soybean, impair the sensory and functional characteristics of the drink made from roasted and ground coffee beans. There is a need to adopt more efficient analytical methods than the microscopy technique currently used. The first chromatographic method used to determine fraud was reported in 1958. This method used paper chromatography to differentiate between coffee and chicory based on the free reducing sugars. As of the 1980s, different methods involving high-performance liquid chromatography and gas chromatography were developed in order to demonstrate geographic authenticity, distinction between species, occurrence of adulteration, and the presence of defective beans by determining the monosaccharides, oligosaccharides, tocopherols, fatty acids, volatiles, diterpenes, sterols, and phenolic substances, among others. As far as the authors know, there are no papers published in the literature that have compiled such an extensive set of information about these chromatographic methods as here. Over the last 2 years, there has been a trend to develop analytical methods for ultra-performance liquid chromatography coupled with tandem mass spectrometry to confirm fraud in coffee, due to high sensitivity and selectivity.

**Key words:** adulteration; coffee; chromatographic methods; carbohydrates; volatile substances.

## Introduction

Coffee fruits are responsible for providing one of the most popular beverages in the world. Considered the second largest commodity in international trade, the global consumption of coffee has doubled

over the last 40 years, mainly due to its sensory and functional properties such as its stimulating and antioxidant characteristics (Domingues *et al.*, 2014; Hall *et al.*, 2015). The strong economic value of this commodity benefits the producer countries, like Brazil,

Vietnam, Colombia, Indonesia, and Ethiopia. Currently, Brazil is the leader in coffee bean production and export, mainly to the USA and the European Union (ABIC, 2018; ICO, 2018).

Considering the economic value of coffee, quality has become one of the most important aspects for the industry. Quality varies according to the species, the geographical region, the roasting process, and also the occurrence of different types of fraud (Franca et al., 2005; Barbin et al., 2014). Reports of fraud by adding other plant sources have been observed since 1851 (THE LANCET, 1851). In Brazil, the most common materials added are corn, soybean, barley, rye, caramel, and coffee husks (Tavares et al., 2012; Oliveira et al., 2015).

Currently, microscopy is the main technique used to control the quality of Brazilian coffee, but other techniques with greater sensitivity and specificity, such as chromatography, can be used for this analysis. Chromatography, through the use of different columns, solvents, and detectors, is able to make detailed investigations into fraud. This article aims to review the main chromatographic methods applied to evaluate coffee quality, especially for the detection of adulterants in coffee samples, and to report on the economic importance of coffee and its quality. No other papers available in the literature have compiled such an extensive set of information about these chromatographic methods and, therefore, this review can be considered an important tool to guide evaluations of coffee commercialized worldwide.

## Technological Aspects and Processing

The coffee tree belongs to the family Rubiaceae and the genus *Coffea*. *Coffea arabica* and *Coffea canephora*, known, respectively, as arabica and robusta, are the main species used for coffee production worldwide (Parras et al., 2007). Its fruit consists of an oval drupe of approximately 10 mm, subdivided into six parts (Esquivel and Jiménez, 2012; Bresciani et al., 2014):

1. pericarp or an external rigid smooth skin, whose colour ranges from green to dark red, depending on the maturation stage;
2. external mesocarp that is a yellowish and fibrous pulp;
3. layer of mucilage and pectin, considered translucent, thin and highly viscous and hydrated;
4. endocarp or parchment, a thin, yellowish layer;
5. perisperm or silverskin, responsible for covering the coffee bean;
6. endosperm or coffee bean.

Different ideal climatic conditions are described for these two principal species: basically arabica coffee is cultivated at higher altitudes and lower temperatures than robusta coffee (Paterson et al., 2014; ICO, 2016).

**Table 1.** Coffee production and export statics

	Total production				
	2014	2015	2016	2017	%
Brazil	52.3	50.4	55.0	51.5	-6.4
Vietnam	26.5	28.7	25.5	28.5	11.6
Colombia	13.3	14.0	14.6	14.0	-4.3
Indonesia	11.4	12.3	11.5	10.8	-6.1
Honduras	5.3	5.8	7.5	8.4	12.0
Global Scale	149.1	152.1	157.7	158.9	0

Data of the main producer countries in million bags of 60 kg (ICO, 2018).

There are two main methods used for processing the coffee bean after harvesting. In the dry process, the beans are dried in the sun, after which the skin, pulp, mucilage, and parchment are removed mechanically. In the wet process, the fruit is initially floated in water to classify and separate the ripe fruits. This is followed by removal of the skin and pulp by mechanical pressing of the fruit in water; the mucilage is removed by controlled fermentation over 12 to 48 h or by mechanical friction; and, finally, the beans are dried and stripped to remove the parchment (Gonzalez-Rios et al., 2007a; Gonzalez-Rios et al., 2007b; Esquivel and Jiménez, 2012).

Coffee is essentially traded internationally as green beans, but is also sold after the roasting stage (Esquivel and Jiménez, 2012). This thermal processing can be divided into three phases: the first corresponds to releasing water vapour and volatile substances of the bean at temperatures higher than 50°C; the second, with increasing temperatures up to 200°C, when volatilizable substances, sulfur compounds, and melanoidins are produced by pyrolysis, and the Maillard and caramelization reactions occur, all of which contribute to the final sensory properties of the product; and the third stage is immediate cooling, stopping these chemical reactions (Franca et al., 2005; Bicho et al., 2012).

The final product tends to have a weight loss between 14% and 23%, due to the loss of water and volatile substances. There is an increase in volume due to cell swelling and the destruction of polymers such as cellulose and lignin. Also the beans have a darker colour (higher melanoidin content) and an aroma with pleasant fresh roasted to even burned notes (Parras et al., 2007; Bagdonaitė et al., 2008).

## Economic Value

Coffee is the second most important commodity in international trade, behind petroleum, mainly due to the increased consumption, which has almost doubled in the last 40 years (Parras et al., 2007; Paterson et al., 2014). According to Barbin et al. (2014), coffee can be considered as the most popular beverage in the world after water.

More than 60 tropical and subtropical countries, located mostly in the “Coffee Belt” (between latitude 25° north and 25° south of the Equator Line), produce the green coffee beans for worldwide consumption (Esquivel and Jiménez, 2012; Narita & Inouye, 2014). According to International Coffee Organization—ICO (2018), the major producer countries are Brazil, Vietnam, Colombia, Indonesia, and Honduras, representing over 63% of world coffee production (Table 1).

According to Instituto Brasileiro de Geografia e Estatística—IBGE (2018), in January 2018, Brazilian production was estimated at nearly 3.2 million tons of coffee beans, with arabica representing 75% (more than 2.4 million tons) and robusta coffee

25% (708.0 tons). Considering only Brazil, which currently is the second largest consumer of the product, there has been a significant increase in the domestic sale of coffee, which over the last 20 years has doubled. Also data referring to Brazilian exports indicate a progressive growth in recent decades, especially to countries like the USA, Germany, Italy, Belgium, and Japan (ABIC, 2018).

As a result of the increased value and consumption of coffee in recent years, the quality of commercialized coffee has become of great importance and there has been increased interest in improving the handling techniques, as well as investing in better quality control of the raw material and the final product.

## Coffee Quality

Quality has become one of the most important aspects for the coffee industry and various factors can affect the final product, such as the different species used, the coffee growing region, and the degree of roasting applied (Franca *et al.*, 2005; Barbin *et al.*, 2014; Oliveira *et al.*, 2015).

One important aspect that has a negative impact on the coffee industry is the occurrence of fraud. Fraud includes the marketing of coffee and coffee products with the addition of low-cost materials and/or the presence of defective beans and even low-quality coffees or beans from different geographical regions to that reported on the product label.

Adulteration of the product is one of the main types of fraud and this was first reported in the literature in 1851, when the British scientific journal *The Lancet* (1851) highlighted the use of chicory root, corn, bean, and potato as adulterants in coffee. Two years later, another paper reported that some commercial coffee contained chicory root in concentrations of up to 50% (w/w), which was tolerated but the consumers were not adequately informed (THE LANCET, 1853).

In Brazil, a significant proportion of roasted coffee commercialized for the domestic market presents adulterants that are added before or during the roasting process, in concentrations of up to 40% (w/w). The most commonly used adulterants are corn, soybean, barley, rye, caramel, and coffee husks (Tavares *et al.*, 2012; Oliveira *et al.*, 2015).

This high level of adulteration is considered a serious threat to the sustainable development of the coffee industry and some international organizations are involved in quality assurance of coffee worldwide, such as ICO, the Institute for Scientific Information on Coffee and the Food and Agriculture Organization (FAO) (Oliveira *et al.*, 2015; Cai *et al.*, 2016). Until the beginning of 2013, the Brazilian Normative Instruction no. 16, of 24 May 2010, of Ministério da Agricultura, Pecuária e Abastecimento (BRASIL, 2010), regulated that roasted coffee beans and roasted and ground coffees could not contain impurities, sediment, and foreign matter greater than or equal to 1.0% (w/w). Consequently, the use of more sensitive and specific analytical techniques is necessary to ensure the quality of coffees commercialized worldwide.

Currently, the main Brazilian laboratories that are authorized to analyse coffee for adulterations use microscopy methods (Tavares *et al.*, 2012). According to Oliveira *et al.* (2015), the first methodologies were based on the removal of the lipid fraction of organic solvents and then these samples were compared with potential adulterants. This initial step also served as the basis for

subsequent methodologies using optical microscopy, scanning electron microscopy, and image analysis (Amboni *et al.*, 1999; Sano *et al.*, 2003). The difficulty to detect the presence of foreign substances is basically due to adulterants that have the same degree of roasting and particle size as the ground coffee. These difficulties require a long time for the analysis as well as the need for professionals qualified in the histological characterization of plants.

Other analytical techniques include infrared spectroscopy, polymerase chain reaction (PCR), and chromatography. Near and mid-infrared spectroscopy is used for quality control in the food industry. The article of Tavares *et al.* (2012) differentiated samples of pure and adulterated coffee with coffee husks using the intensity of spectral bands at specific wavelengths and principal component analysis (PCA). This method reduced the time of analysis and increased reliability.

Methods based on the replication of DNA molecules also have various advantages such as high specificity and sensitivity compared with conventional methods, because the DNA molecules remain stable during the coffee roasting process. The use of PCR in real time using fluorescent reagents allows the quantification of DNA initially present in the samples (Oliveira *et al.*, 2015). Ferreira *et al.* (2016) developed a methodology by Real-Time PCR to detect barley, corn, and rice in ground roasted coffee and soluble coffee using specific nucleic acid sequences, which was successfully applied to commercial samples from different countries.

Chromatography is currently one of the most versatile analytical techniques used to determine different types of fraud. Organic compounds with different polarities and molecular weights in concentrations up to trace level can be detected and quantified through the combination of columns, mobile phases, and detectors.

## Chromatographic Methods to Investigate Fraud in Coffee

Various different methodologies have been developed to evaluate the quality of commercialized coffee in recent decades since Skarka and Radej (1958) applied paper chromatography to differentiate coffee and chicory by quantifying the free reducing sugars. Tables 2 and 3 show, respectively, the main methods used to determine adulteration and other fraud types in commercial coffee samples.

Due to its versatility, various techniques of chromatography have been employed to investigate coffee fraud, such as high-performance liquid chromatography (HPLC) with different types of separation (normal phase, reverse, and ion exchange) and detection (absorption in ultraviolet and visible region—UV/Vis, fluorescence, pulsed amperometric detector—PAD, and refractive index—RI); gas chromatography (GC) with a capillary column and flame ionization detector (FID), thermionic detector (TID), or mass spectrometry (MS); and the latest advances which include HPLC or ultra-performance liquid chromatography (UPLC) coupled with detection by mass spectrometry.

### High-performance liquid chromatography

The first attempt to develop a method to detect fraud in coffee by HPLC was at the beginning of the 1980s. Still based on adulteration with chicory root, Smith (1981) separated and quantified the

**Table 2.** Main methods developed to determine adulteration in coffee

Reference	Analyte	Method
Skarka and Radej (1958) Smith (1981)	Free reducing sugars 5-Hydroxymethylfurfural and caffeine	Paper Chromatography High-performance liquid chromatography (HPLC) in normal phase and detection by absorption in Ultraviolet (UV) region
Clifford <i>et al.</i> (1987)	5-Hydroxymethylfurfural	HPLC in reverse phase and detection by absorption in ultraviolet (UV) region
Blanc <i>et al.</i> (1989)	Arabinose, fructose, galactose, glucose, mannose, xylose	HPLC in normal phase and, post derivatization, detection by absorption in visible region
Bernal <i>et al.</i> (1996)	Arabinose, fucose, fructose, galactose, glucose, mannitol, mannose, rhamnose, sucrose	HPLC by ion exchange and pulsed amperometric detector
Jham <i>et al.</i> (2007)	$\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -Tocopherol	HPLC in normal phase and detection by fluorescence
Jham <i>et al.</i> (2008)	Palmitic, stearic, oleic, linoleic, linolenic and arachidic Acid	Gas chromatography (GC) in capillary column and detection by flame ionization
Garcia <i>et al.</i> (2009)	Mannitol, arabinose, galactose, glucose, xylose, mannose, fructose	HPLC by ion exchange and pulsed amperometric detector
Oliveira <i>et al.</i> (2009)	Volatile and semivolatile substances	GC in capillary column and detection by mass spectrometry
Santiago <i>et al.</i> (2012)	Maltose, raffinose, stachyose, kestose, nystose, 1- $\beta$ -fructofuranosyl-nystose	HPLC in reverse phase and detection by refractive index
Pauli <i>et al.</i> (2014)	Arabinose, fructose, galactose, glucose, mannose, xylose	HPLC by ion exchange and pulsed amperometric detector
Domingues <i>et al.</i> (2014)	Arabinose, galactose, glucose, mannose, xylose	HPLC by ion exchange and detection by pulsed amperometric and, post derivatization, by absorption in visible region
Cai <i>et al.</i> (2016)	Oligosaccharides	ultra performance liquid chromatography (UPLC)-hydrophilic interaction liquid chromatography (HILIC) and detection by high resolution mass spectrometry
Tavares <i>et al.</i> (2016)	$\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -Tocopherol	HPLC in normal phase and detection by fluorescence

levels of caffeine and 5-hydroxymethylfurfural (HMF), one of the Maillard reaction products, using a Shandon column filled with silica gel Hypersil™, 5% isopropanol in dichloromethane as the mobile phase at low pressure (160 psi), and detection by absorption at a wavelength of 280 nm. This study evaluated only three samples of instant coffee and one sample of “French” coffee, a product consisting of 49% (w/w) chicory and which presented an HMF content of at least three times higher than pure coffee (Figure 1). The author proposed using the ratio between the HMF and caffeine levels as a marker of distinction among products, but he indicated that the extraction method would not be suitable for the quantification of HMF. Another paper, by Clifford *et al.* (1987), also emphasized HMF as a marker for coffee adulteration but with a different methodology using a column containing Spherisorb 5 ODS, a mobile phase in the gradient mode with a formic acid 0.5% (v/v) aqueous solution and acetonitrile and quantification at 313 nm.

Although HMF was the initial component for comparative analysis, the main methods nowadays to detect adulteration in coffee samples by HPLC involve the determination of the carbohydrate profile. Initially, the use of a detector by absorbance in the UV/vis regions was a limiting factor, and post-column derivatization steps of the samples were necessary to include chromophore groups in carbohydrate molecules (Oliveira *et al.*, 2015).

An example of this strategy was described by Blanc *et al.* (1989), in which pure and adulterated soluble coffees were differentiated through the determination of their free and total

carbohydrate profiles, after acid hydrolysis. These authors prepared the soluble coffee standards from light-roasted coffee beans by a process similar to the industrial processes. They also prepared samples of a mix with 1% (w/w) coffee husks. The carbohydrate analysis was carried out with an HPLC in normal phase, with a stationary phase such as silica previously impregnated with ammonia solution modified and a mobile phase in isocratic elution with acetonitrile and phosphate solution (80:20 v/v), a post-column derivatization with Tetrazolium Blue solution and detection by spectrophotometry in the visible region, at a wavelength of 530 nm. A method by GC with a fused silica capillary column and helium (He) as the carrier gas also was employed to analyse maltose and sucrose. Based on the specific carbohydrate profile for the pure and adulterated instant coffee, the results of a large number of commercial samples ( $n = 122$ ) confirmed that high levels of total xylose, maltose, and sucrose can be, respectively, indicative of the presence of coffee husks, maltodextrins, and caramelized sugars.

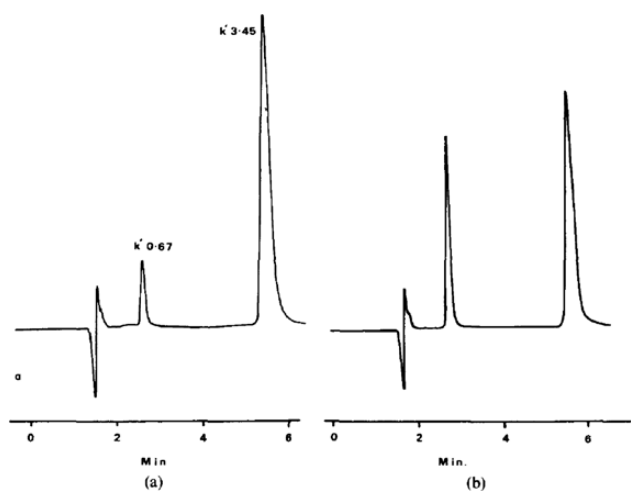
An official HPLC method to determine carbohydrate in instant coffee is recommended by ISO 11292 (1995), using ion-exchange columns and a pulsed amperometric detector. Bernal *et al.* (1996) employed it to verify adulteration in instant coffee. These authors used a Carbo-Pac PA1 column and a Carbo-Pac Guard pre-column, both Dionex™, and a mobile phase in a gradient mode with nano-filtered water and a NaOH 0.2 mol.L<sup>-1</sup> solution. This method is highlighted because of its low detection limits, which were found between 1.0 ppm and 2.5 ppm for the

**Table 3.** Main methods used to investigate other types of coffee frauds

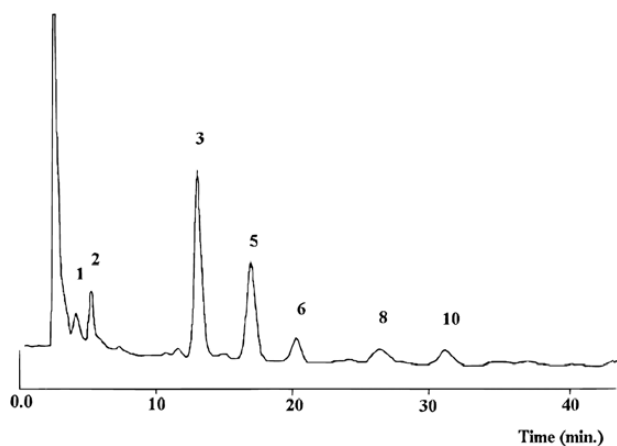
Reference	Analyte	Method	Fraud type
Clifford and Jarvis (1988)	Chlorogenic acids	High-performance liquid chromatography (HPLC) in reverse phase and detection by absorption in ultraviolet (UV) region	Geographical authenticity
Correia <i>et al.</i> (1995)	Phenolic substances	HPLC in reverse phase and detection by absorption in ultraviolet (UV) region	Geographical authenticity
Hashim and Chaveron (1995)	Methylpyrazines	Gas chromatography (GC) in capillary column and thermionic detector	Geographical authenticity
Lercker <i>et al.</i> (1995)	Diterpenic alcohols and sterols	GC in capillary column and detection by flame ionization and by mass spectrometry	Differentiation among species
Carrera <i>et al.</i> (1998)	Sterols	GC in capillary column and detection by flame ionization and by mass spectrometry	Differentiation among species
Martín <i>et al.</i> (1998)	Caffeine	HPLC in reverse phase and detection by absorption in ultraviolet (UV) region	Differentiation among species
Freitas and Mosca (1999)	Volatile substances	GC in capillary column and detection by flame ionization and by mass spectrometry	Geographical authenticity
Valdenebro <i>et al.</i> (1999)	Sterols	GC in capillary column and detection by flame ionization and by mass spectrometry	Differentiation among species
González <i>et al.</i> (2001)	$\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -Tocopherol	HPLC in normal phase and detection by fluorescence	Differentiation among species
Martín <i>et al.</i> (2001)	Fatty acids	GC in capillary column and detection by flame ionization	Differentiation among species
Kamm <i>et al.</i> (2002)	16-O-Methylcafestol and sterols	On-line HPLC in normal phase and GC in capillary column and detection by flame ionization	Differentiation among species
Murkovic and Derler (2006)	Carbohydrates and amino acids	HPLC by ion exchange and detection by pulsed amperometric and, post derivatization, by fluorescence	Differentiation among species
Agresti <i>et al.</i> (2008)	Volatile substances	GC in capillary column and detection by mass spectrometry	Presence of defective coffee beans
Akiyama <i>et al.</i> (2008)	Volatile substances	GC in capillary column and detection by mass spectrometry and by olfactometry	Geographical authenticity
Risticvic <i>et al.</i> (2008)	Volatile and semi-volatile substances	GC in capillary column and detection by mass spectrometry	Geographical authenticity
Toci and Farah (2008)	Volatile substances	GC in capillary column and detection by mass spectrometry	Presence of defective coffee beans
Alves <i>et al.</i> (2009)	$\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -Tocopherol	HPLC in reverse phase and detection by absorption in ultraviolet (UV) region and by fluorescence	Differentiation among species
Pacetti <i>et al.</i> (2012)	Diterpenic alcohols	GC in capillary column and detection by flame ionization	Differentiation among species
Romano <i>et al.</i> (2014)	Fatty acids	GC in capillary column and detection by flame ionization	Differentiation among species
Toledo <i>et al.</i> (2014)	Volatile substances	GC in capillary column and detection by flame ionization and by mass spectrometry	Differentiation among coffee types
Jumhawan <i>et al.</i> (2015)	Metabolite fingerprinting	GC in capillary column and detection by flame ionization	Differentiation among coffee types
Mehari <i>et al.</i> (2016)	Phenolic substances	ultra performance liquid chromatography (UPLC) in reverse phase and detection by absorption in ultraviolet (UV) region and by mass spectrometry	Geographical authenticity

analysed substances. Coffees adulterated with cereals and 5% chicory extract showed higher levels of free glucose and fructose than pure samples (Figure 2).

The same methodology with modifications in the mobile phases and programmed pulses was applied recently in arabica roasted and ground coffee by Garcia *et al.* (2009), Domingues *et al.* (2014), and



**Figure 1.** Chromatograms of extracts of (a) coffee powder and (b) coffee/chicory powder, separated on Hypersil™, solvent 5% isopropanol/dichloromethane and detector at 280 nm, which the majorities peaks is HMF ( $k'$  0.67) and caffeine ( $k'$  3.45). \*Reprinted (adapted) with permission from Smith (1981). Copyright © 1981 Published by Elsevier Ltd.



**Figure 2.** Chromatogram by high-performance liquid chromatography (HPLC)-PAD of a soluble coffee. Peaks: 1 = mannitol; 2 = fucose; 3 = arabinose; 5 = galactose; 6 = glucose; 8 = xylose; 10 = fructose. Coffees adulterated with cereals and 5% chicory extract showed higher levels of free glucose (peak 6) and fructose (peak 10). \*Reprinted (adapted) with permission from Bernal et al. (1996). Copyright © 1996 Published by American Chemical Society.

Pauli et al. (2014) to investigate the addition of different vegetable materials. The first paper managed to distinguish mixtures of coffee, coffee husks, and corn through chemometric methods based on the carbohydrate content. The other studies concluded statistically that glucose could be a chemical marker for triticale and wheat, mannose for açai seeds, and fructose for soybean. Although the detector employed is highly selective and sensitive, the chromatographic method has a run time of 72.6 min, because it employs an anionic column, which requires regeneration at each run for a good resolution and quantification at the desired levels of legislation. Including the sample preparation steps, it can be considered a long-time analysis and therefore impair the routine application for inspection laboratories. Another less time-consuming alternative, however, which presents lower chromatography resolution, is still described by Domingues et al. (2014). It used the same extraction method as ISO 11292:1995, but employed a cationic column (Aminex

HPX-87P, Bio-Rad) at 85°C, which does not require regeneration between the runs and its detection was done by UV-vis spectrum with post-column reaction with 4-aminobenzoyl-hydrazide (ABH).

The use of RI detectors can also be applied to determine adulterants. Santiago et al. (2012) developed a quantification method in roasted and ground coffee samples for the carbohydrates: maltose (indicative of the addition of corn), raffinose, and stachyose (indicative of soybeans) and 1-kestose, nystose, and  $\beta$ -fructofuranosyl-nystose (marker for barley and wheat middling) by HPLC in reverse phase, with a Agilent® Zorbax Carbohydrate column and a mobile phase in isocratic mode with acetonitrile and ultra-purified water. The analysis, from analytical standards, obtained a good chromatographic resolution, unlike the samples when the elution of contaminants with maltose and kestose were observed. To overcome this problem, an initial analysis with the mobile phase containing 75% (v/v) acetonitrile and 25% (v/v) water was carried out for the separation of these peaks, and then another analysis with an increase in the percentage of water to 30% (v/v) for the separation of the remaining analytes. However, this chromatographic method was not successfully applied to commercial ground and roasted coffee due to its limit of detection (LOD) and, therefore, it was not recommended for these types of commercial samples.

Other analytes were used to verify these fraud types in coffee, such as tocopherol, which has great potential as a marker of adulteration, according to Jham et al. (2007). Using the HPLC method recommended by AOAC, in normal phase (Inertsil® silica column and mobile phase consisting of hexane and 2-propanol, at a rate of 99.5:0.5) and fluorescence detection with excitation and emission wavelengths of 290 nm and 330 nm, respectively, these authors highlighted the  $\gamma$ -tocopherol contents that contributed significantly to the detection of the addition of corn in Brazilian arabica coffee samples rather than other tocopherols ( $\alpha$ -,  $\beta$ -, and  $\delta$ -tocopherol), with a detection limit of 0.5  $\mu\text{g}\cdot\text{mL}^{-1}$  for  $\gamma$ -tocopherol. The application of this method indicated, on the basis of linear regression for corn contamination and  $\gamma$ -tocopherol levels, among six commercial samples collected in Viçosa—Minas Gerais, one coffee with an 8.9% (w/w) addition of corn. Nine years later, Tavares et al. (2016) also tested the known potential of tocopherols profile for adulteration detection with coffee by-products. These authors prepared samples with husks and cleaned husks (without parchment), using a medium-dark roast level, as well as maize for distinction. The hexane extracts were injected into the HPLC in normal phase, with a Supelcosil™ LC-SI column and isocratic mobile phase *n*-hexane and 1,4-dioxane (98:2), a photodiode arrangement detector and fluorescence detector as described by Jham et al. (2007). The results were statistically treated and demonstrated that this adulteration can be perceived at low amounts (5% w/w) and distinguished at 10% (w/w) for maize and 20% (w/w) for coffee by-products. This methodology was simpler than the one reported in the previous article.

To investigate other types of fraud, liquid chromatography has also been used. In the 1980s and 1990s, two articles, based on the classic knowledge that chlorogenic acids (CGA) are present in coffee, applied HPLC with a column containing Spherisorb® 5 ODS stationary phase and detection by absorption in the UV region (280 and 313 nm) to try a differentiation between the robust coffee from Angola and other growing regions. The first, published by Clifford and Jarvis (1988), indicated that the levels of CGA in samples from the species *C. canephora* are influenced by the geographical region and reported that four substances derived from the CGA showed restricted occurrence in Angolan coffees. Seven years later, Correia et al. (1995) proved that two substances, one of them identified as

caffeoyl-tyrosine, were only detected in clarified aqueous extracts of Angolan robusta coffees rather than Angolan arabica coffees and robust coffees from other countries, and therefore can be used as markers for authenticity. Both articles showed initial data to authenticate Angolan robusta coffee. However, there are few details about the type of quantification employed and the definition of validation parameters.

The differentiation between arabica and robusta species has also featured in many articles with emphasis on different analytes. Among several chemical analyses performed, Martín *et al.* (1998) used the caffeine content, HPLC in reverse phase (Lichrosorb RP-18 column and isocratic elution with methanol 20% v/v aqueous solution at pH 4.5), quantification at a wavelength of 254 nm and applied a statistical treatment to differentiate between the two species. Robusta green coffee showed a maximum content of 5.9% (w/w) on a dry basis, whereas the arabica green coffee had 1.8% (w/w). Also Murkovic and Derler (2006), in their study concerning the amount of Maillard reaction precursors in coffee, differentiated the green beans of these two species for their contents of sucrose (higher in arabica coffee), alanine, and asparagine (greater concentrations in robusta coffee), through HPLC by anionic exchange and PAD for carbohydrates, and in reverse phase with, post-derivatization with 6-amino-*N*-hydroxysuccinimidyl-carbamate quinolyl, fluorescence detection for amino acids. These papers only studied green beans, and therefore, it would be necessary to investigate whether these substances could be used to differentiate roasted coffees, due to the effects of the thermal roasting process.

Some years before the work of Jham *et al.* (2007), the tocopherols had been studied for the discrimination of this species by other authors. González *et al.* (2001) tested the profile of tocopherols and also triglycerides as an authentication parameter by HPLC, respectively, in normal phase (Lichrosphere Si-60 column and as mobile phase *n*-hexane and propan-2-ol, 99:1 v/v) and fluorescence detection similar to Jham *et al.* (2007), and in reverse phase (Superspher 100 RP-18 Merck column and as mobile phase acetonitrile and acetone, 1:1 v/v) and RI detector. Based on 32 samples, these authors managed to differentiate *C. arabica* and *C. canephora* through the both profiles. However, the statistical tests, PCA and linear discrimination analysis (LDA), confirmed that the tocopherols profile constitutes better parameters than triglycerides for this purpose. The article of Alves *et al.* (2009) showed similar results: they proved that *C. arabica*, when compared with *C. canephora*, has higher levels of  $\alpha$ -tocopherol ( $2.7 \pm 0.4 \text{ mg} \cdot 100 \text{ g}^{-1}$  against  $1.7 \pm 0.3 \text{ mg} \cdot 100 \text{ g}^{-1}$ ) and  $\beta$ -tocopherol ( $8.0 \pm 0.9 \text{ mg} \cdot 100 \text{ g}^{-1}$  against  $2.1 \pm 0.2 \text{ mg} \cdot 100 \text{ g}^{-1}$ ). However, these levels tend to reduce 10%, on average, after roasting the beans, and, furthermore, the data interpretation, through ANOVA, *t*-tests, and cluster analysis (CA), allows the discrimination of both species (either green or roasted) according to the tocopherols profile. These studies differ mainly by their extraction methodologies, in which the first was based on the conventional Soxhlet method per 8 h with 18 g of sample and the other applied a method for exhaustion, extracting overnight at 4°C and a smaller sample mass (150 mg). The chromatographic methodology used by Alves *et al.* (2009) was applied in the study of Tavares *et al.* (2016), previously related in this present article.

### Gas chromatography

Methodologies involving GC techniques have also been tested to determine fraud in coffee samples. The main chemical marker to detect fraud, in general, is 16-*O*-methylcafestol (16-OMC) and was first reported in the article of Speer (1989). This cyclic diterpene

alcohol (Figure 3) shows heat stability and is considered absent in arabica coffee, which facilitates the quantification of the robusta coffee percentage in roasted coffee. There is an HPLC method validated by the German Institute for Standardization—DIN 10799 (1999), but, in recent years, many authors have employed GC analysis to detect and quantify the levels of this compound. Pacetti *et al.* (2012) developed a new predictive model to quantify the content of robusta in blends used for the Italian Espresso coffee, through the cubic polynomial function with the absolute GC area of 16-OMC and the other diterpene kahweol. To carry out this, a large study with 82 samples of these species using two roasting processes and mixtures with different amounts of coffee species was made. The unsaponifiable fraction of oils extracted from roasted coffees were analysed by GC using a column CP-TAP Varian coated with phenyl and methylpolysiloxane (1:1 w/w), He as the carrier gas and FID. The PCA test indicated that the results in percentage of chromatographic area of these terpenes can be used as an indicator for coffee species. The percentage of robusta in eight commercial roasted blends was predictive by this model ( $R^2 = 0.998$ ) and no significant difference (two-tailed *t*-test,  $P > 0.05$ ) was determined based on label information. Although the article also concluded that these compounds are not degraded during the analytical procedure and this method was validated for both species, the results concerning such information were not available in that article.

Still in the 1990s, Lercker *et al.* (1995) conducted a preliminary study determining the composition of diterpenic alcohols, such as 16-OMC, and sterols in the same unsaponifiable fraction of green and roasted coffee oils by GC-FID, under the same conditions as the previous article, and GC-MS by chemical ionization (CI) with methane as the reagent gas and an ion trap (IT) analyser (Figure 4). Some years later, this principle was used to distinguish, on the basis of the profile of sterols, *C. arabica* and *C. canephora* green and roasted beans, respectively, by Carrera *et al.* (1998) and Valdenebro *et al.* (1999). In these papers, the applied methodology consisted of an initial separation of the unsaponifiable fraction by thin-layer chromatography (TLC), sequence conversion into trimethyl silyl (TMS) with a derivatizing reagent, and analysis by GC-FID in a column coated with a bonded stationary phase HP-5 (5% diphenyl and 95% methylpolysiloxane) and Hydrogen ( $\text{H}_2$ ) as the carrier gas. According to Carrera *et al.* (1998) and their studies of 31 green coffee with PCA and CA, the samples may be easily classified based on the content of sitostanol and  $\Delta^5$ -avenasterol. For roasted beans, a group of samples ( $n = 40$ ), including preparations and commercial blends, was analysed and the results, through principal component regression,

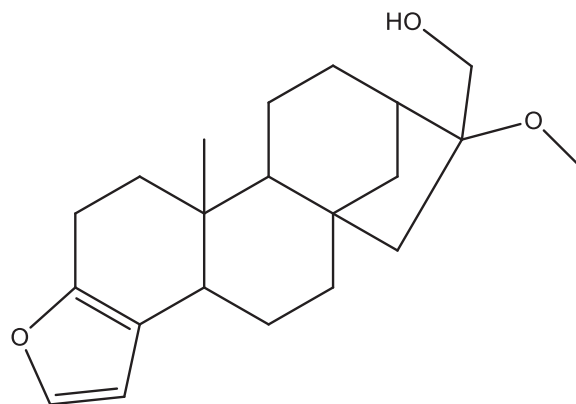
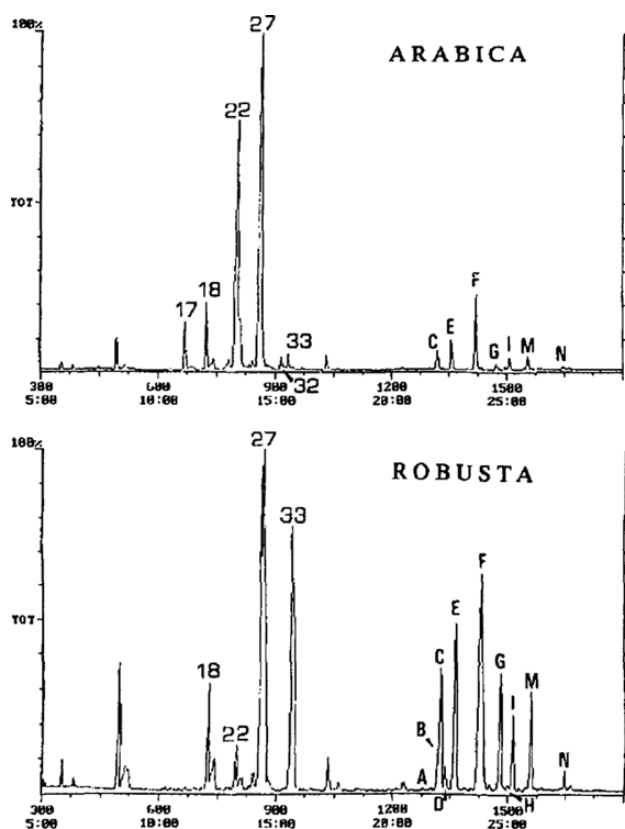


Figure 3. The structural formula of 16-*O*-methylcafestol.



**Figure 4.** Gas chromatography (GC)-ion trap MS traces of the unsaponifiable matter (after diazomethane and silylation treatment): arabica coffee and robusta coffee. Peaks: 22 = kahweol; 27 = cafestol; 33 = 16-*O*-methylcafestol; A = cholesterol; C = campesterol; D = 24-methylencholesterol; E = stigmasterol; F =  $\beta$ -sitosterol; G =  $\Delta^5$ -avenasterol; I = cycloartenol; M = 24-methylcycloartanol. \*Reprinted (adapted) with permission from Lercker et al. (1995). Copyright © 1995 Friedr. Vieweg & Sohn Verlagsgesellschaft mbH. Published by Springer.

indicated  $\Delta^5$ -avenasterol as the most adequate chemical descriptor for predicting the content of the both species of roasted coffee samples due to the good linearity and reduction of the noise caused by other analytes. Valdenebro et al. (1999) claimed that this application in nine commercial coffees also showed excellent results, but there were no statistical tests to confirm this conclusion, as the more recent article of Pacetti et al. (2012).

A rapid and simultaneous methodology involving the detection of the previously mentioned substances (16-OMC and sterols) in green coffee was developed by Kamm et al. (2002), using a fully automated online LC-GC instrument Dualchrom 3000. The authors eliminated some stages of sample preparation, such as oil saponification (basic hydrolysis), preliminary separation by TLC, and derivatization with TMS, which were considered tedious processes. Their methodology consists in the transesterification of the oil, pre-separation of the fractions of interest by HPLC, and online transfer to GC. For the HPLC analysis, a silica gel column Hypersil™ and a solution of 2-propanol 0.8% (v/v) in *n*-hexane as the mobile phase was used. After the initial separation, two fractions (I and II) were redirected to the GC-FID system with a fused-silica capillary CP-Sil8CB connected in series with an uncoated phenyldimethyl silylated fused-silica capillary and  $H_2$  as carrier gas. The fraction I was calculated based on internal standards and the fraction II that contained 16-OMC was injected into GC-MS with electron impact ionization

(EI) to confirm its identity and calculated its contents via external standards (dynamic range between 0.6  $\mu\text{g/ml}$  and 153.9  $\mu\text{g/ml}$ ;  $R^2 = 0.999$ ). These results also reported 16-OMC and  $\Delta^5$ -avenasterol as key markers for this fraud, but the specific equipment used, the low number of samples and the lack of statistical treatments impair the implementation of this methodology for routine analysis.

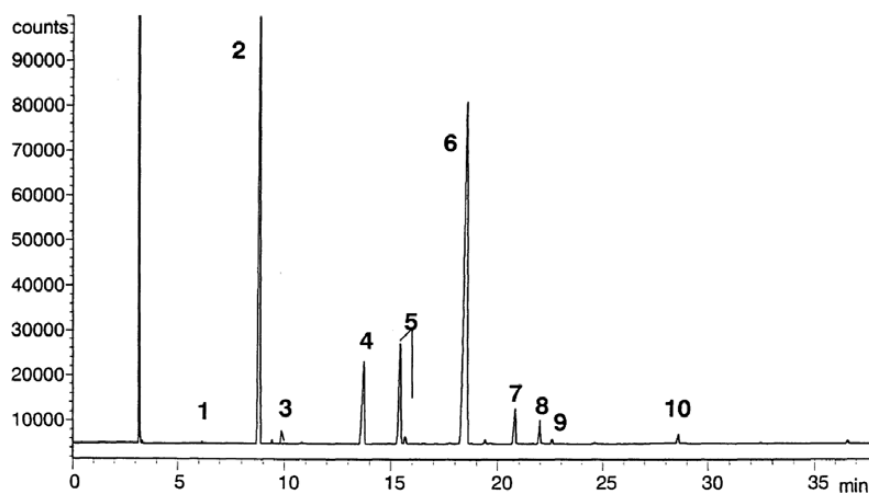
Fatty acids (FA) have also been widely used in research for this fraud investigation. At the beginning of the previous decade, Martín et al. (2001) applied the detection of methyl ester derived from fatty acids (FAME) to attempt to differentiate between the *C. arabica* ( $n = 27$ ) and *C. canephora* species ( $n = 13$ ). The authors used a GC-FID with a SP-2380 Supelco® capillary column and  $H_2$  as the carrier gas to evaluate the levels of ten of the FA from the heptanic extracts, after esterification reaction with potassium hydroxide (KOH) in methanol (Figure 5). Based on PCA, it was possible to highlight oleic (C18:1), linolenic (C18:3), myristic (C14:0), and linoleic acid (C18:2) as markers to distinguish between the two species. By internal normalization, linoleic acid showed the highest quantities in all samples (average percentage of 44% of the total FA). Thirteen years later, Romano et al. (2014) based their work on a similar methodology for the differentiation of 19 coffee blends. The samples consisted of 13 commercial products with their label information and six mixtures prepared in the laboratory. The authors reported similar results to the previous article in terms of the FA levels, such as the concentration of linoleic acid, and they emphasized parameters that are influenced significantly by the percentage of arabica coffee in blends, such as the linolenic acid content, the total level of monounsaturated fatty acids (MUFA), the ratio between the total concentration of MUFA and saturated fatty acids (SFA) and between the content of stearic (C18:0) and oleic acid.

Other types of fraud have also been investigated by GC, and usually with arabica coffee, valued for its sensory characteristics, and, recently, solid phase microextraction (SPME) and statistical analysis, mainly by PCA, has been used. Geographical authentication was first attempted and published by Hashim and Chaveron (1995). This study was related to levels of methylpyrazines with different roasting degrees. The levels of these compounds and the ratios of 2-methylpyrazine and 2,6-dimethylpyrazine and 2-methylpyrazine and 2,5-dimethylpyrazine of commercial roasted arabica coffees from three different origins were evaluated with GC-FID using a fused silica Chrompack capillary column and He as the carrier gas. The authors reported no significant difference between the samples; however, the low number of samples used should be emphasized.

Freitas and Mosca (1999) employed a GC-MS with EI ionization and IT analyser to identify the analytes responsible for arabica and robusta roasted coffee aromas, previously separated and detected by GC-FID, with a SuOx-01 capillary column and He as the carrier gas. Using roasted coffee samples imported from eight different countries (Brazil, Costa Rica, Honduras, Ivory Coast, Angola, Uganda, India, and Cameroon) and provided by Nestlé Portugal SA, the authors obtained the geographical differentiation from specific substances detected in some samples, such as furan, 3-pentyl (Brazil),  $\gamma$ -butyrolactone and methyl-pyrazine (Costa Rica), and pyrazine-3-ethyl-2,5-dimethyl (Ivory Coast). The study was not able to identify some compounds, and therefore, the results were not conclusive for the proposed aim.

Risticvic et al. (2008) characterized the volatile and semivolatile profile of arabica coffee beans from different geographical origins (Brazil, Colombia, Costa Rica, Guatemala, Ethiopia, and Indonesia) by SPME and GC with an SLB-5 column, consisting of 5% diphenyl and 95% dimethylpolysiloxane, He as the carrier gas and detection





**Figure 5.** Chromatogram by gas chromatography (GC)-FID of the methyl esters of fatty acids in a coffee sample. Peaks: 1 = myristic acid (C14:0); 2 = palmitic acid (C16:0); 3 = palmitoleic acid (C16:1 *cis, trans*); 4 = C18:0, (5) C18:1 (*v9cis, v7cis*), (6) C18:2, (7) C18:3, (8) C20:0, (9) C20:1, (10) C22:0. \*Reprinted (adapted) with permission from [Martín et al. \(2001\)](#). Copyright © 2001 Published by Elsevier Science B.V.

by MS-EI and the mass analyser time of flight (TOF). Initially, the authors made a comprehensive study based on a multivariate experimental design and statistical tests such as ANOVA, PCA, and Doehlert Design. They used the best SPME coating for application in coffee beans, which in this case is a fibre consisting of divinylbenzene, carboxen, and polydimethylsiloxane (DVB/CAR/PDMS). Also they optimized the experimental variables that affect the extraction efficiency. The results with their coffee samples showed that it was possible to discriminate between Brazilian and Colombian samples by PCA, which demonstrated a great potential for their classification study. However, parameters such as the climatic conditions in terms of cultivation and the thermal roasting processes were not associated in the article.

Following the same research strategy, [Akiyama et al. \(2008\)](#) applied SPME with the same coating to differentiate the aroma from freshly brewed Ethiopian arabica coffee from the beans produced in Guatemala and Tanzania. The GC analysis, with fused silica capillary column DB-WAX and He as a carrier gas, used a different detector by olfactometry (O) that distinguished the samples mainly through the detection of 4-(4'-hydroxyphenyl)-2-butanone. This compound was quantified by internal normalization at levels of 1.63 ppb in lightly roasted coffee, using GC-MS under same conditions as GC-O and significantly higher ppb levels than other samples (Tukey's HSD test,  $P < 0.01$ ). Also the sensorial analyses provided an important contribution to characterize the aroma of Ethiopian coffee.

In addition to the use of HPLC, a few researchers have investigated the adulteration of coffee with addition of vegetable matter by GC. To identify the addition of corn, [Jham et al. \(2008\)](#) tested, after tocopherols by HPLC-fluorescence, the possibility to detect FA profiles based on the same methodology used by [Martín et al. \(2001\)](#). First, the authors evaluated the ratios between six FAs (palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids) identified in Brazilian arabica coffee varieties in an analysis lasting only 6 min and they defined the ratio of linoleic and stearic acid as the most significant difference between coffee and corn (1.0% and 15.7% of average area). Despite the good correlation of this ratio with the percentage of roasted corn added in pure coffee ( $R^2 = 0.996$ ), this study was not a success, because the commercial coffee samples, previously analysed using the tocopherol methodology, did

not show any significant difference from pure coffees by ANOVA ( $F$ -test,  $P > 0.05$ ), preventing the use of these analytes as markers of adulteration with corn.

[Oliveira et al. \(2009\)](#) employed a methodology using SPME-GC-MS, similar to the [Risticcevic et al. \(2008\)](#), with this objective. The authors performed a comparison of the main substances responsible for the aroma of roasted coffee and roasted barley, at different roasting degrees, and, differently from the work by [Jham et al. \(2008\)](#), this method was efficient to check adulteration with barley at levels as low as 1% (w/w). Through the PCA analysis and a clear separation of the plots from roasted coffee and roasted barley, the viability of the method was emphasized in the published article, especially for more severe conditions of the thermal process. Research involving the identification of the volatile compounds of roasted barley and other vegetable matrices would be a continuation to this paper.

GC-MS can also be applied to differentiate between different types of coffees such as the sensorial quality and presence of defects. [Toledo et al. \(2014\)](#) investigated blends of freshly ground roasted arabica coffee certified with roasted arabica coffee of low quality, through SPME using a polymer ionic liquid sorbent previously synthesized and GC-FID with an SLB-5ms Supelco® capillary column, filled with polydiphenyldimethylsiloxane and 5% (w/w) diphenylsiloxane, and He as the carrier gas. Among the analytes identified by GC-MS-EI, 11 volatile substances, including pyrazines, pyrazoles, ketones, furans, pyridines, and methoxyphenols, were highlighted as chemical markers for coffees of different qualities. This article differs from the others that used SPME as it applied different polymer ionic liquid sorbents.

[Jumhawan et al. \(2015\)](#) compared detection by FID and MS to authenticate Kopi Luwak coffee or civet coffee, the highest price coffee that is obtained from the excretions of civets (*Paradoxurus hermaphroditus*), a small Asian mammal whose gastrointestinal tract changes the chemical composition of coffee beans and confers a distinct and much appreciated sensory aspect to the beverage. Certified and commercial Kopi Luwak coffee, as well as regular coffee and blends of both types ( $n = 37$ ), was used for the experiments. These authors used a GC with a CP-SIL 8 CB low bleed capillary column, consisting of polydimethylsiloxane with 5% (w/w) phenolic substances, and He as the carrier gas,

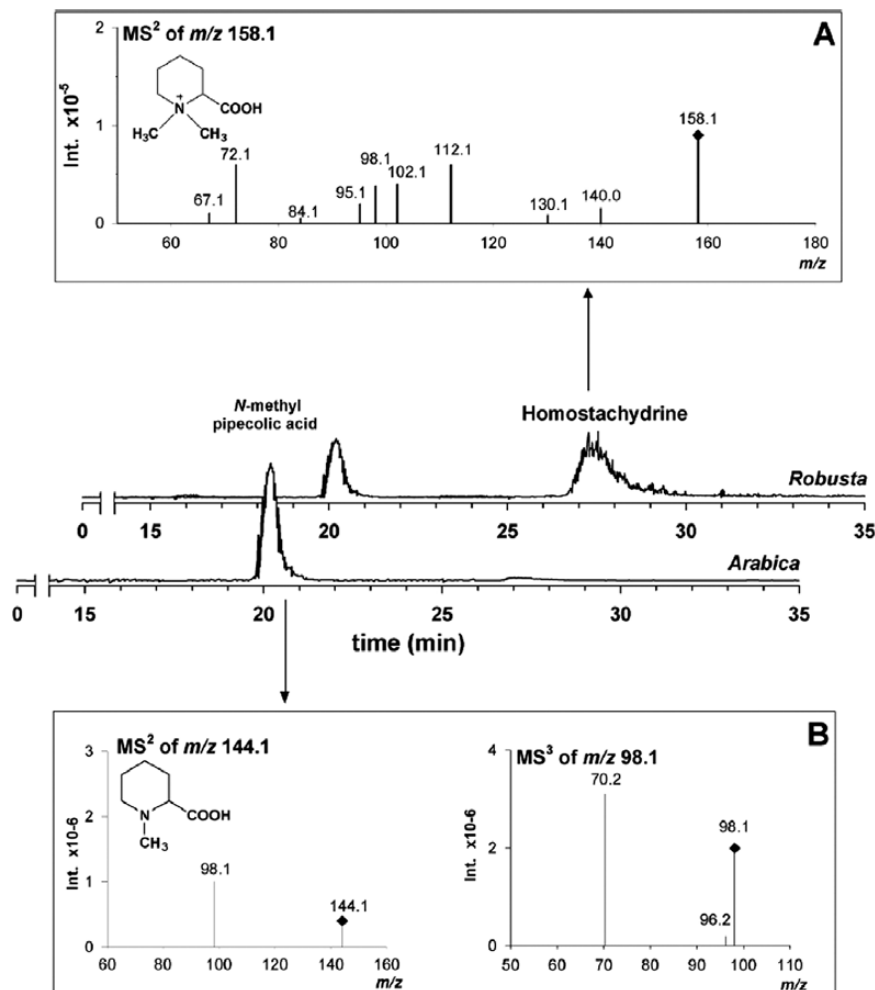
and statistical treatment by PCA and orthogonal projection to latent structures-discriminant analysis (OPLS-DA). According to the authors, the peaks by FID, such as citric and malic acid that contribute significantly to quality evaluation of this coffee type, showed higher intensity by MS and, therefore, a higher sensitivity and thus indicating the possibility of adopting GC-FID for routine analysis of authentication in the food industry.

Studies to differentiate healthy and defective beans, different maturation points or beans in the fermentation state at harvest of coffees often consumed in Brazil, were investigated in two studies by Agresti *et al.* (2008) and Toci and Farah (2008). The first authors discriminated the volatile fraction of defective beans (black, immature, and sour) and non-defective beans, after the roasting process, by SPME with DVB/CAR/PDMS and GC-MS-EI under conditions similar to the previous article. Through PCA and CA, the results indicated that black and sour beans can be associated with fermentation of immature and healthy beans, respectively. Toci and Farah (2008), applying the same methodology, advanced this line of research, and suggested some potential chemical markers, among 97 substances identified in the raw and roasted defective Brazilian arabica beans such as 2-methylpyrazine and 2-furyl-methanol acetate (raw black-immature); butyrolactone (raw sour

beans); benzaldehyde and 2,3,5,6-tetramethylpyrazine (raw defective beans in general); and pyrazine, 2,3-butanediol *meso*, 2-methyl-5-(1-propenyl)pyrazine, hexanoic acid, 2-methoxy-4-ethyl-phenol, and 1-methyl-4-[(1-methylethyl)thio]benzene (roasted defective beans in general). However, no statistics were disclosed by these authors; thus, further studies with a larger number of samples are needed to confirm these results.

#### Latest advances of fraud analysis in coffee

Various studies developed in the last year have employed liquid chromatography coupled to detection by MS, ensuring analyses with greater sensitivity, selectivity, and reliability. The study of betaines present in *Coffea* genus by Servillo *et al.* (2016) showed the possibility of using this distinct compound as an authentication marker for the presence of robusta coffee in commercial blends. The compound called homostachydrine or pipercolic acid betaine is a derivative from *N*-methylpipercolic acid. It was detected by HPLC, with a Supelco Discovery C8 column and mobile phase formic acid 0.1% aqueous solution, coupled to MS-ESI with a mass analyzer quadrupole (Q) and IT (Figure 6). The analysis of certified coffee showed, through external standard calibration, homostachydrine levels of 1.5 ppm and 31.0 ppm for arabica and



**Figure 6.** High-performance liquid chromatography (HPLC)-MS<sup>n</sup> analysis of extracts of green arabica and robusta beans, with representative MS<sup>2</sup> extracted ion chromatograms conducted in MRM mode. (A) MS<sup>2</sup> fragmentation patterns of homostachydrine ( $t_r = 27.4$  min), isolating at m/z 158.1. (B) MS<sup>2</sup> (left) and MS<sup>3</sup> (right) fragmentation patterns of *N*-methylpipercolic acid ( $t_r = 20.2$  min), isolating at m/z 144.1 (MS<sup>2</sup>) and at m/z 98.1 (MS<sup>3</sup>). \*Reprinted (adapted) with permission from Servillo *et al.* (2016). Copyright © 2016 Published by Elsevier Ltd.

robusta samples, respectively. The potential use of this marker is also because of some characteristics such as the minimal sample treatment for the analysis, when compared with the 16-OMC extraction; the absence of variation in its content under similar conditions to the industrial roasting process (maximum of 210°C for 30 min); and a good correlation between declared percentages of robusta coffee in commercial roasted ground coffee blends tested by this methodology and estimated percentage by inverse regression line.

Research involving UPLC-MS is being developed for fraud analysis in coffee. The article of Mehari *et al.* (2016) investigated the profile of phenolic substances from 100 green coffee samples from different regions of Ethiopia by UPLC in reverse phase (Acquity BEH C<sub>18</sub> Waters UPLC® column and mobile phase composed of a gradient mode with acetonitrile and formic acid 0.1% aqueous solution) coupled to a photodiode array detector and tandem MS-ESI in negative mode and QTOF. The analysis of the clarified extract in methanol identified 11 chlorogenic acids, among 18 detected substances in a run time of only 9 min, using the spectrum in the UV region, the ratio of mass/charge, and the fragmentation by MS-MS (Figure 7). The most important results pointed out the relation between high levels of 5-*O*-caffeoylquinic acid (5-CQA) and the samples of low sensory quality (46% to 59% of the total CGA), making it possible also to distinguish, by the content of the 4,5-*O*-dicaffeoylquinic acid, calculated in reference to 5-CQA, between coffee beans cultivated around the city of Harar, located in the eastern region and considered of higher quality and price compared with other Ethiopian coffees. The implementation of this strategy in monitoring of the coffee market depends on the future studies with roasted coffee, due to the known reductions in CGA levels in the thermal processing of green beans.

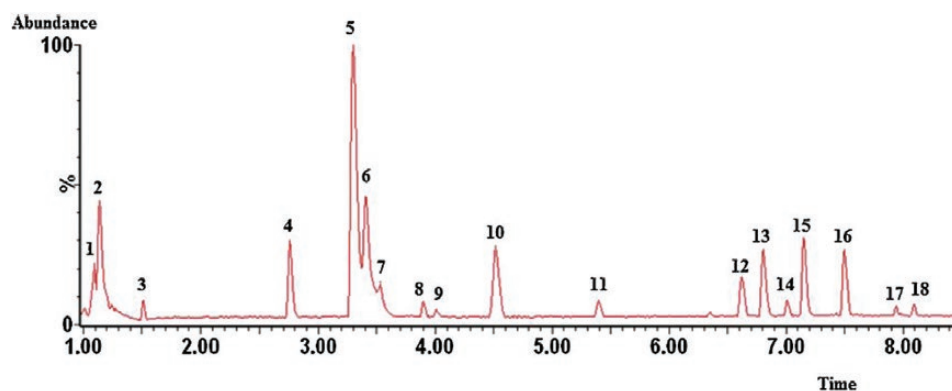
In addition to this article, investigations into coffee adulteration have returned to carbohydrates, such as in the previously mentioned articles by Santiago *et al.* (2012), Domingues *et al.* (2014), and Pauli *et al.* (2014), because these are usually adopted by public inspection agencies. The monosaccharide and oligosaccharide profiles can be determined by UPLC-MS for each adulterant. The application of UPLC in hydrophilic interaction liquid chromatography (HILIC) and high-resolution mass spectrometry (HRMS)

by Cai *et al.* (2016) also demonstrates the possibility of using this technique for quality control in the coffee industry. With 111 substances detected in a run time of 15 min, these authors determined the oligosaccharide profiles in coffee beans, soybeans, and rice, after derivatization with 2,4-bis-(diethylamino)-6-hydrazine-1,3,5-triazine, and demonstrated by OPLS-DA that 8 and 5 of the substances originally present in rice and soybean, respectively, can be used as chemical markers to detect fraud in coffee. Through the results of five ground coffees plotted on a contour map generated by a series of mixtures composed of rice and soybean, it was possible to estimate for one sample that the level of rice was approximately 10%. According to the authors, this methodology exhibited significant potential as a powerful tool for coffee qualification.

This last method seems promising for the number of compounds analysed in time, although only rice and soybean were tested, adulterants less common than corn and husks, which has profiles very similar to coffee. Thus, it is necessary for more accurate evaluation to verify its efficiency, as to find qualitatively distinct markers for the different adulterants and quantitatively in relation to ion suppression.

## Conclusion

Thus, the different research that has been developed over the last 30 years to investigate fraud in coffee has contributed to improve coffee quality and the sustainability of the coffee industry. Currently, there is no consensus on which is the most suitable method for certain types of fraud, but some methodologies have been routinely applied such as carbohydrate analysis by HPLC and, mainly, pulsed amperometric detector, suggested by ISO 11292 (1995), SPME for volatile compounds, quantification of 16-*O*-methylcafestol in coffee blends, among others. However, analyses with the most accurate results and low sample preparation and analysis time are extremely important in combating fraud. UPLC-MS analysis seems to be promising, due to low analysis time, greater sensitivity, and selectivity, but future studies should be performed with other common adulterants, to ensure qualitative and quantitative results imposed by the legislation and concerning routine analysis.



**Figure 7.** Typical chromatogram by ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS-MS) of an Ethiopian green coffee extract, indicating the presence of eighteen phenolic compounds. Peaks: 4 = 3-*O*-caffeoylquinic acid (3-CQA); 5 = 5-*O*-caffeoylquinic acid (3-CQA); 6 = 4-*O*-caffeoylquinic acid (3-CQA); 8 = *cis*-5-*O*-caffeoylquinic acid (*cis*-5-CQA); 9 = 5-*O*-*p*-coumaroylquinic acid (5-*p*CoQA); 10 = 5-*O*-feruloylquinic acid (5-FQA); 12 = 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA); 13 = 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA); 14 = *cis*-3,5-di-*O*-caffeoylquinic acid (*cis*-3,5-diCQA); 15 = 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA). \*Reprinted (adapted) with permission from Mehari *et al.* (2016). Copyright © 2015 Published by Elsevier Inc.

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Conflict of interest statement. The authors declare that there is no conflict of interest.

## References

- ABIC – Associação Brasileira da Indústria de Café. (2018). Indicadores de Desempenho da Cafeicultura Brasileira. <http://abic.com.br/estatisticas/indicadores-da-industria/> accessed 6 March 2018.
- Agresti, P. D. C. M., Franca, A. S., Oliveira, L. S., Augusti, R. (2008). Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. *Food Chemistry*, 106: 787–796.
- Akiyama, M., et al. (2008). Characterization of headspace aroma compounds of freshly brewed arabica coffees and studies on a characteristic aroma compound of ethiopian coffee. *Journal of Food Science*, 73: C335–C346.
- Alves, R. C., Casal, S., Alves, M. R., Oliveira, M. B. (2009). Discrimination between arabica and robusta coffee species on the basis of their tocopherol profiles. *Food Chemistry*, 114: 295–299.
- Amboni, R. D. M. C., Francisco, A. D., Teixeira, E. (1999). Utilização de microscopia eletrônica de varredura para detecção de fraudes em café torrado e moído. *Food Science and Technology*, 19: 311–313.
- Bagdonaite, K., Derler, K., Murkovic, M. (2008). Determination of acrylamide during roasting of coffee. *Journal of Agricultural and Food Chemistry*, 56: 6081–6086.
- Barbin, D. F., Felicio, A. L. S. M., Sun, D.-W., Nixdorf, S. L., Hirooka, E. Y. (2014). Application of infrared spectral techniques on quality and compositional attributes of coffee: an overview. *Food Research International*, 61: 23–32.
- Bernal, J. L., Del Nozal, M. J., Toribio, L., Del Alamo, M. (1996). HPLC Analysis of Carbohydrates in Wines and Instant Coffees Using Anion Exchange Chromatography Coupled to Pulsed Amperometric Detection. *Journal of Agricultural and Food Chemistry*, 44: 507–511.
- Bicho, N. C., Leitão, A. E., Ramalho, J. C., Lidon, F. C. (2012). Use of colour parameters for roasted coffee assessment. *Food Science and Technology*, 32: 436–442.
- Blanc, M. B., Davis, G. E., Parchet, J. M., Viani, R. (1989). Chromatographic profile of carbohydrates in commercial soluble coffees. *Journal of Agricultural and Food Chemistry*, 37: 926–930.
- BRASIL – MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. (2010). *Instrução Normativa nº 16, de 24 de maio de 2010 – Regulamento Técnico para o Café Torrado em Grão e para o Café Torrado e Moído*. <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualizarAtoPortalMapa&chave=794116535> accessed 6 March 2018.
- Bresciani, L., Calani, L., Bruni, R., Brighenti, F., Del Rio, D. (2014). Phenolic composition, caffeine content and antioxidant capacity of coffee silver-skin. *Food Research International*, 61: 196–201.
- Cai, T., Ting, H., Jin-Lan, Z. (2016). Novel identification strategy for ground coffee adulteration based on UPLC-HRMS oligosaccharide profiling. *Food Chemistry*, 190: 1046–1049.
- Carrera, F., León-Camacho, M., Pablos, F., González, A. G. (1998). Authentication of green coffee varieties according to their sterolic profile. *Analytica Chimica Acta*, 370: 131–139.
- Clifford, M. N., Jarvis, T. (1988). The chlorogenic acids content of green robusta coffee beans as a possible index of geographic origin. *Food Chemistry*, 29: 291–298.
- Clifford, M. N., Shutler, S., Thomas, G. A., Ohiokpehai, O. (1987). The chlorogenic acids content of coffee substitutes. *Food Chemistry*, 24: 99–107.
- Correia, A. M. N. G., Leitão, M. C. A., Clifford, M. N. (1995). Caffeoyl-tyrosine and Angol II as characteristic markers for Angolan robusta coffees. *Food Chemistry*, 53: 309–313.
- DIN 10779. (1999). *Analysis of coffee and coffee products – Determination of 16-O-methylcafestol content of roasted coffee – HPLC-method*. Deutsches Institut für Normung e. V., Berlin.
- Domingues, D. S., et al. (2014). Detection of roasted and ground coffee adulteration by HPLC and by amperometric and by post-column derivatization UV-vis detection. *Food Chemistry*, 146: 353–362.
- Esquivel, P., Jiménez, V. M. (2012). Functional properties of coffee and coffee by-products. *Food Research International*, 46: 488–495.
- Ferreira, T., Farah, A., Oliveira, T. C., Lima, I. S., Vitorio, F., Oliveira, E. M. (2016). Using real-time PCR as a tool for monitoring the authenticity of commercial coffees. *Food Chemistry*, 199: 433–438.
- Franca, A. S., Mendonça, J. C. F., Oliveira, S. D. (2005). Composition of green and roasted coffees of different cup qualities. *LWT – Food Science and Technology*, 38: 709–715.
- Freitas, A. M. C., Mosca, A. I. (1999). Coffee geographic origin—an aid to coffee differentiation. *Food Research International*, 32: 565–573.
- Garcia, L. M., Pauli, E. D., Cristiano, V., da Camara, C. A., Scarmínio, I. S., Nixdorf, S. L. (2009). Chemometric evaluation of adulteration profile in coffee due to corn and husk by determining carbohydrates using HPAEC-PAD. *Journal of Chromatographic Science*, 47: 825–832.
- González, A. G., Pablos, F., Martín, M. J., León-Camacho, M., Valdenebro, M. S. (2001). HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters. *Food Chemistry*, 73: 93–101.
- Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.-P., Schorr-Galindo, S. (2007a). Impact of “ecological” post-harvest processing on the volatile fraction of coffee beans: I. Green coffee. *Journal of Food Composition and Analysis*, 20: 289–296.
- Gonzalez-Rios, O., Suarez-Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.-P., Schorr-Galindo, S. (2007b). Impact of “ecological” post-harvest processing on coffee aroma: II. Roasted coffee. *Journal of Food Composition and Analysis*, 20: 297–307.
- Hall, S., et al. (2015). A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. *Food Research International (Ottawa, Ont.)*, 76: 626–636.
- Hashim, L., Chaveron, H. (1995). Use of methylpyrazine ratios to monitor the coffee roasting. *Food Research International*, 28: 619–623.
- IBGE - Instituto Brasileiro de Geografia e Estatística. (2018). Levantamento Sistemático da Produção Agrícola – Janeiro de 2018. <https://www.ibge.gov.br/estatisticas-novportal/economicas/agricultura-e-pecuaria/9201-levantamento-sistemático-da-produção-agrícola.html?&t=resultados> accessed 6 March 2018.
- ICO - International Coffee Organization. (2016). Guidelines for the Prevention of Mould Formation in Coffee. <http://dev.ico.org/documents/ed1988e.pdf> accessed 6 March 2018.
- ICO - International Coffee Organization. (2018). Trade Statistic Tables. [http://www.ico.org/trade\\_statistics.asp?section=Statistics](http://www.ico.org/trade_statistics.asp?section=Statistics) accessed 6 March 2018.
- Jham, G. N., Berhow, M. A., Manthey, L. K., Palmquist, D. A., Vaughn, S. F. (2008). The use of fatty acid profile as a potential marker for Brazilian coffee (*Coffea arabica* L.) for corn adulteration. *Journal of Brazilian Chemical Society*, 19: 1462–1467.
- Jham, G. N., Winkler, J. K., Berhow, M. A., Vaughn, S. F. (2007). Gamma-tocopherol as a marker of Brazilian coffee (*Coffea arabica* L.) Adulteration by corn. *Journal of Agricultural and Food Chemistry*, 55: 5995–5999.
- Jumhawan, U., Putri, S. P., Yusianto, Bamba, T., Fukusaki, E. (2015). Application of gas chromatography/flame ionization detector-based metabolite fingerprinting for authentication of Asian palm civet coffee (kopi luwak). *Journal of Bioscience and Bioengineering*, 120: 555–561.
- Kamm, W., Dionisi, F., Fay, L. B., Hischenhuber, C., Schmarr, H. G., Engel, K. H. (2002). Rapid and simultaneous analysis of 16-O-methylcafestol and sterols as markers for assessment of green coffee bean authenticity by online LC–GC. *Journal of American Oil Chemical Society*, 79: 1109–1113.
- Lercker, G., Frega, N., Bocci, F., Rodriguez-Estrada, M. T. (1995). High resolution gas chromatographic determination of diterpenic alcohols and sterols in coffee lipids. *Chromatographia*, 41: 29–33.

- Martín, M. J., Pablos, F., González, A. G. (1998). Discrimination between arabica and robusta green coffee varieties according to their chemical composition. *Talanta*, 46: 1259–1264.
- Martin, M. X., Pablos, F., González, A. G., Valdenebro, M. X., León-Camacho, M. (2001). Fatty acid profiles as discriminant parameters for coffee varieties differentiation. *Talanta*, 54: 291–297.
- Mehari, B., Redi-Abshiro, M., Chandravanshi, B. S., Combrinck, S., Atlabachew, M., McCrindle, R. (2016). Profiling of phenolic compounds using UPLC-MS for determining the geographical origin of green coffee beans from Ethiopia. *Journal of Food Composition and Analysis*, 45: 16–25.
- Murkovic, M., Derler, K. (2006). Analysis of amino acids and carbohydrates in green coffee. *Journal of Biochemical and Biophysical Methods*, 69: 25–32.
- Narita, Y., Inouye, K. (2014). Review on utilization and composition of coffee silverskin. *Food Research International*, 61: 16–22.
- Oliveira, E. M. M., Farah, A., Freitas-Silva, O., de Souza, A. M., dos Santos, M. T. F., Santiago, M. C. P. A. (2015). Chapter 105: Overview of currently applied techniques for detection of adulterants in coffee and potential use of DNA-based methods as promising new analytical tools. In: Preedy V. R. (ed.) *Coffee in Health and Disease Prevention*. Academic Press, San Diego, pp. 953–961.
- Oliveira, R. C. S., Oliveira, L. S., Franca, A. S., Augusti, R. (2009). Evaluation of the potential of SPME-GC-MS and chemometrics to detect adulteration of ground roasted coffee with roasted barley. *Journal of Food Composition and Analysis*, 22: 257–261.
- Pacetti, D., Boselli, E., Balzano, M., Frega, N. G. (2012). Authentication of italian espresso coffee blends through the GC peak ratio between kahweol and 16-O-methylcafestol. *Food Chemistry*, 135: 1569–1574.
- Parras, P., Martínez-Tomé, M., Jiménez, A. M., Murcia, M. A. (2007). Antioxidant capacity of coffees of several origins brewed following three different procedures. *Food Chemistry*, 102: 582–592.
- Paterson, R. R. M., Lima, N., Taniwaki, M. H. (2014). Coffee, mycotoxins and climate change. *Food Research International*, 61: 1–15.
- Pauli, E. D., et al. (2014). Detection of ground roasted coffee adulteration with roasted soybean and wheat. *Food Research International*, 61: 112–119.
- Risticvic, S., Carasek, E., Pawliszyn, J. (2008). Headspace solid-phase microextraction-gas chromatographic-time-of-flight mass spectrometric methodology for geographical origin verification of coffee. *Analytica Chimica Acta*, 617: 72–84.
- Romano, R., Santini, A., Le Grottaglie, L., Manzo, N., Visconti, A., Ritieni, A. (2014). Identification markers based on fatty acid composition to differentiate between roasted Arabica and Canephora (Robusta) coffee varieties in mixtures. *Journal of Food Composition and Analysis*, 35: 1–9.
- Sano, E. E., Assad, E. D., Cunha, S. A. R., Correa, T. B. S., Rodrigues, H. R. (2003). Quantifying adulteration in roast coffee powders by digital image processing. *Journal of Food Quality*, 26: 123–134.
- Santiago, M. C. P. A., Godoy, R. L. O., Licurgo, F. M. S., Rosa, J. S., Pacheco, S., Nascimento, L. S. M. (2012). Adaptations in HPLC method for fraud determination in coffee. Presented at the 7<sup>o</sup> Encontro Nacional de Cromatografia da Sociedade Portuguesa de Química, Porto, Portugal, Jan 9–11, 2012.
- Servillo, L., et al. (2016). Homostachydrine (pipercolic acid betaine) as authentication marker of roasted blends of *Coffea arabica* and *Coffea canephora* (robusta) beans. *Food Chemistry*, 205: 52–57.
- Skarka, B. S., Radej, Z. (1958). Proof of adulteration of coffee with substitutes by means of paper chromatography. *Průmysl potravin*, 4.
- Smith, R. M. (1981). Determination of 5-hydroxymethylfurfural and caffeine in coffee and chicory extracts by high performance liquid chromatography. *Food Chemistry*, 7: 41–45.
- Speer, K. (1989). 16-O-Methylcafestol – ein neues Diterpen im Kaffee. *Z Lebensm Unters Forsch*, 189: 326–330.
- Tavares, K. M., Lima, A. R., Nunes, C. A., Silva, V. A., Mendes, E., Casal, S., Pereira, R. G. F. A. (2016). Free tocopherols as chemical markers for Arabica coffee adulteration with maize and coffee by-products. *Food Control*, 70: 318–324.
- Tavares, K. M., Pereira, R. G. F. A., Nunes, C. A., Pinheiro, A. C. M., Rodarte, M. P., Guerreiro, M. C. (2012). Espectroscopia no infravermelho médio e análise sensorial aplicada à detecção de adulteração de café torrado por adição de cascas de café. *Química Nova*, 35: 1164–1168.
- THE LANCET. (1851). The Analytical Sanitary Commission. Coffee and its adulterations. *The Lancet*, 57: 21–26.
- THE LANCET. (1853). The Analytical Sanitary Commission. Coffee and its adulterations. *The Lancet*, 61: 418.
- Toci, A. T., Farah, A. (2008). Volatile compounds as potential defective coffee beans' markers. *Food Chemistry*, 108: 1133–1141.
- Toledo, B. R., Hantao, L. W., Ho, T. D., Augusto, F., Anderson, J. L. (2014). A chemometric approach toward the detection and quantification of coffee adulteration by solid-phase microextraction using polymeric ionic liquid sorbent coatings. *Journal of Chromatography A*, 1346: 1–7.
- Valdenebro, M. S., León-Camacho, M., Pablos, F., González, A. G., Martín, M. J. (1999). Determination of the arabica/robusta composition of roasted coffee according to their sterolic content. *The Analyst*, 24: 999–1002.