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Chromatographic Methods for Coffee Analysis: A Review

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Abstract

Coffee has been one of the most commercialized food products and most widely researched beverage in the world for decades. It is considered a functional food, primarily due to its high content of compounds that exert antioxidant and other beneficial biological properties. This review summarized the data from analysis of coffee components, both volatile constituents and non-volatile high-molecular weight compounds performed by various chromatographic methods. A list of compounds identified by gas chromatography with mass spectrometry which define the aroma of coffee is provided. Publications on the measurement of methylxanthines (caffeine, theobromine, and theophylline), chlorogenic acids, diterpenes, sugars, amino acids, gamma-aminobutyric acid, dibasic acids, anions, and other compounds by HPLC and UHPLC-MS are reviewed. An overview of publications on the determination of organic contamination in coffee (PAHs, acrylamides, mycotoxins, etc.) and ways to reduce contamination through production technology and brewing methods are presented. Finally, an overview of the literature on authentication assessment for different grades of coffee grown in different regions is provided.

Keywords: coffee, chemical composition, chlorogenic acid, caffeine, GC, HPLC

1. Introduction

Coffee has been studied for many years and the research varied depending on the needs: pests, nutrition, pruning, harvesting, processing and quality. Recent studies have shown that the moderate intake of coffee reduces body fat and decreases oxidative damage-related diseases, such as type 2-diabets, cardiovascular, Alzheimer's and Parkinson's disease (Bakuradze et al., 2011; Freedman et al., 2012, O'Keefe et al., 2013). Erdem et al. (2016) indicated that cinnamic acid derivatives have been related with different biological effects including anti-inflammatory, antioxidant, anticarcinogenic, or neuroprotective activities. Coffee has a complex chemical composition and the compounds which include volatile and non-volatile compounds, diterpenes, sugar, amino acids and organic pollutants are important for coffee flavor, quality and health effects. This review article will focus on the chemical composition of coffee and their analytical techniques majorly by different chromatographic methods. We will also introduce some compounds found from different coffee processing procedures and their analytical techniques.

2. Chromatographic Methods Used to Determine the Chemical Composition of Coffee

Coffee contains over two thousand components—from volatile low-molecular weight to high-molecular weight compounds (Morton and Macleod, 1986). Various chromatographic methods have been used to separate and identify all kinds of mixtures, from gases and volatile compounds to high-molecular weight compounds, as well as mixtures of organic and inorganic ions (cations and anions). Therefore, almost all components of coffee can be detected by chromatographic methods. The components in a mixture are first separated on columns by different adsorption rates, and then the separated components are registered at the column outlet with detectors.

Gas chromatography has been used to separate and identify volatile and semi-volatile compounds in coffee. It is important that these compounds do not decompose during the evaporation process. Compounds with molecular

weights up to 500-600 can be identified by gas chromatography. Liquid chromatography has been used to separate and identify non-volatile high-molecular weight compounds at temperatures close to room temperature. Therefore, even unstable compounds can be determined by liquid chromatography. For example, six quality markers in coffee, which are caffeine, trigonelline, nicotinic acid, N-methylpyridinium, 5-caffeoylquinic acid, and 5-hydroxyfurfural were simultaneously detected for three coffee matrices—green, roasted, and soluble—with the limit of quantification 0.069-0.71 μg/ml by HPLC-DAD. This technique is useful for routine determination of the thermal degradation rate during the roasting process (Gant, Leyva, Gonzalez & Maruenda, 2015). Ion chromatography and ion exchange chromatography methods utilize columns filled with ion exchangers to separate ion mixtures. In thin layer chromatography, separation occurs not on columns but on plates made of glass, metal, or polymer coated with a thin layer of adsorbent material. Capillary electrophoresis techniques employ quartz capillaries with an inner diameter of 0.1 mm, or less. Mixtures may be separated due to the electrophoretic mechanism alone (capillary zone electrophoresis), or due to electrophoretic and chromatographic mechanisms at the same time. In the latter case, a layer of adsorbent is applied on the internal walls of the capillary, or sorbent (adsorbent) is added to the mobile phase.

Gas chromatography with mass spectrometry (GC-MS) and liquid chromatography with mass spectrometry (HPLC-MS) have recently come into wide use to identify the components of the separated compounds (i.e., to conduct qualitative analysis). Table 1 provides a list of chromatography methods used to analyze coffee (based on publications).

Table 1. Chromatographic methods used for the analysis of coffee

No.	Methods	References
1.	Gas chromatography (GC)	Lercker et al, 1995; Kolling-Speer et al., 1999; Holscher and Steinhart, 1992; Sanz et al., 2001; Bicchi et al., 1997.
2.	Gas chromatography with mass spectrometry	Dirinck et al., 2001; Costa-Freitas et al., 2001.
3.	Two-dimensional gas chromatography	Ryan et al., 2004
4.	Gas chromatography in coupled with head-space analysis	Semmeirich and Grosh, 1995; Maetzu et al., 2001; Procida et al., 1997; Bücking and Steinhart, 2002; Rocha et al., 2004.
5.	Pyrolysis gas chromatography	Harada et al., 1987
6.	High performance liquid chromatography (HPLC)	de Andrade et al., 1995; Casal et al., 2002; Bispo et al., 2002; Meger et al., 1996; Dias et al., 2010.
7.	HPLC-mass spectrometry	Kurzrock and Speer, 2001; Ventura et al., 2003.
8.	HPLC-MS/MS	Trugo, 1984
9.	Gel-filtration chromatography	Daglia et al., 2004.
10.	Micellar chromatography	Perez-Martinez et al., 1995
11.	Ion chromatography	Gennano and Abrigo, 1992
12.	Thin layer chromatography	Levi, 1975

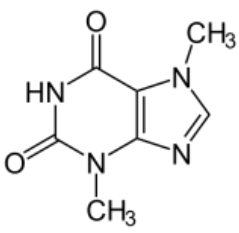
In addition to individual chromatographic methods, coffee has been analyzed by various combinations of chromatography methods, such as HPLC and GC, capillary electrophoresis, spectrophotometric methods, and ‘electronic nose’ and ‘electronic tongue’ systems. Table 2 provides a list of compounds which were identified in coffee (except the volatile compounds). Various estimates suggest that coffee contains 2000-3000 compounds. Table 2 shows only useful components that determine nutritional value of coffee: sugars, amino acids, fatty acids, vitamins, antioxidants, trace elements etc.

Table 2. List of compounds identified in coffee and typical analytical methods

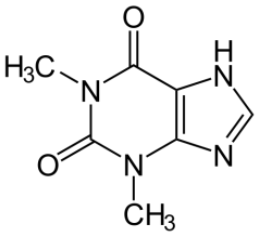
No.	Compounds	Typical concentration	Comment	Analytical methods	References
1	Chlorogenic acids	0.6-26.4%	More than 10 types of acids	HPLC, LC/MS	Trugo et al., 1984; Clifford, 2000; Mullen et al., 2011
2	Caffeine, theobromine, theophylline	3-350μg/mL		HPLC, LC/MS	Wanyika et al., 2010; Bispo et al., 2002; Eanyika et al, 2010
3	Trigonelline	3-10 mg/g		HPLC, LC/MS	Ky et al., 2001; Casal et al., 2000
4	Carbohydrates	3.41-9.43%	Saccharose, glucose, fructose, arabinose, galactose	IC	Knopp et al., 2006
5	Amino acids	4.4-1075 mg per 100g coffee powder	16 amino acids	IC, HPLC, LC/MS	Nakhmedov et al., 1984; Bytof et al., 2005
6	Vitamins	less than 3mg	Vitamin B_1, riboflavin (B_2), nicotinic acid (PP), pyridoxine (B_6), tocopherol (E), vitamin B_{12}	HPLC	Clarke, 1985; O'Driscoll, 2014
7	γ-aminobutyric acid	30-1860 mg/kg		HPLC	Bytof et al., 2005, Kramer et al., 2010
8	Serotonin	about 10 mg/g dry weight	'happiness hormone'	HPLC	Kele and Ohmacht, 1996
9	Organic acids	about 1%	Citric, malic, oxalic, acetic acids, etc.	HPLC	Kele and Ohmacht, 1996; Mabrok and Deatheroge, 1956
10	Anions	phosphates 0.2%, sulfates 0.1%	Fluoride, chloride, nitrate, sulfate, phosphate	IC	Mabrok and Deatheroge, 1956
11	Oxyaromatic acids	3-6%	Ferulic, n-coumaric, 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic, sinapic acids	HPLC	Clarke, 1985; Clifford, 2000; Murata et al., 1995
12	Tannins	3.6-7.7%		Spectrophotometry	Clarke, 1985; Savolainen, 1992
13	Polysaccharides	over 12%	Cellulose, pectic substances, fibers	IC, HPLC	Clarke, 1985; Moreira et al., 2012
14	Melanoidins	5-60 g/100g	Dark brown natural coloring agent	HPLC, DPPH assay	Clarke, 1985; Moreira et al., 2012; Pérez-Hernández et al., 2012
15	Mineral substances	3-4.5%	Potassium, magnesium, calcium, sodium, iron, manganese, zinc, copper	ICP/MS	Clarke, 1985; Abdulmadjid et al., 2017

3. Structural Formulas and Primary Compounds in Coffee

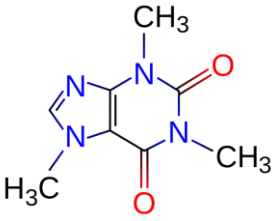
The structures of primary compounds in coffee are presented below (Fig. 1): caffeine, theobromine, theophylline; Trigonelline; caffeic, ferulic, protocatechuic acids; chlorogenic acids; gamma-isobutyric acid and diterpenes.



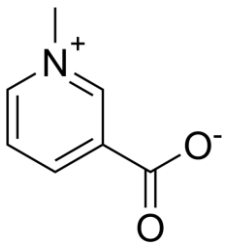
Theobromine



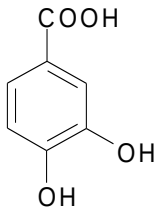
Theophylline



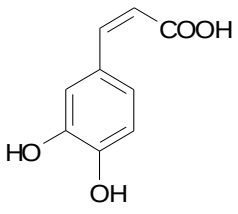
Caffeine



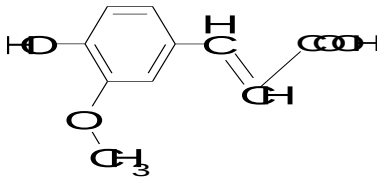
Trigonelline



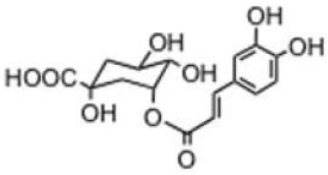
Protocatechuic acid



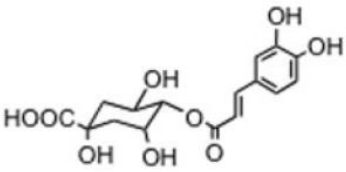
Caffeic acid



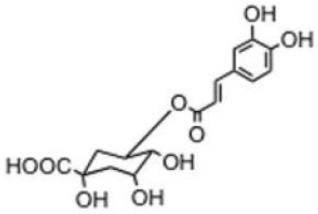
Ferulic acid



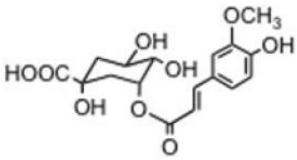
3-O-caffeoylquinic acid



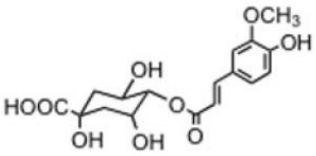
4-O-caffeoylquinic acid



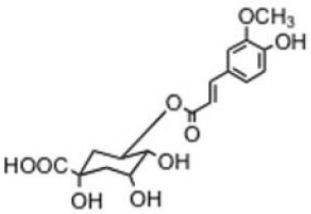
5-O-caffeoylquinic acid



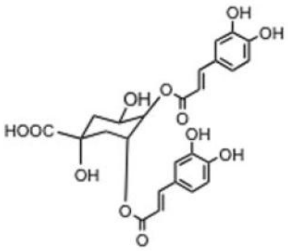
3-O-feruloylquinic acid



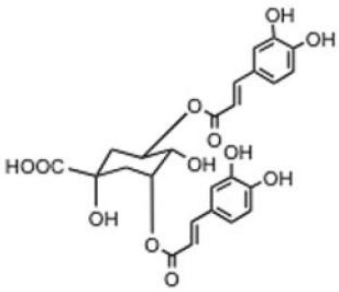
4-O-feruloylquinic acid



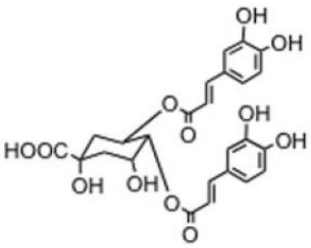
5-O-feruloylquinic acid



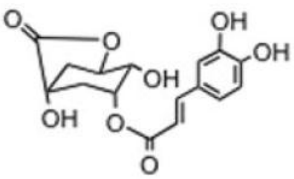
3,4-O-dicaffeoylquinic



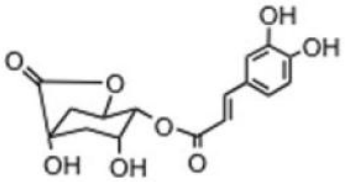
3,5-O-dicaffeoylquinic



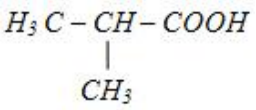
4,5-O-dicaffeoylquinic



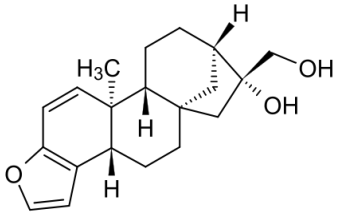
3-O-caffeoyl-1,5-quinate



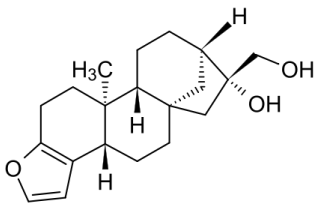
4-0-caffeoyl-1,5-quinate



Gamma-isobutyric acid



Kahweol



Cafestol

Figure 1. The structures of primary compounds in coffee

As one of the major primary compounds, chlorogenic acid was first isolated from coffee beans in 1908, and their structure was determined in 1932. Chlorogenic acids are mono- or diesters of quinic and cinnamic acids. Coffee contains more than fifteen chlorogenic acids along with their derivatives (lactones). Recent research indicated that the chlorogenic acid profiles of whole coffee fruits were influenced by the extraction procedures (Mullen et al., 2011; Craig et al., 2016)

Coffee is one of the richest sources of chlorogenic and other oxyaromatic acids. Due to its health effects, chlorogenic acid consumption rate can be up to 1 g per day (Murata, Okada & Homma, 1995). Eleven chlorogenic acids have been detected by three-dimensional HPLC. Farah et al. (2008) studied chlorogenic acids in extracts of green and decaffeinated coffee and identified 9 major and 17 minor components. Shan et al. (2016) simultaneous determined different chlorogenic acids in green coffee bean extracts with effective relative response factors. Köseoglu Yilmaz & Kolak (2017) used a new solid phase extraction method (hydrophilic-lipophilic balance cartridges) combined with HPLC analysis to perform chlorogenic acid analysis recently. According to some publications, coffee is a major nutritional source of oxyaromatic acids for its consumers (Clifford, 2000; Murata, Okada, & Homma 1995). Besides chlorogenic acid isomers (main isomer is 5-caffeoylquinic acid) and their diesters, some other hydroxycinnamic acid conjugates were also identified (Clifford, 2000) in coffee. The recognized chlorogenic acids and their derivatives, as well as their content in coffee are provided in Tables 3, 4 and 5.

Table 3. List of main chlorogenic acids (Clifford, 2000)

No.	Proper Chemical Names of Acids	Simplified Chemical Names of Acids
1.	Caffeoyl-3-quinic	Chlorogenic
2.	4,5-dicaffeoylquinic	Isochlorogenic A
3.	3,4-dicaffeoylquinic	Isochlorogenic B
4.	3,5-dicaffeoylquinic	Isochlorogenic C
5.	Caffeoyl-5-quinic	Neochlorogenic

Table 4. List of main chlorogenic acids and their derivatives contained in coffee (Clifford, 2000)

No.	Acid Name	Typical value, %
1	5-0-caffeoylquinic	26.4
2	4-0-caffeoylquinic	17.3
3	3-0-caffeoylquinic	16.0
4	5-0-feruloylquinic	14.9
5	4-0-feruloylquinic	4.9
6	3-0-feruloylquinic	4.4
7	5-0-n-coumaroylquinic	0.7
8	4-0-n-coumaroylquinic	0.9
9	3,4-0-dicaffeoylquinic	1.3
10	3,5-0-dicaffeoylquinic	0.6
11	3-0-caffeoylquinic acid lactone	7.5
12	4-0-caffeoylquinic acid lactone	5.1

Table 5. Content of the main chlorogenic acids in extracts of green and decaffeinated coffee (Farah et al., 2008)

No.	Chlorogenic Acids and Their Derivatives	μmol per 0.4g
1	5-caffeoylquinic	119.8 ±0.23
2	3-caffeoylquinic	103.3 ±0.14
3	4-caffeoylquinic	97.4 ±1.2
4	3,4-dicaffeoylquinic	16.8 ±0.01
5	4,5-dicaffeoylquinic	16.2 ±0.32
6	3,5-dicaffeoylquinic	10.2 ±0.06
7	5-feruloylquinic	22 ±0.02
8	3-feruloylquinic	20.7 ±0.69
9	4-feruloylquinic	16.4 ±0.30
10	5-n-coumaroylquinic	1.7 ±0.01
11	3-n-coumaroylquinic	1.1 ±0.28
12	diferuloylquinic (1 isomer)	1.3 ±0.01
13	caffeoylferuloylquinic acid (6 isomers)	11.8 ±1.33
14	caffeoyltryptophan	10.5 ±0.15
15	4-caffeoylquinate	0.3 ±0.01
16	3- feruloylquinate	0.3 ±0.01
17	4- feruloylquinate	0.1 ±0.03
18	3,4-dicaffeoylquinate	0.03 ±0.004

4. Composition of Volatile Components in Coffee

In the early twentieth century little was known about coffee aromatic components. Later, different compounds such as methylamine, ammonia, trimethylamine, pyrrole, pyridine, acetone, formic and valeric acids, furfural, furfuryl alcohol were identified by classic chemical methods.

In the 1920s, 29 more compounds were detected with the financial support of the Government of Switzerland (Morton and McLeod, 1986).The most typical compounds were furfuryl mercaptan, α-diketones and alkylpyrazines.

Twenty-seven extra compounds were identified between 1929 and 1962. From 1963 to 1982, more than 600 components were identified by gas capillary chromatography. Different classes of compounds (Table 6) which determine aroma of roasted coffee were identified by Morton & MacLeod (1986). Primary compounds which determine coffee aroma have been study by Ky et al. (2001) (Table 7).

Table 6. Identified classes of compounds which determine aroma of roasted coffee (Morton & MacLeod, 1986)

No.	Classes of Compounds	Number of identified compounds
1.	Furans	99
2.	Pyrazines	79
3.	Ketones	70
4.	Pyrroles	67
5.	Hydrocarbons	50
6.	Phenols	42
7.	Esters	29
8.	Aldehydes	28
9.	Thiazoles	28
10.	Oxazoles	27
11.	Amines and nitrogen-containing compounds	24
12.	Thiophenes	26
13.	Acids	20
14.	Alcohols	20
15.	Sulfides and sulfur-containing compounds	16
16.	Pyridines	13
17.	Lactones	8
18.	Other compounds	9
	Total:	655

Table 7. Primary compounds which determine coffee aroma (Ky et al., 2001)

No.	Compounds	No.	Compounds
1.	β-myrcene	31.	2-thiophenecarbaldehyde
2.	limonene	32.	3-methyl-2-thiophenecarbaldehyde
3.	n-cumene	33.	5-methyl-2-thiophenecarbaldehyde
4.	naphthalene	34.	2-acetylthiophene
5.	1-methylnaphthalene	35.	2-propionylthiophene
6.	2-methylnaphthalene	36.	2-acetyl-3-methylthiophene
7.	2-ethylnaphthalene	37.	2-acetyl-5-methylthiophene
8.	biphenyl	38.	1-(2-thienyl)-1,2-propanedione
9.	3-methylbiphenyl	39.	phenyl formate
10.	methylol	40.	methyl 2-thiophenecarboxylate
11.	α-terpineol	41.	4,5-dihydro-2(3H)thiophenone
12.	2-methylbenzaldehyde	42.	4,5-dihydro-2(2H)thiophenone
13.	2-heptanone	43.	2-methyl-4,5-dihydro-3(2H)-thiophenone
14.	2-undecanone	44.	2,4-dimethyloxazole
15.	3-methyl-2-hydroxy-2-cyclo-pentane-1-one	45.	4,5-dimethyloxazole
16.	geranylacetone	46.	2,4,5-trimethyloxazole
17.	β-damascenone	47.	2-ethyl-4,5-dimethyloxazole
18.	catechol	48.	2-methylbenzoxazole
19.	hydroquinone	49.	2-phenyloxazole
20.	guaiacol	50.	5-acetyl-2- methyloxazole
21.	n-methylguaiacol	51.	5-acetyl-2,4-dimethyloxazole
22.	n-ethylguaiacol	52.	methanethyl
23.	n-vinylguaiacol	53.	dimethylsulfide
24.	eugenol	54.	dimethyl disulfide
25.	isoeugenol	55.	dimethyl trisulfide
26.	thiophene	56.	3,3-dimethyl-1,2-dithiolane
27.	3-methylthiophene	57.	3,3-dimethyl-1,2-dithiolane-4-one
28.	4-ethyl-2-methylthiophene	58.	2-methyl-3-oxa-8-thiabicyclo[3,3,0]-1,4-octadiene
29.	benzothiophene	59.	2,4-dimethyl-3-oxa-8-thiabicyclo[3,3,0]-1,4-octadiene
30.	thiopheno [3,2-δ] thiophene		

In one study (Maeztu et al., 2001), aroma of espresso coffee from different botanical varieties and types of roast were investigated. Aroma components were determined by gas chromatography with mass spectrometry, volatile components were extracted by static headspace. Seventy-seven compounds were identified in all samples (Table 8). Among them, 13 key odorants have the greatest effect on coffee aroma (Maeztu et al., 2001) (Table 9). Chromatographic analyses were comparable with the sensory flavor profile. Aldehydes were found to correlate with a fruity aroma, diones with buttery aroma, and pyrazines with earthy burnt aroma.

Similar studies were conducted on samples of coffee Arabica (Colombia) and coffee Robusta (Indonesia) (Bücking & Steinhart, 2002). Analyses were performed by gas chromatography, flame ionization detection (FID), mass spectrometry, olfactometry, and specialized headspace.

These investigations showed that approximately 30 volatile compounds (Table 10) were substantially responsible for the coffee aroma. Most typical volatile compounds are provided in the literature (Vitenberg & Ioffe, 1984). The study also investigated how milk added to coffee influenced coffee aroma. The aroma intensity decreased due to milk lipids, proteins, and carbohydrates, however, aroma profile remained the same.

In recent years, a combination of GC-GC-FID with solid-phase microextraction was used for analysis of volatile compounds. In one study (Ryan et al., 2004), 44 volatile compounds were identified by two-dimensional chromatography (the first column with a polar phase and the second with a non-polar phase). Coffee was also analyzed by a time-of-flight mass spectrometer in this study. The coffee aroma was found to contain various classes of chemical compounds (ketones, tyrosines, furans, phenols, pyrroles, etc.). Bressanello et al. (2017) correlated the chemical composition of the coffee volatile fraction to its sensory properties. The chemical information concerning coffee aroma and flavor obtained with HS-SPEM of the ground coffee and in-solution

Table 8. Volatile compounds identified in aroma of coffee espresso (Maeztu et al., 2001)

No.	Compound name	No.	Compound name
	Alkenes		Ketones
1.	1,3-pentadiene	31.	2-propanone
	Sulfur compounds	32.	2-butanone
2.	Methanethiol	33.	2,3-butanedione
3.	Dimethylsulfide	34.	3-hexanone
4.	Dimethyl disulfide	35.	2,3-pentanedione
	Aldehydes	36.	3,4-hexanedione
5.	Acetaldehyde		Alcohols
6.	Propanal	37.	2-methyl-1-propanol
7.	2-methylpropanal	38.	2-methylbutan-1-ol
8.	Butanal	39.	3-methyl-3-buten-1-ol
9.	3-methylbutanal	40.	3-methyl-2-buten-1-ol
10.	2-methylbutanal	41.	2-ethyl-1-hexanol
11.	Hexanal		Thiophenes
	2-methyl-2-butanal	42.	Thiophene
12.	Esters	43.	2-methylthiophene
	Formic acid methyl ester		Pyrroles
13.	Acidic acid methyl ester	44.	1-methylpyrrole
	Acidic acid ethyl ester	45.	1-ethyl-1H-pyrrole
14.	Propionic acid methyl ester	46.	2,5-dimethylpyrrole
	1-hydroxy-2-propanone acetate		Pyridines
15.	Furans	47.	Pyridine
	Furan	48.	2-methylpyridine
16.	3-methylfuran	49.	3- ethylpyridine
	2-methylfuran		Pyrazines
17.	2,5-dimethylfuran	50.	Pyrazine
18.	2-vinylfuran	51.	2-methylpyrazine
19.	2-vinyl-5-methylfuran	52.	2,5-dimethylpyrazine
20.	2-methoxymethylfuran	53.	2,6-dimethylpyrazine
21.	2-methyltetrahydrofurol-3-one	54.	Ethylpyrazine
22.	2-furancarboxaldehyde	55.	2,3-dimethylpyrazine
23.	2-furfurylmethylsulfide	56.	N-propylpyrazine
24.	furfuryl formate	57.	2-vinylpyrazine
25.	2-acetylfuran	58.	2-methyl-6-vinylpyrazine
26.	2- furfurylfuran		Thiazoles
27.	Furfuryl alcohol	59.	1,3-thiazole
28.	Furfuryl acetate	60.	4-methylthiazole
29.	5-methylfurfural		Acids
30.	2-furfurylfuran	61.	Acetic acid
			Lactones
		62.	γ-butyrolactone
			Phenolic compounds
		63.	2-methoxyphenol (guaiacol)

Table 9. Relative ratio of key odorants in coffee espresso (Maeztu et al., 2001)

Compound name	% (relative)	
	Arabica	Robusta
Sulfur compounds		
Methanethiol	0.13 ±0.01	0.08 ±0.01
Aldehydes		
Acetaldehyde	0.36 ±0.02	0.35 ±0.04
Propanal	0.32 ±0.07	0.50 ±0.07
2-methylpropanal	1.80 ±0.25	2.55 ±0.35
3-methylpropanal	1.25 ±0.11	2.33 ±0.34
3-methylbutanal	2.61 ±0.29	3.33 ±0.56
Hexanal	0.05	0.06
Ketones		
2,3-butanedione	0.42 ±0.04	0.36 ±0.04
2,3-pentanedione	0.63 ±0.04	0.42 ±0.03
Pyrazines		
2-ethyl pyrazine	0.10 ±0.02	0.17 ±0.01
2-ethyl-6-methylpyrazine	0.06 ±0.01	0.13 ±0.02
2-ethyl-3,5-dimethylpyrazine	0.04 ±0.01	0.07 ±0.01
Phenolic compounds		
Guaiacol	0.11 ±0.01	0.09 ±0.01

Table 10. Identified coffee odorants (Bücking and Steinhart, 2002)

No.	Compound Name	Type of Aroma
1.	Methanethiol	putrefactive
2.	Dimethylsulfide	putrefactive
3.	2-methylpropanal	aroma of cocoa
4.	2,3-butanedione	buttery
5.	3-methylbutanal	malt-like
6.	2-methylbutanal	fruity
7.	2,3-pentanedione	oily
8.	Hexanal	aroma of leaves
9.	2,3-methylbutyric acid	sweetish
10.	3- methyl-2- buten-1 thiol	putrefactive
11.	Methional	potato-like
12.	2-furfurylthiol	aroma of roasted coffee
13.	1-octen-3-one	mushroom-like
14.	2,3,5-trimethylpyrazine	frying
15.	3-mercapto-2-methylbutyl-formate	putrefactive
16.	phenylacetaldehyde	honey-like
17.	2-ethyl-3,5-dimethylpyrazine	earthy/frying
18.	Guaiacol	phenolic/burnt
19.	2-isopropyl 3-methoxypyrazine	earthy/frying
20.	(E)-2 nonenal	cucumber-like
21.	2-isobutyl-2- methoxypyrazine	aroma of sweet pepper

SBSE/SPME sampling combined with GC-MS to evaluate their compatibility with the cupping evaluation for quality control purposes. Yang et al. (2016) found that different temperature profiles for roasting can affect volatile aroma compounds associated with roast coffee defects (light, scorched, dark, baked and underdeveloped) by GC-MS with headspace solid phase micro extraction.

Volatile thiols are among the compounds that have the greatest impact on the flavor of coffee. Due to their extremely low odor thresholds, they have a significant sensory impact even at very low concentration. Thiols are formed during coffee roasting and are the key odorants influencing the sensory characteristics of coffee. Gas chromatography coupled to mass spectrometry is most frequently used technique for the analysis of coffee thiols.

On-column injection at low temperature has been applied in order to prevent thermal degradation of the thiols (Czerny, Mayer & Grosch, 1999, Dulsat-Serra, Quintanilla-Casas & Vichi, 2016)

5. Determination of Non-Volatile Components in Coffee

5.1 Simultaneous Determination of Caffeine, Theobromine, and Theophylline

Methylxanthines, i.e. 1,3,7-trimethylxanthine (caffeine), 3-dimethylxanthine (theobromine), and 1,3-dimethylxanthine (theophylline) in coffee were determined by reversed-phase chromatography HPLC with UV detector at 273 nm and a Bondesil C_{18} column (15×0.4 cm, particle size 5 μm) (Bispo et al., 2002). A mixture of methanol-water-acetic acid or ethanol-water-acetic acid in a ratio of 20:75:5 was used as an eluent. Optimum flow rate of the eluent was 0.7 mL min^{-1}. The detection limit achieved under these conditions was $1 \cdot 10^{-11}$g. Separation time was 10 min, the yield sequence was theobromine, theophylline, and caffeine. In the same study, the content of methylxanthines was determined in some beverages (coffee, tea, cocoa, and mate) and in human urine after consumption as well. Caffeine was determined to be within 0.1 pg (350 μg mL^{-1}), theobromine within 0.1 pg (32 μg mL^{-1}), and theophylline within 0.1 pg (47 μg mL^{-1}).

Table 11 shows the values of methylxanthines contained in various drinks and urine, as determined by HPLC.

Table 11. Concentrations of methylxanthines in beverages and urine (Bispo et al., 2002)

No.	Analyzed medium	Concentration, μg mL^{-1}		
		Caffeine	Theobromine	Theophylline
	Beverages			
1.	Roasted coffee	350.0	17.0	$<1 \cdot 10^{-7}$
2.	Decaffeinated coffee	26.0	13.0	47.0
3.	Instant coffee	122.0	12.0	15.0
4.	Black Tea	217.0	12.0	$<1 \cdot 10^{-7}$
5.	Cocoa	4.0	17.0	$<1 \cdot 10^{-7}$
6.	Mate	62.0	32.0	21.0
	Urine			
7.	Coffee	3-71	$1 \cdot 10^{-7}$	$1 \cdot 10^{-7}$

The International Olympic Committee (IOC) banned the use of beverages in such amounts which results in excess of the maximum permitted urinary caffeine level of 12 μg mL^{-1}. Caffeine at high concentrations leads to various disorders, such as increased secretion of acid gastric juice, kidney dysfunctions, nervous system disorders, arrhythmias, etc. The IOC does not restrict the use of theobromine and theophylline because these compounds have been used to treat asthma.

In one of the most recent studies (Wanyika, Gotebe, Gitu, Ngumba & Maritim, 2010), caffeine content was determined in certain instant coffee (Africafe, Nescafe and Dormans) by HPLC with a spectrophotometric detector at a wavelength of 278 nm determined. The 250×4.6 mm column with ODS sorbent was used; methanol-acetic acid-water (20:0.1:79.9) as mobile phase and flow rate was 1 mL min^{-1}. The results are Dormans 1.64%, Africafe 3.42% and Nescafe 3.12%. The caffeine content was also determined by the spectrophotometric method—the results were about twice as high. Demissie et al. (2016) used UV/VIS spectrometer to determine caffeine in green coffee beans from hararghe, Ethiopia, using beer-lamberts's law and integrated absorption coefficient techniques.

Caffeine is known to be a pharmacologically active compound which stimulates the central nervous system. Caffeine does not accumulate in the body and is excreted in the urine within a few hours after consumption.

In one study (Rajkovic et al., 2004), quality of coffee was analyzed by different analytical methods, and the content of caffeine, heavy metals and aflatoxins was determined. Caffeine was additionally determined by HPLC in accordance with the recommendations of US Food and Drug Administration. The conditions used were: 150×2.1 mm column with Symmetry Shield RP18, particle size 3 μm; acetonitrile-water (30:70) as eluent, flow rate 0.3 cm^3 min^{-1} (Rajkovic, Vukoivc & Moriera, 2004). 1.38%, 1.59%, and 1.61% of caffeine was detected in three different coffee samples. The total content of caffeine, phenolic compounds, and chlorogenic acids is shown in Table 12 (Trugo, de Maria, Moriera & Petracco, 1995).

Table 12. Composition of coffee samples (methanolic extracts) (Trugo et al., 1995)

Coffee samples	Total content of phenolic compounds, %	Content of caffeine, %	Content of chlorogenic acids, %
Instant coffee 1	15.14	2.36	23.8
Instant coffee 2	14.50	2.99	23.0
Light roasted coffee	5.41	1.37	16.9
Medium roasted coffee	5.25	1.62	19.2
Dark roasted coffee	5.70	1.48	15.5

Using ion chromatography and ion exchange chromatography, both inorganic (chloride, nitrate, sulfate, phosphate) and organic anions (acetate, formate, tartrate, oxalate, citrate) were determined in decaffeinated coffee on AS19 capillary column (250×0.4 mm), gradient eluent KOH, 30℃, eluent flow rate 10 μL min^{-1}, conductometric detector and IonPak ICE-AS6 column, conductometric detector separately (Mabrok & Deatheroge, 1956).

5.2 Determination of Diterpenes (Kahweol and Cafestol) in Coffee

Many studies related to the effects of coffee consumption on human health are aimed at investigating its lipid composition such as coffee oil (Boekschotem, Engberink & Katan, 2003). Coffee oil contains pentacyclic diterpene alcohols. Coffee diterpenes are primarily esterified by various fatty acids. Fourteen cafestol derivatives and twelve kahweol derivatives were detected (Kurzrock & Speer, 2001). These compounds are partially preserved during the roasting process (Roos, Van Der Weg & Urgert, 1997). But they are also dehydrated which results in the formation of dehydrated derivatives along with other decomposition products, such as kahweol, cafestol, isokahweol, and dihydroisokahweol. Cafestol was found in both coffee Arabica and in coffee Robusta whereas kahweol was found only in coffee Arabica (Roos, Van Der Weg & Urgert, 1997). These diterpenes may contribute to the decomposition of toxic substances and provide a protective effect, particularly against aflatoxin B1 (Cavin, Holzhaeuser & Scharf, 2002). Moreover, they were noted to have anticarcinogenic, antioxidant, anti-inflammatory, and hepatoprotective properties (Kim, Hwang & Jeong, 2007; Lee, Choi & Jeong, 2007; Lee & Jeong, 2007). These beneficial effects of coffee oil create great opportunities for its use in the food and cosmetics industries. The use of coffee oil as a sunscreen agent in cosmetics has already been patented (Grollier & Plessis, 1998). However, the diterpenes, mainly cafestol, were also reported to have an undesired effect which tends to increase cholesterol levels (Kurzrock & Speer, 2001; Kim Hwang & Jeong, 2009; Urgert, Van Der Weg & Kosmeijer-Schuil, 1995). A reliable analytical method is necessary for the determination of synthetic metabolic pathways of these diterpenes. In addition, the determination of diterpene content can be used for classification of coffee mixtures, in particular as a means of establishing the difference between Arabica and Robusta.

Diterpene content in coffee drinks strongly depends on the way they were prepared. Espresso coffee may contain 5-10 times as much diterpenes as in filtered coffee (Urgert, Van Der Weg & Kosmeijer-Schuil, 1995; Ruiz del Castrillo, Herraiz & Blanch, 1999). The Arabica beans have the highest concentration of cafestol—11 mg per 100 g (Kölling-Speer, Strohschneider & Speer, 1999). In one study, diterpene content was determined in different parts of the coffee bean tissues (pericorp, perisperm, endosperm) (Dias, Campanha & Veira, 2010). Several methods were used for analysis and identification of diterpenes, i.e., Raman spectroscopy (Rubayiza & Meurens, 2005), GC (Urgert, Van Der Weg & Kosmeijer-Schuil, 1995; Ruiz del Castillo, Herraiz & Blanch, 1999; Pettitt, 1987), and HPLC (Kurzrock & Speer, 2001; Ruiz del Castillo, Herraiz & Blanch, 1999; Pettitt, 1987; Araujo & Sandi, 2006). The HPLC was used most often because this method allows the direct determination of components without derivatization, avoiding the decomposition of other lipid compounds. In HPLC, the 250×4.6mm reversed-phase columns filled with C_{18}, 5 μm, were used. Acetonitrile-water mixture at a ratio of 55-45% were used as eluents. A UV detector was used for detection; the absorption maximum was set at 290 nm for kahweol and at 230 nm for cafestol. Prior to the analysis, the diterpenes were extracted from coffee beans using organic solvents, such as n-hexane, diethyl ether, petroleum ether, followed by saponification by an alcoholic KOH solution. Diterpene compounds were also determined in Arabica coffee by HPLC-DAD (Erny, Moeenfard & Alves, 2015). In Arabica and Robusta coffee beverages, previously unknown compounds affecting their flavor were identified by high resolution HPLC-MS (Nascimento et al., 2015).

5.3 Determination of Sugars in Coffee

The content of low molecular weight sugars was investigated in green Arabica coffee beans which were obtained after flesh of the berries was separated from the seeds by wet or dry methods (Knopp, Bytof & Selmar, 2006).

Low content of fructose and glucose was noted in washed coffee beans (wet method). The content of these sugars was higher when coffee beans were dry processed. Laboratory models of these processing methods have confirmed the above results. Table 13 shows the content of monosaccharides in coffee samples of several manufactures from various countries. As can be seen from these data, saccharose typically has the highest content level.

Table 13. Concentration of the most common sugars in commercial green coffee samples (Knopp, Bytof & Selmar, 2006)

Type of green coffee and processing method	Country/Province	Content of sugars, %		
		Saccharose	Fructose	Glucose
Arabica (dry)	Brazil 1	9.25	0.14	0.04
	Brazil 2	8.70	0.15	0.04
	Brazil 3	7.60	0.02	0.01
	Ethiopia 1	8.26	0.17	0.04
	Ethiopia 2	6.30	0.01	0.01
	Mexico	9.64	0.05	0.03
	Honduras	6.92	0.05	0.03
Arabica (wet)	Colombia 1	8.76	0.06	0.04
	Colombia 2	8.07	0.01	0.01
	Colombia	7.16	0.20	0.01
	El Salvador	9.89	0.05	0.02
	Peru	8.21	0.05	0.02
	Kenya	9.31	0.06	0.03
	Cameroon	5.87	0.04	0.01
Robusta (dry)	Vietnam	3.15	0.16	0.10
	Ivory Coast	3.27	0.18	0.03
	Uganda	4.55	0.11	0.03
	Indonesia	4.85	0.18	0.06

Saccharose content is significantly lower in the Robusta than the Arabica. In the Arabica coffee, the saccharose content remains basically the same and does not depend either on the processing method or location where it was grown. A similar conclusion can be drawn with respect to glucose. However, the fructose content does depend both on the processing method and the location where it was grown. The fructose content in the Robusta coffee is higher compared with the Arabica, yet the glucose content is approximately the same in both grades. Typical content of sugars is provided in Table 14. Coffee processing technology can be ascertained by the content of fructose and glucose.

Table 14. The content of different sugars in the Arabica coffee beans (Brazil, Province Acaia) obtained by the dry processing method (Knopp, Bytof & Selmar, 2006)

Kind of Sugar	Content, %
Saccharose	7.07
Fructose	0.39
Glucose	0.23
Raffinose	0.06
Stachyose	0.04
Galactose Arabinose Rhamnose Mannose	0.01
Total:	7.79%

The content of sugars was determined by ion exchange chromatography on the Dionex PA20 column with amperometric detector at gold electrodes; NaOH solution as eluent and the flow rate was 0.5 mL min^{-1}.

5.4 Determination of Amino Acids in Coffee

Amino acids in coffee have been determined by a classic ion exchange chromatography in the form of

derivatives as well as by a direct method with amperometric detection (Nakhmedov, 1984). Amino acids were also separated by HPLC and ion-pair chromatography in the form of derivatives with ortho-phthalic dialdehyde by other researchers (Bytof et al., 2005). The following amino acids were identified in green coffee: glutamic acid, aspartic acid, serine, histidine, glycine, arginine, alanine, tyrosine, methionine, valine, norvaline, tryptophan, phenylalanine, isoleucine, leucine, lysine, etc with concentration range 4.4-1075 mg per 100g of coffee dry powder. The content of protein amino acids from different process methods are different. It is higher in dry processed coffee than wet processed coffee.

As a non-protein amino acid, Gamma-aminobutyric acid (γ-aminobutyric acid, GABA) is an inhibitory neurotransmitter which reduces brain activity, especially during sleep, and reduces depression. The World Health Organization believes that by 2020 depression will be the second most common disease after cardiovascular diseases. γ-aminobutyric acid (GABA) is contained in the Gabaron tea grade which was specially cultivated in Japan. GABA is also contained in coffee.

Drinks containing GABA are considered to be medicinal, they have the following basic biological properties:

- Help cure stress-related insomnia and improve sleep quality;
- Reduce risk of cardiovascular diseases;
- Have anti-hypertensive properties;
- Help cure alcoholism (alcoholics have decreased GABA content in the blood);
- Help in treatment of diabetes, Alzheimer's disease and Parkinson's disease;
- Retard the aging process.

In one study, the impact of coffee technological processing on the GABA content was investigated (Bytof, Knopp & Schieberle, 2005). Significant amounts of GABA were found in green coffee obtained by dry method. Coffee beans produced by the wet method had significantly less GABA.

GABA and other amino acids were determined by high performance liquid chromatography (de Andrade, Pinheiro & Lopes, 1995; Granvogl & Schieberle, 2007; Casal, Oliveira & Ferreira, 2002, Bispo et al., 2002; Meger, Ngiruwonsanga & Henze, 1996; Dias, Campanha & Veira, 2010). It was reported that GABA is produced in plants by α-decarboxylation of glutamic acid.

5.5 Determination of Heavy Metals

As for heavy metals, in accordance with the Regulation of Health Food, coffee may contain only trace amounts. In one study, less than 0.1 μg kg^{-1} of lead and less than 0.05 μg kg^{-1} of arsenic was detected by atomic absorption spectrophotometry (Rajkovic, Vukovic & Demin, 2004). Maximum permissible concentration of these elements is below 1μg kg^{-1}.

6. Determination of Organic Pollutants in Coffee

There are some compounds undesirable for flavor and bioactivity of the brew which occur due to inappropriate harvesting, weather conditions during processing or improper storage of coffee. Most of these compounds are microbial by products such as ochratoxin and specific biogenic amines. Acrylamide and polycyclic aromatic hydrocarbons could be formed by high roasting temperature.

6.1 Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

The PAHs were determined in coffee by HPLC with different detection methods and GC-MS. (Shi et al., 2016; Guatemala-Morales et al., 2016; Ventura et al., 2003; Pissinatti et al., 2015). Seven types of PAHs were detected in coffee by HPLC with fluorimetric detection (Ventual et al., 2003) (benzo(a)anthracene, benzofluoranthene, benzo(a)pyrene, benzoperylene, dibenzoanthracene, indenopiren). Separation was performed on a 100×4.6mm column filled with Chrom Sep with gradient elution. Excitation wavelengths of the fluorimetric detector were set at 274, 296, and 300 nm, emission wavelengths were 414, 406, and 470 nm, respectively. The PAH detection limit was in the range of 0.2 ng kg^{-1}, the linear range was between 0.2-10 μg L^{-1}. Ten polyaromatic hydrocarbons were determined in the roasted coffee by GC-MS on 24 commercial samples at levels of 1.00-11.29 μg kg^{-1} (Pissinatti et al., 2015).

The concentrations of PAHs in different grades of coffee are different, with lower content in green coffee. Benzo(a)pyrene was not found in green coffee at all. The most amount of benzo(a)pyrene was determined in

instant coffee and in roasted coffee. It was shown that the content of benzo(a)pyrene depends on the roasting degree (Kayali-Sayadi et al., 1999; Badolato, et al., 2006). Typically, benzo(a)pyrene served as an indicator of the overall contamination of coffee with PAHs.

6.2 Determination of Acrylamide in Coffee

Acrylamide exerts a carcinogenic effect (Tarcke, Rydberg, Karlsson & Tomnqvist, 2002). Acrylamide is synthesized when carbohydrate-rich foods are heated (Taeymans et al., 2004). One of the possibilities is that acrylamide synthesis occurs via Maillard reaction when amino acids react with carbonyl compounds during heating. Significant amounts of acrylamide can be formed during coffee roasting process, therefore coffee may become a source of acrylamide in people's every day diet. Reliable and quick method to measure acrylamide level in coffee is needed to optimize the technology of making coffee with minimal acrylamide level. As is well-known, some compounds which are formed in the roasting process give coffee unmatched aroma and pleasant taste. At the same time, some undesirable and even harmful compounds could also be formed in the roasting process.

Measuring acrylamide level in coffee is a challenging task because it requires pre-extraction from a complex matrix. Gas chromatography, liquid chromatography, GC-MS (Surma, Sadowska-Rociek, Cieślik & Sznajder-Katarzyńska, 2017; Ono et al., 2003; Biedermann et al., 2002) and LC-MS (Khan et al., 2017; Becalski, Lau, Lewis & Seaman 2003; Roach, Andrzejewski, Gay, Nortrup & Musser, 2003; Zyzak et al., 2003) are used most often. The acrylamide contents are different in some commercial coffee from several manufactures in different countries, with 42-338 ng g^{-1} in instant coffees and about 50ng g^{-1} in filtered coffee.

The effect of temperature and roasting time on the acrylamide content in coffee was also studied (Senyuva & Gökmen, 2005). The same sample was roasted at 150 ℃, 200 ℃, and 225 ℃ for 30 min. At 150 ℃, the acrylamide content was increasing during all roasting period, whereas at 200 ℃ and 225 ℃, the acrylamide content was increasing only during the first 10 minutes, after that, the acrylamide content was continuously decreasing during the remaining roasting time (up to 30 min). The level of acrylamide cut down to 5%.

Coffee also changed its color during roasting process. Nonlinear correlation between the color of coffee, as measured by Minolta's CM-3600d spectrophotometer, and acrylamide content in it was noted for 9 types of coffee. The highest acrylamide level, 338 ng g^{-1}, was noted in Jacobs Monarch coffee.

LC-MS method used for measuring acrylamide was quite fast, reliable, and accurate (Senyuva & Gökmen, 2005). Prior to the analysis, samples were extracted with methanol and purified.

6.3 Determination of ochratoxin in coffee

Ochratoxin is one of the carcinogenic mycotoxins which may develop in foods and beverages under inadequate production processes and storage conditions. In many countries, the maximum allowable levels of ochratoxin are set within 2-50 ng g^{-1}.

The EU Commission plans to establish standards for ochratoxin levels in green and roasted coffee. AOAC current method for the determination of ochratoxin in coffee was first published in 1975 (Levi, 1975). This method is based on the thin-layer chromatography with an insufficiently sensitive detection (maximum permissible concentration 20 ng g^{-1}).

It has been planned to develop a new method for the determination of ochratoxin in coffee. In recent years, dozens of articles were published on this subject. In one study, it was suggested to use reversed-phase HPLC with fluorimetric detection. The presence of ochratoxin in coffee was confirmed by mass spectrometry. The detection limit was 0.1 ng g^{-1}. Twenty samples of coffee from different countries, such as Brazil, Colombia, Zimbabwe, India, and Indonesia, were analyzed. Extraction was carried out with 1% $NaHCO_3$. A polymer-based column (Oasis MAX) which employs both reversed-phase and ion-exchange mechanisms of retention was used in this study. Using such a state-of-the-art technique, ochratoxin in the amounts exceeding the MPC was not detected either in green or in roasted coffee.

Another publication describes a method for measuring an ochratoxin level in green coffee by immunoaffinity column cleanup followed by HPLC separation and analysis (Vorgas, Dos Santos & Pittet, 2005). This technique was tested in 8 countries as part of preparation for establishing it as the EU official method.

A new HPLC-MS-MS method was developed for simultaneous determination of 21 mycotoxins in coffee beverages. Mycotoxins were detected at the μg kg^{-1} level. Ochratoxin—a mycotoxin regulated in coffee in Europe—was measured in two samples at the maximum allowable level (García-Moraleja, Font, Mañes & Ferrer, 2015).

Eight types of aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2, GM, GM_2) could be formed out of the three structural variants of aflatoxins. Determining aflatoxin B_1 is the most important goal since it is the precursor of all types of aflatoxins (Rajkovic, Vukovic & Demin, 2004).

Aflatoxins were determined by HPLC with fluorimetric detection at the detection limit of 2.5 μg kg^{-1}, excitation wavelength of 366 nm and emission wavelength 460nm. In all samples of Grand and Don coffee varieties (Golex Product), the total amount of B_1 and G_1 aflatoxins was less than 2.5 μg kg^{-1} whereas the maximum permissible concentration is 5.0 μg kg^{-1} (Regulation of Health Food).

The harmful substances in coffee can be eliminated or reduced to a minimum (below MPC) by adherence to the adequate technology for gathering and processing green coffee beans, and proper storage conditions of the finished product. It is worth to mention people should avoid to consume coffee or tea along with foods containing significant amount of nitrates since hydrogen cyanide may be formed (Seto et al., 2008).

7. Identification of Coffee

Identifying characteristics of coffee at different stages of its production are determined by: the shape and size of coffee beans, its organoleptic characteristics, element compositions (Krivan et al., 1993; Martin et al., 1998 and 1999; Weckerle et al., 2002; Serra et al., 2001; Wieser et al., 2002; Rodrigues et al., 2010), aroma analysis (Dirinck et al., 2001), physical and chemical composition (Rocha et al., 2004; Martin et al., 1998; Ky et al., 2001; Casals et al., 2000; Guerrero et al., 2001; Carrera et al., 1998; Valdenbro et al., 1999 ; Bertrand et al., 2005; Martin, et al., 2001; Gonzales et al., 2001; Anderson et al., 2002; Kemsley et al., 1995; Dupuy et al., 1995; Suchanek et al., 1996; Downey et al., 1996). For identification of botanical species of coffee beans, assessment of the outward anatomy and morphology may sufficient. Arabica and Robusta coffee beans differ in shape and size. Green Arabica coffee beans have an elongated shape 6-15mm long. Green Robusta coffee beans have rounded shape 4-9mm long. After roasting, the bean size is increased by 25-50%.

Coffee Arabica has richer aroma than coffee Robusta. Arabica coffee tastes slightly sour whereas Robusta coffee has a more bitter taste. Experts can organoleptically—by taste and aroma—distinguish natural roasted Arabica and Robusta coffee.

Modern physicochemical methods, primarily chromatography, provide the most reliable criteria for identification of coffee authenticity at all production and preparation stages because the main varieties of coffee Arabica and Robusta differ in content of sugars, caffeine, theobromine, theophylline, trigonelline, chlorogenic acids and other compounds (Table 15).

Table 15. Overall typical chemical composition of Arabica and Robusta coffee

No.	Compounds	Arabica	Robusta
1	Water-soluble extractive substances	19-20%	24-27%
2	Chlorogenic acids (more than 10)	5.5-8%	9-11%
3	Caffeine	0.6-1.2%	1.8-3%
4	Theophylline	1-4 %	10-100 ppm
5	Theobromine	1.3-2.5 %	0.1-0.67%
6	Trigonelline	1-1.2%	0.6-0.7%
7	Diterpene glycosides	290-340 mg/kg	10-45 mg/kg
8	Carbohydrates.		
	Polysaccharides, cellulose	Over 12%	Over 12%
	Monosugars	0.7-1%	0.7-1%
9	Protein substances (by amino nitrogen)	1.55-1.63%	1.36-1.72%
10	Amino acids (free)	Over 1.6%	Over 1.6%
11	Tannins	3.6-7.7%	2.2-6.6%
12	Free fatty acids (linoleic, palmitic, oleic, myristic, linolenic, etc.)	0.5-3%	1.2-3.8%
13	Organic acids (citric, malic, maleic, oxalic, acetic, etc.)	About 1%	About 1%
14	Vitamins B1, B2, B6, pantothenic acid, nicotinic acid (PP), tocopherol (E).	Less than 3 mg	Less than 3 mg
15	Mineral substances (Na, Mg, K, Fe, Mn, Zn, etc.)	3-4.5%	3-4.5%

Note: Coffee contains polyamines and gamma-aminobutyric acid. The table shows average values—the actual values depend on coffee variety and growing area.

Recently the elemental detection of green bean of arabica and robusta coffee from Gayo Highland, Aceh-Indonesia, has been identified by using fundamental Nd-YAG Laser for distinguishing the characteristics of both coffees. It is noticed that the order of elements concentration from highest to lowest are Ca>K>CN>Na>C for arabica and K>Ca>CN>Na>N for robusta. The ratio of intensities of these elements to C intensity could be used as a marker to discriminate kind of coffee for the purpose of authentication (Abdulmadjid, Meilina, Hedwig & Kurniawan, 2017).

The state-of-the-art equipment, namely, multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) and isotope ratio mass spectrometer (IRMS), were used to determine coffee authenticity and its origin (Bertrand, Etienne, Lashermes, Guyot & Dovrieux, 2005). Sixty samples from twenty different geographical areas were analyzed. The $^{87}Sr/^{86}Sr$ isotope ratio and average value of the $^{18}O_2$ isotope were determined in the samples. The measurement results showed a high degree authenticity of coffee origin, especially for countries in South America.

Different compounds from coffee have been used to assess the grade of coffee and its origin. Samples of coffee (fruit, beans, husks) from China, India, and Mexico were analyzed with regards to the content of chlorogenic acids, caffeine, and total polyphenols (Mullen et al., 2013). Among chlorogenic acids (69 chlorogenic acids are currently identified in green coffee beans), 5-O-caffeoylquinic acid was prevalent. The analysis was performed by UHPLC coupled with a QE Orbitrap MS. The samples' antioxidant capacity was also determined. The content of chlorogenic acids in coffee from India and Mexico was similar (18.8 and 22.9 mg g^{-1} in Arabica coffee beans; 27.3 and 27.4 mg g^{-1} in Robusta coffee beans, respectively), while it was significantly lower in coffee beans from China (1.7 and 7.2 mg g^{-1}). Also, coffee from China contained a third to a half of the amount of polyphenols in coffee from India and Mexico, although the total content of flavonoids was about the same. Antioxidant activity measured by FRAP was 2-2.5 times lower in coffee from China than in coffee from India and Mexico (112, 227 and 267 μmol g^{-1}, respectively, for Arabica coffee). Coffee husk turned out to be a rather rich source of procyanidins, flavanols, and flavonols—about 115-130, 18-30, 260+ μg g^{-1}, respectively. Mehari et al., (2015) simultaneously determined the alkaloids in green coffee beans from Ethiopia using chemometric method to evaluate the geographical origin of coffee. High performance liquid chromatography was applied and the limits of detection for the method were established. Study showed that the application of linear discriminant analysis provided 75% correct classification of samples into the respective production regions, with a 74% prediction success rate. The moderate classification efficiency obtained when using alkaloid data demonstrated the potential of using this class of compounds in discriminant models for determination of the geographical origin of green coffee beans from Ethiopia.

8. Conclusion

Coffee is one the most popular beverage and consumed by millions of people all over the world. Numerous factors throughout the coffee production chain, from plant to cup, have been shown to have effects on the color, aroma, and flavor of coffee. This article reviews publications on qualitative and quantitative determination of volatile and non-volatile compounds in green and roasted coffee which determine its aroma, taste and quality, majorly by chromatographic methods.

Using these methods, the following antioxidants and nutrients were identified in coffee: oxyaromatic acids, chlorogenic acids, vitamins, amino acids, sugars, diterpene glycosides, trace minerals, etc. Knowing the chemical composition of coffee will facilitate the study of coffee impact on human health and disease prevention.

It has been reliably proven by now that moderate consumption of coffee reduces cardiovascular risk, prevents the development of type 2 diabetes, protects the liver and even helps combat liver cirrhosis, reduces the risk of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and protects against asthma. In general, coffee intake decreases oxidative stress which precedes many dangerous diseases.

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