CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE
Faculty of Tropical AgriSciences

Monitoring of volatile substances in the coffee

Master's thesis

Prague 2018

Supervisor:

Ing. Klára Urbanová, Ph.D.

Author:

Bc. Sabina Kožichová

DECLARATION

I, Sabina Kožichová, hereby declare that this thesis entitled **"Monitoring of volatile substances in the coffee"** is my own work and all the sources have been quoted and acknowledged by means of complete references.

In Prague, 26th April 2018

…….....

Sabina Kožichová

ACKNOWLEDGEMENTS

Foremost, I would like to thank my supervisor, Ing. Klára Urbanová, Ph.D., for her patience, kindness, continuous encouragement, and positive attitude. Her office door was always open whenever I ran into a trouble spot in my research and needed an advice or consultation. Without her knowledge, experience, and guidance successful completion of this diploma thesis would be hardly possible.

Besides my supervisor, my sincere thanks also go to Ing. Alexander Kandakov, Ph.D. for his helpful advice, and to Ing. Andrea Jindrová, Ph.D. for making time for the consultation on the statistical part of my diploma thesis.

Last but not least, I would like to express my sincere gratitude to my friends, classmates, boyfriend, and family for providing me with unfailing support throughout my years of study. Thanks to them everything always seemed easier and achievable.

ABSTRACT

Coffee is one of the major socioeconomic crops with a direct impact on the development of producing countries. Aroma, resulting from a complex mixture of volatile substances, is the most important quality and price determinant. The chemical changes occurring in the volatile profiles of roasted *Coffea arabica* beans during storage under two different conditions (room temperature, refrigerator) for the period of 15 weeks have been characterized by means of HS-SPME in combination with GC-MS. A total of 65 volatile compounds, including 7 potent odourants, were identified and quantified in samples of four diverse geographical origins. No off-flavour compounds were generated during the research in any of the samples. The only new substances emerged were dodecane and p-cymene. The complete loss of 3-ethyl-1,2-cyclopentanedione was detected in samples stored in the refrigerator. The total loss of another substance did not occur, but the overall considerable volumetric decrease of volatile substances in roasted coffee beans over time has been confirmed. Significant drops in volume have occurred, beside others, in the case of 2-methylbutanal, 3-methylbutanal, and 2,3-pentanedione, which are ranked among the important odourants. Only minimum of substances remained completely inert to the different storage temperatures throughout the research. In general, study demonstrated decreasing rate of loss of the volatile substance when storing the samples in the refrigerator. Minor fluctuations in reactions to storage conditions were also noted in accordance to coffee's origin. Disturbance of the balance of potent volatile compounds was assessed as the main cause leading to coffee quality degradation.

KEY WORDS:

Coffea arabica, volatile compounds, SPME, GC-MS, storage, temperature, aroma

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AC	Alternate current
AL	Aluminium
CAR	Carboxen
CW	Carbowax
DC	Direct current
DVB	Divinylbenzene
EFTA	European Free Trade Association
EPA	Environmental Protection Agency
ESI	Electrospray ionization
EU	European Union
FAO	Food and Agriculture Organization
GC	Gas chromatography
GDP	Gross domestic product
GLC	Gas–liquid chromatography
GSC	Gas–solid chromatography
HPLC	High performance liquid chromatography
HS	Headspace
ICO	International Coffee Organization
IM	Identification method
KI	Kovats Index
LLE	Liquid–liquid extraction
LLME	Liquid–liquid micro extraction
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser desorption/ionization
MS	Mass spectrometry
MSD	Mass selective detector
NIH	National institutes of Health
NIST	National Institute of Standard and Technology
PA	Polyacrylate
PDMS	Polydimethylsiloxane
PE	Polyester
PET	Polyethylene
PFE	Pressurised fluid extraction
PTFE	Polytetrafluorethylene
QMF	Quadrupole mass filter

RF	Radio frequency
RT	Room temperature
RSD	Relative standard deviation
SCF	Supercritical Fluid Extraction
SCOT	Support-coated open tubular
SDE	Simultaneous distillation−extraction
SFE	Supercritical Fluid Extraction
SL	Significance level
SPE	Solid phase extraction
SPME	Solid phase microextraction
TOF	Time-of-flight
TPR	Templated Resin
US	United States
U.S.A.	United States of America
UV	Ultraviolet
WITS	World Integrated Trade Solution
WCOT	Wall-coated open tubular

1 INTRODUCTION

Coffee is the most traded tropical agricultural commodity (Smělíková & Zdráhal 2011). The earnings from its exports have a positive strong impact on the overall economic situation and development of the producing countries, which are particularly developing and least developed countries (Al-Abdulkader et al. 2017). A positive effect of coffee drinking on the human body, along with a pleasant taste and aroma, makes coffee one of the most popular beverages in the world. In 2016, 11 million hectares of land was used for coffee growing (FAO 2018). Its production, processing and distribution employ millions of people worldwide. Therefore, coffee is one of the major socioeconomic crops with a direct impact on access to food, education, medical services, housing and the other basic needs (Smělíková & Zdráhal 2011).

With increasing affordability of the beverage, demand for coffee is constantly rising (FAO 2015; ICO 2017). The price of coffee is reflected by a quality of the bean, which depends mainly on the plant species, growing conditions and processing method. The quality of coffee is judged, in particular, in terms of its aroma. Aroma is one of the most important properties of coffee, having a main role in purchase decision making process. It is the result of a mixture of volatile substances, of which about 1,000 have been identified in coffee so far (Yeretzian et al. 2003). However, revealing of the specific effect of all these substances on the taste and smell of coffee is still in its beginning stages. Other uncertainties are associated with the presence of volatile substances in coffee in relation to the coffee's properties and storage conditions (Toledo et al. 2016). Clarification of the effect of storage conditions on the composition of volatile substances in *Coffea arabica* L. beans has led to the idea of starting this research.

2 LITERATURE REVIEW

2.1 COFFEE'S ORIGIN AND CONSUMPTION

The exact origin of coffee remains unclear to this day. Researchers trace that the first coffee tree appeared in the forests of Ethiopia (Amamo 2014). It is assumed that before the coffee began to be prepared in today's well-known manner, the coffee beans were initially only chewed in their raw form. The first credible mention of coffee drinking comes from Mocca, city of Yemen, and dates back to the late 14th or early 15th century (Schivelbusch 1992; Topik 2000). Soon, the beverage began to enjoy great popularity and its drinking gradually spread all over the world (Wolf et al. 2008).

Nowadays, coffee is the second most popular beverage, right after tea (Caprioli 2015; Batista et al. 2016). In 2010, more than 500 billion cups were consumed worldwide (Farah 2012). The latest data released by the International Coffee Organization (ICO) refer to the 9,078 billion kilograms of coffee consumed annually worldwide. The largest amount of coffee is consumed by residents of the United States (US), Brazil, Italy, Japan and Germany (Farah 2012). The highest coffee consumption per capita is in Scandinavian countries and Germany (Smělíková & Zdráhal 2011). Considering only the weight of the basic raw materials for making these two beverages, 80 % more coffee is consumed than tea. But while we use about 45 grams of coffee beans to produce one liter of coffee, only 10 grams of tea leaves are needed to produce one liter of tea. For this reason, to compare the quantity consumed, it is much more logical to measure the volume in liters. Then the tea prevails over the coffee. For every cup of coffee there are three cups of tea drunk. Choosing a preferred drink is affected by many aspects. Of course, tradition is important, but income also has its influence. It turns out that people from high income classes usually prefer drinking coffee over drinking tea, while people from lower income classes drink more tea than coffee (Grigg 2002).

Consumption of coffee is continually increasing (Butler 1999; Maeztu et al. 2001; Illy & Viani 2005; ICO 2017). Due to the swelling middle classes of the developing world and their rising incomes coffee is becoming more a more affordable (FAO 2015).

2.2 PRODUCTION, TRADE AND ECONOMY

In 2014, 8,790,005 tonnes of green coffee were produced worldwide. Over the past 50 years the production has risen considerably. Compared to 1994, the amount of coffee produced more than doubled (FAO 2017). Coffee is currently grown by 70 countries (FAO 2015; Iwasa et al. 2015). The five largest coffee producers are Brazil, Vietnam, Colombia, Indonesia, and Ethiopia (ICO 2017; Statista 2017). The top three are responsible for more than half of world production, headed by Brazil, which accounts for almost one third of the world's coffee production (FAO 2017). A century ago, 90 % of all coffee was grown in Brazil (Barter 2016).

Nowadays, 90 % of coffee comes from developing countries (Feleke & Walters 2005). According to Grigg (2002), it is even 100 % of the world's coffee that is being produced in developing countries. The reason is that most of the developed countries do not have suitable conditions for coffee growing. However, these countries are the most important consumers. Only 25 % of the world's coffee consumption takes place in coffee producing countries (Feleke & Walters 2005). The rest of the coffee becomes a part of the business. Coffee is exported mainly in its unprocessed form. Around 7 million tonnes of green coffee are exported annually, while the amount of roasted coffee exported per year usually does not exceed one tonne (FAO 2017). In smaller quantities, coffee extracts, essences, husks, skins and concentrates are also exported (Pokorná & Smutka 2010; FAO 2017). The most important coffee importers are European Union (EU), US, Japan and Canada. Russia and Switzerland share the fifth place in the ranking (US Department of Agriculture 2017). In 2014, the green coffee imported into the EU and EFTA was valued at 7.9 billion euros (Netherlands Ministry of Foreign Affairs 2016).

From the above figures and information, it is clear that coffee plays a major role in the economy of many developing countries. It is a cash crop and the world’s most valuable traded foodstuff (Barter 2016). Some sources claim that coffee is, right after the crude petrol, the most important traded commodity of the world markets (Nair 2010; Pokorná & Smutka 2010; Patay et al. 2016). About 500 million people are involved in the

international coffee trade (Batista et al. 2016). Coffee cultivation is a vital source of income for many people in the developing world. The livelihood of around 125 million people worldwide is dependent on coffee. Only a small percentage of coffee comes from large-scale plantations owned by multinational companies. 80 % of the world's coffee is produced by 25 million smallholder farmers (Fairtrade Foundation 2017). In Indonesia, it is even 96 % of the overall coffee productions that comes from the smallholders (Brata 2007). The livelihood of about 2,053,000 people in Central America is based on coffee production. Due to the successful pursuit of greater diversification of exported products, coffee is no longer contributing to the GDP of Central American states as much as it used to be 30 to 40 years ago, but it still remains a very important commodity (Avelino et al. 2015). It is the top exported product of Honduras, second most exported product of Nicaragua and San Salvador and third of Guatemala. Outside of Central America, coffee is second to gold among legal non-petroleum exports in Colombia (Barter 2016; WITS 2017).

The coffee market is inherently unstable. Farmers face many risk factors such as climate change, diseases, farmland losses and constant wide fluctuations in prices. Urban migration and aging workforce are also increasingly becoming common problems (Fairtrade Foundation 2017; Global Washington 2017).

2.3 TYPES OF COFFEE

Coffee plant belongs to the family Rubiaceae, genus *Coffea*. It is a perennial evergreen shrub or tree that can grow up to 3 to 12 meters high. However, for easier harvesting, the plants are regularly pruned and kept at a maximum height of 2.5 meters (Belitz et al. 2009). Highly scented white flowers mature into deep red or purple fruits, so-called cherries, that usually contain two seeds called coffee beans. Detailed structure of coffee bean is presented in Figure 1. The fruits ripen irregularly which makes harvesting difficult (Masarirambi et al. 2009). For this reason coffee is still predominantly picked selectively by hand (Batista et al. 2016).

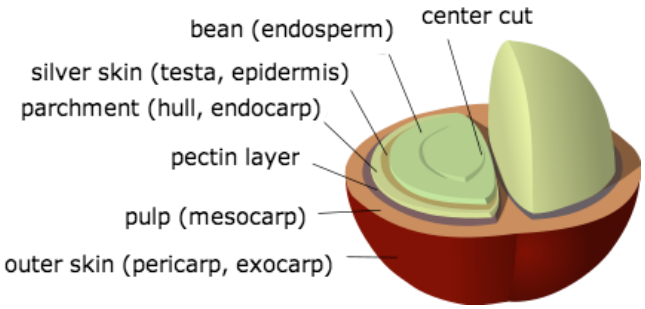


Figure 1. Coffee bean structure
(Source: National Coffee Association of U.S.A. 2018a).

Coffea species grow in warm and humid climate of tropics and subtropics. Equatorial regions with the altitude from 1,300 to 1,600 meters provide the most suitable conditions. Nevertheless, coffee is grown especially at an altitude of 200 to 1,200 meters at temperatures around 18-22°C (Rohwer 2002; Tesfaye 2013). The lifespan of a coffee plantation can be over fifty years, but most of the plantations have an average lifespan about thirty years (Wintgents 2009; Bunn et al. 2014).

According to Clarke (2003) the *Coffea* genus comprises more than 80 species. Other sources mention even more than 100 identified *Coffea* species (Kochko et al. 2010; Davis et al. 2011; Batista et al. 2016). Three best known are *Coffea arabica* L. (Arabic coffee), *Coffea canephora* L. (Robusta coffee) and *Coffea liberica* L. (Liberian coffee). Only first two are grown on a large scale and economically important as commercial products (Patay et al. 2016; Herrera & Lambot 2017). While *C. arabica* accounts for about 60 % of the world's production, remaining 40 % of coffee comes from *C. canephora* (FAO 2015; US Department of Agriculture 2017). *C. arabica* is very sensitive to heat and prefers partial shade. The shade should not cover more than 50 % of the cultivated area. In Central America, *Ingа* spp. and *Grevillea robusta* are the most commonly used shade trees, planted with the density ranging from 83 to 100 trees per hectare at higher altitude or 156 to 204 trees per hectare at lower elevations. In Africa, farmers mostly use *Albizzia* spp. for these purposes, while *Erythrina indica* is typical shade tree for India. Majority of shade trees in Indonesia and Papua New Guinea belongs to the genus Casuarina (Illy & Viani 2005).

Annual mean air temperature for *C. arabica* should range from 18°C to 23°C. *C. canephora* generally tolerates higher temperatures and grows best in sun, but it is more susceptible to cold. It grows best at temperature among 22°C to 26°C (Wintgents 2009; Paes de Camargo 2010; Toledo et al. 2016). *C. canephora* is limited to grow at lower latitudes than *C. arabica*, because it needs a climate with little intra-seasonal variability (Bunn et al. 2014). *C. canephora* prefers lower altitudes, usually up to 800 meters above the sea level, while most suitable conditions for *C. arabica* are at higher altitudes from around 1,000 to 2,100 meters, as for example in hilly terrains of Central America and Colombia (Paes de Camargo 2010; Toledo et al. 2016). *C. canephora* gives greater yields and it is easier to grow, as it is less demanding and more resistant to pests and diseases (Farah 2012; Toledo et al. 2016).

The overall quality assessment of coffee is based on its organoleptic quality as well as physical properties. The organoleptic quality describes coffee's overall taste, flavour, sweetness, aroma or fragrance and acidity, while the physical quality includes features such as shape, weight, thickness, width, length and colour (Leroy et al. 2006). Arabic coffee is generally considered to be of higher quality than Robusta coffee. Therefore, Arabic coffee is better valued in the markets and it is more expensive. In June 2017 the average price of Arabica on the US market was 144.10 US cents per pound, while you could buy one pound of Robusta for 104.83 US cents. In October 2014, the price for Robusta was even less than half the price of Arabica (IndexMundi 2017). The price difference often leads to the fraudulent mixing *of C. canephora* coffee beans with *C. arabica* coffee beans. The mixture is then sold at the same price as pure Arabica (Schievano et al. 2014). Aroma of Robusta coffee is raw and earthy whereas Arabica's is mild. Even so, most customers are unable to recognize the difference and never reveal the truth (Knysak 2017). And of course, a coffee made from carefully harvested and properly processed Robusta seeds will always be of the better quality than a coffee made from Arabica seeds which has been handled improperly (Farah 2012).

2.4 CHEMICAL COMPOSITION

The chemical composition of coffee beans is a complex combination of a large number of defined and still undefined substances. The basic constituents of coffee seeds are polysaccharides, proteins, lipids and waxes, caffeine, chlorogenic acids, quinic and caffeic acids, water, and minerals, primarily potassium, magnesium, calcium, phosphorus, manganese and iron (Franca et al. 2005; Petriková & Patočka 2006). Other naturally occurring minerals in coffee are sodium, copper, zinc, rubidium, strontium, vanadium, cobalt, nickel, barium, boron. Minor amount of B, C, P and PP vitamins can be also found (Patay et al. 2016). It is not possible to precisely pin down the composition of a coffee bean in general. The individual constituents contained in seeds and their amounts vary greatly. The final composition of a green coffee seed depends mainly on its genetic and physiologic aspects, such as botanical species, variety, or degree of maturation. For example, Arabica coffee normally contains lower amounts of antioxidants, less chlorogenic acids and about two times less caffeine than Robusta coffee (Ky et al. 2001; Campa et al. 2004; Farah 2012). Secondly, chemical compositon is also influenced, to a lesser extent, by extrinsic factors such as soil pH, soil composition and its fertilization, agricultural practices, drying method, processing and post-harvest techniques, climate and storage conditions (Farah & Donangelo 2006; Selmar et al. 2006; Perrone et al. 2009). Several scientists believe that higher altitudes and shady conditions have also a positive effect on coffee quality. Only a small number of studies inquire into this phenomenon. It is not yet clear, whether the two factors directly lead to a better coffee quality, or whether it is just the fact that they dramatically reduce air temperature. Lower temperatures slow down the ripening process, resulting in increased accumulation of aroma precursors (Vaast et al. 2006; Joët et al. 2010).

Roasting also significantly changes the chemical composition and physical properties of the seed. The temperature of roasting and the exposure time are the most influential factors (Gloess et al. 2014). Less important, but still insignificant, factors influencing the chemical composition are the air-flow speed, roaster type, amount of coffee in the roaster

and other roasting variables. From a processing point of view roasting is a relatively easy step. The great number of chemical reactions that occur in this process is, on the contrary, so complicated that it has not yet been fully understood (Farah 2012). Easily perceived physical changes associated with roasting are volume increase, weight loss, colour change and decrease of density (Noor et al. 2015). Soluble fiber is partially degraded and incorporated into melanoidins. The degradation of sucrose and polysaccharides rise the levels of aliphatic acids (formic, acetic, glycolic, and lactic), which may increase the brew acidity (Clarke & Macrae 1985; Ginz et al. 2000). Most of the chlorogenic acids are degraded due to their thermal instability (Farah 2012). A degree of roasting affects the amount of losses. Sever roasting can cause degradation of almost 100 % chlorogenic acids (Trugo & Macrae 1984). A variety of compounds including nicotinic acid (3 %) and volatile compounds such as pyrrols (3 %), pyridines (46 %), pyrazines, and methyl nicotinate are produced by degradation of trigonelline (Flament et al. 1968; Trugo & Macrae 1984). Although, caffeine as well as lipid fraction are relatively stable components, small losses may also occur (Farah 2012). Water evaporation is the main reason for weight loss. Its vaporization together with release of CO_2 and formation of volatile components leads to the already mentioned increase in volume (Dutra et al. 2001). Key components that are attributing to the coffee flavour and thus significantly influencing the coffee quality are described in Table 1. The table also contains information on the behavior of individual components during roasting.

Table 1. Changes of the key coffee components after roasting

Component	Flavour attribute	Influence of roasting	References
Caffeine	Perceived strength, body and bitterness	Stable	(Oestreich-Janzen 2010)
Trigonelline	Overall aromatic perception, bitterness	60-90 % degraded	(Clarke & Macrae 1985)
Chlorogenic acids	Acidity, astringency and bitterness	59.7-98 % degraded	(Trugo & Macrae 1984)
Sucrose	Flavour precursor	Disappear	(Grosch 2001)
Lipids	Flavour carriers, texture and mouthfeel	Stable	(Oestreich-Janzen 2010)
Water		>80 % vaporized	(George et al. 2008)

Following Table 2 lists the basic components of the green coffee seeds and roasted coffee beans of the two most common coffee species – *Coffea arabica* L. and *Coffea canephora* L. The table shows percentages of each component in the seed/bean. Numbers are not illustrative of extreme possibilities. They represent the most common values or values around which the percentage typically fluctuates.

Table 2. Chemical composition of green seeds and roasted beans of *Coffea arabica* L. and *Coffea canephora* L.

	Concentration (g/100g)				Source
	Robusta	**Robusta**	**Arabica**	**Arabica**	
Carbohydrates/fiber					
Polysaccharides	37.0	48.0-55.0	31.0-33.0	34.0-44.0	a
Sucrose	1.6	0.9-4.0	4.2	6.0-9.0	a
Reducing sugars	0.3	0.4	0.3	0.1	a
Pectins	2.0	2.0	2.0	2.0	a
Lignin	3.0	3.0	3.0	3.0	a
Nitrogenous compounds					
Protein/peptides	7.5-10.0	11.0-15.0	7.5-10.0	10.0-11.0	a
Free amino acids	-	0.8-1.0	-	0.5	a
Caffeine	2.4-2.5	1.5-2.5	1.1-1.3	0.9-1.3	a
Trigonelline	0.3-0.7	0.6-0.7	0.2-1.2	0.6-2.0	a
Nicotinic acid	0.014-0.025	-	0.016-0.026	-	a
Lipids					
Coffee oil	11.0	7.0-10.0	17.0	15.0-17.0	a
Diterpenes	0.2	0.2-0.8	0.9	0.5-1.2	a
Minerals (as oxide ash)	4.7	4.4-4.5	4.5	3.0-4.2	a
Water	1.5-5.0	8.5-12.0	1.5-5.0	8.5-12.0	a
Acids and esters					a
Chlorogenic acids	3.3-3.8	6.1-11.3	1.9-2.5	4.1-7.9	a
Aliphatic acids	1.6	1.0	1.6	1.0	a
Quinic acid	1.0	0.4	0.8	0.4	a
Melanoidins	25.0	-	25.0	-	a
Volatile compounds	0.1	traces	0.1	traces	b

(Sources: [a]Farah 2012, [b]Oestreich-Janzen 2010)

2.4.1 CAFFEINE

Caffeine (methylxanthine, specifically 1,3,7-trimethylxanthine) is a heat stable purine alkaloid that is synthesized in the immature coffee fruits and young leaves of seedlings and gradually accumulated in these organs during the maturation process

(Ashihara et al. 2008; Farah 2012). In the case of fruit, caffeine is synthesized in outer part, pericarp, from which it is translocated into the endosperm. Eight months after flowering caffeine reaches its final value. Root system and brown parts of shoots do not usually contain any caffeine (Zheng & Ashihara 2004). Caffeine biosynthesis is a four step process starting with the methylation of xanthosine. Slow degradation of caffeine also occurs in the plant. The basis for caffeine catabolism is the separation of the three methyl groups and the formation of xanthine that is further degraded to CO_2 and NH_3 (Ashihara et al. 2008). The molecular structure of caffeine is shown in Figure 2.

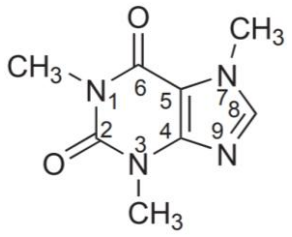


Figure 2. Molecular structure of caffeine
(Source: Oestreich-Janzen 2010).

The reason for the presence of caffeine in plants is explained by two theories. According to the first one, “chemical defence theory”, caffeine, as a toxin which can be lethal in certain doses to many living organisms, acts as a protection of young soft tissues against herbivores and pathogens. The second theory, called “allelopathic or autotoxic function theory”, is based on the fact that after the abscission caffeine still remains in the leaves. Its release into the soil inhibits germination of seeds around the parent plant and thus regulates spacing and suppresses competitive plants (Anaya et al. 2006; Ashihara et al. 2008). Caffeine is considered as a major pharmacologically active compound in the coffee. It is soluble in hot water. It has a form of white crystalline substance and bitter taste (George et al. 2008; Farah 2012). However, it is responsible only for small percentage of perceived bitterness of the beverage and thus plays minor role in flavour (Flament et al. 1968). Caffeine intake results in stimulation of the central nervous system, peripheral vasoconstriction and myocardial stimulation. People do not drink coffee just because of its delicious flavour, but also because of caffeine's ability to enhance cognition

and mood, support wakefulness and produce stimulatory effects (Lieberman et al. 2002; Haskell et al. 2005).

2.4.2 TRIGONELLINE

Trigonelline (1-methylpyridinium-3-carboxylate) is a pyridine alkaloid synthesized by enzymatic methylation of nicotinic acid also known as niacin or vitamin B_3. As with caffeine, biosynthesis occurs in fruits and leaves (Ashihara 2006; Ashihara et al. 2011). Trigonelline can be found in all organs of coffee plant, including roots (Ashihara 2015). While caffeine is still present in detached senescent leaves in relatively high amounts, trigonelline is almost completely absent in them (Ashihara 2006). Another difference from caffeine is thermal instability of trigonelline. At higher temperatures it degrades and is demethylated back to nicotinic acid (Farah 2012). From a biological aspect, trigonelline helps plants to survive and grow thanks to its regulatory functions (Garg 2016). It also serves as a reservoir of nicotinic acid (Ashihara 2006). In terms of coffee drinking, its importance is primarily associated with the development of characteristic flavour, as trigonelline and some of its thermolytic products, including pyrazine, furans, pyrroles and alkyl-pyridines, undoubtedly contribute to aroma and taste (Wei & Tanokura 2015). Trigonelline promotes glucose utilization and stimulation of respiratory activity (Riedel et al. 2014). Firgure 3 shows molecular structure of trigonelline and its precursor, nicotinic acid.

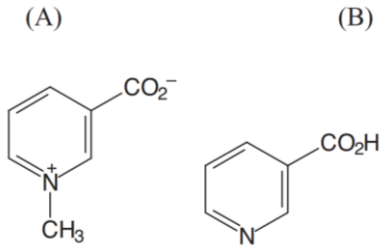


Figure 3. Molecular structures of (A) trigonelline and (B) nicotinic acid
(Source: Farah 2012)

2.4.3 CHLOROGENIC ACIDS

Chlorogenic acids are known to have DNA protective functions and many other positive effects such as anticancer, antibacterial and anti-inflammatory activities (Henry-Vitrac et al. 2010; Lou et al. 2011; Xu et al. 2012).

2.4.4 VOLATILE COMPOUNDS

Among the factors influencing the perception of the taste of food, the aroma is unequivocally dominating. Both academic and popular literature, mention that 75 to 90 % of what we consider to be 'taste', in fact, derives from the sense of smell. Even though the number needs to be taken with a caution, since it can be slightly tricky to indicate the exact value of the perception of the senses, it is clear that the influence of the aroma on the taste is undeniable (Spence 2015). Roasted coffee is one of the most aromatic food products with more than 900 volatile compounds identified (Flament 2002). While Yeretzian et al. (2003) speak even of up to 1000 of volatile substances identified in the roasted coffee, Franca et al. (2005), are more restrained and mention just over 800 of them. The most common classes of volatile compounds (in order of abundance) are furans, pyrazines, ketones, pyrroles, phenols, hydrocarbons, acids and anhydrides, aldehydes, esters, alcohols, sulfur compounds, and others (Flament 2002). From which sulfur compounds, such as thiols, and pyrazines has the strongest influence on coffee flavour (Nijssen et al. 1996).

Customers often base their purchase decisions of food on aroma, which makes it one of the main food attributes (Toledo et al. 2016). However, the question remains which of the hundreds of substances and their corresponding precursors relevantly contribute to the flavour and to what extent (Franca et al. 2005). Current assumptions estimate that human nose can sense to varying degrees only about 5 % of volatile compounds derived from coffee, so probably only a small fraction of a large number of substances is capable to impact coffee's aroma (Yeretzian et al. 2003). Not only the odour compound itself but also its concentration plays a huge role. Each substance has its unique threshold concentration which, when exceeded, causes the substance to be perceived.

Concentrations well above the threshold lead to the "bloom out" of the odour, which can then become identifiable or dominant (Toledo et al. 2016). Normal concentration levels are ranging from parts per million (ppm) to parts per trillion (ppt) (Grosch 2001; Buffo & Cardelli-Freire 2004).

Some of the common coffee volatile substances, including the description of their fragrance, are listed in Table 3.

Table 3. Odour description of the volatile compounds naturally occurring in roasted coffee beans

Aroma compound	Odour description	References
Indole	Burnt, mothball	5
Octanoic acid	Sweet cheesy	5
4-Ethylguaiacol	Smoky, spicy, phenolic	1, 4, 5
Phenol	Smoky	5
Difurfuryl ether	Coffee-like, toasted odour	5
1-(1-H-pyrrol-2-yl)ethanone	Nutty, musty	5
Maltol	Caramel	5
1-Furfurylpyrrole	Hay-like, mushroom-like, green	5
2-Furfuryl methyl disulphide	Coffee-like	5
Hexanoic acid	Fatty-rancid, acrid-acid	5
Butanoic acid	Sour	5
Furfuryl alcohol	Burnt	5
Pyrrole	Nutty, hay-like, herbaceous	5
2-Acetylfuran	Balsamic-sweet	5
Acetic acid	Sour	5
2-Furfural	Bread, almond, sweet	5
Trimethyl pyrazine	Nutty, roasted	5
2-Ethyl-6-methylpyrazine	Roasted, hazelnut-like, peanuts	3, 5
Dimethyl trisulphide	Onion	5
2,3-Dimethylpyrazine	Nutty, roasted, green, hazelnut	2, 5
2-Ethylpyrazine	Nutty, roasted, peanuts	3, 5
2,6-Dimethylpyrazine	Nutty, sweet, fried	5
2,5-Dimethylpyrazine	Nutty, roasted, grassy, corn, hazelnut	2, 5
Dihydro-2-methyl-3-furanone	Sweet, roasted	5
2-Methylpyrazine	Nutty, roasted, chocolate	5
Furfuryl methyl ether	Nutty, coffee grounds-like, rich, phenolic	5
Pyridine	Bitter, astringent, roasted, burnt	5
2,3-Hexandione	Buttery, cheesy, sweet, creamy	5
3-Methylthiophene	Ash	5
Hexanal	Grassy, green oily, butter rancid	3, 5
2-vinylfuran	Ethereal, rum, cocoa note	5

Dimethyl disulphide	Onion	5
2,3-Pentadione	Oily buttery	3, 4, 5
2,3-Butanedione	Buttery, cheesy	1, 2, 3, 4, 5
2,5-Dimethylfuran	Ethereal	5
2-Methylbutanal	Malty	5
3-Methylbutanal	Malty	5
1-Octen-3-one	Mushroom-like	1
2-Hydroxy-3-methyl-2-cyclopenten-1-one	Sweet, caramel	2
Propanal	Roasted, fruity	3,4
2-Methylpropanal	Malty, fruity	3,4
3-Methylpropanal	Roasted cocoa	3
2-e 4-Methylbutanal	Buttery	2, 3, 4
(E)-2-nonenal	Buttery	2
Methional	Cooked potato	1, 6
Methanenthiol (mercaptan)	Cooked potato	3, 4
4-Methyl-2-buteno-1-thiol	Smoke, roasted	2, 4
Furfuryl mercaptan	Meat	1, 4
5-Dimethyl-trisulfide	Sulfur	1, 4
2-Furfurylthiol	Roasted, toast	1, 4, 6
2-Furanmethanethiol	Smoke, roasted	2
2-(methylthiol)propanal	Soy sauce	2
2-(Methylthio-methyl)furan	Smoke, roasted	2
3,5-Dihydro-4(2H)-thiophenone	Smoke, roasted	2
2-Acetyl-2-thiazoline	Roasted	1
4-Methylbutanoic acid	Sweet, acid	1, 2
(E)-1-(2,6,6-Trimethyl-1-cyclohexa-1,3-	Cooked apple, sweet, fruity	1, 2, 4
2-Ethyl-furaneol	Caramel	1
2,5-Dimethyl-4-hydroxy-3(2H)-furanone	Caramel, sweet	1, 2, 4, 6
4,5-Dimethyl-3-hydroxy- 2(5H)-furanone	Spicy, toast	1, 4, 6
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	Spicy, honey	1, 6
2-Ethyl-4-hydroxy-5-methyl-4(5H)-furanone	Sweet, caramel	2
2-Methoxyphenol (guaiacol)	Phenolic, roasted, plastic	1, 2, 3, 4, 6
4-Methoxyphenol	Phenolic	1, 2
2-Methoxy-4-vinylphenol	Clove	1
4-Ethenyl-2-methoxyphenol	Phenolic, cloves	2, 6
3-Methylindole	Coconut	1
4-Hydroxy-3-methoxybenzaldehyde	Vanilla	1, 4, 6
2,3-Diethyl-5-methylpyrazine	Hazelnut, roasted	1, 2, 4
2-Ethyl-3,5-dimethylpyrazine	Earth, hazelnut, roasted	1, 2, 3, 4
3-Ethyl-2,5-dimethylpyrazine	Earth	1
3-Isopropyl-2-methoxypyrazine	Earth	1
3-Isobutyl-2-methoxypyrazine	Earth	1
2-Etenyl-3,5-dimethylpyrazine	Earth	1, 4
2-Etenyl-3-ethyl-5-methylpyrazine	Earth	1, 4

6,7-dihydro-5H-ciclopentapyrazine	Hazelnut, roasted	2
6,7-Dihydro-5-methyl-5H-	Hazelnut, roasted, cotton candy	2, 6
3-Mercapto-3-methylbutyl formate	Cat, green, cassis	1, 2, 4
3-Mercapto-3-methylbutanol	Hazelnut, roasted	2
β-damascenone	Fruit	6
2,4,5-Trimethylthiazole	Plastic	6
Limonene	Lemon, minty, orange	7
Linalool	Flowery	7

(Sources: [1] Sanz at al. 2002; [2] Akiyama et al. 2005; [3] Maeztu et al. 2001; [4] Czerny et al. 1999. [5] Flament 2002; [6] Deibler et al. 1998 [7] Qiao et al. 2008)

Among substances that are likely to negatively contribute to the final coffee aroma is, for example, pyrazine with its characteristic sweet, pungent and slightly ammoniacal odour (Toci & Farah 2008). Another undesirable compound is hexanoic acid whose acrid-acid, heavy, fatty rancid aroma notes resemble the smell of sweat (Flament 2002). Burnt, roasted or typical soy sauce flavour is attributed to 2-methoxy-4-ethyl-phenol (4-ethylguaiacol) that occurs exclusively in defective beans (Maga 1978). Identification of 2-phenyl-1-ethanol, methyl 2-phenylacetate, 2-isoamyl-6-methylpyrazine, 1H-pyrrole, 4-methylthiazole and 1H-pyrrole-2-carbonitrile is also characteristic for defective beans (Czerny, & Grosch 2000; Agresti et al. 2008). 2,3-butanediol has been associated with so called stinkers – healthy looking coffee beans that inadvertently ferment which leads to the production of an unpleasant flavour (Flament 2002; Bee et al. 2005;).

Composition of volatile substances varies with species and cultivars. Generally, *arabica* species contain higher concentrations of 4,5-dimethyl-3-hydroxy-2(5H)-furanone (sotolon), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (abhexon), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), and 2,3-pentanedione, while Robusta is richer in phenol and phenol derivatives, such as 2-methoxyphenol, 4-ethylguaiacol, and 4-ethenyl-2-methoxyphenol (Pypker & Brouwer 1969; Blank et al. 1991; Freitas et al. 2001; Mondello et al. 2005; Akiyama et al. 2005; Korhonová et al. 2009). Deeper investigation of differences between cultivars is needed. Mathieu et al. (1996) published one of a few studies devoted to this topic and observed substantial differences between fresh berries of different cultivars of *arabica* and *robusta* species.

A deeper research is also required to clarify the influence of coffee's geographical origin on the content of its volatile substances. Several studies comparing samples of identical coffee originating from different countries illustrate differences in the content of some volatiles, but in general we cannot draw any provable conclusions from these studies as they are not considering many other relevant parameters, such as postharvest treatment, sun/shade grown, altitude and various others (Toledo et al. 2016). For example Cheong et al. (2013) compared roasted coffee beans obtained from 4 coffee varieties coming from 3 countries – China (Yunnan province, annual mean rainfall of 1500 mm, temperate climate, *C. arabica* L. cv. Catimor), Thailand (Doi Chang city, annual mean rainfall of 1415 mm, hot temperate climate, *C. arabica* L. cv. Catimor) and Indonesia (Sidikalang city, annual mean rainfall of 2536 mm, tropical climate*, C. arabica* L. cv.Typica, two samples with different postharvest processing). Although, the result revealed huge similarity between the aromatic profiles of coffees coming from Thailand and China, there is no evidence that similar climatic conditions are the cause, as they also share the same cultivar. Despite sharing the same origin location and cultivar two samples of Indonesian coffees presented slight differences in aroma profiles, probably caused by different postharvest treatment of two samples. Another article, published in 2012 by Bertrand et al., studied the influence of altitude and temperature on the fruit development. The authors summarized that Arabica coffee produced in warm climate and at lower altitude evince lower aroma quality. There is no clear relationship identified between coffee aroma quality and annual rainfall (Toledo et al. 2016).

Method of postharvest processing technique also substantially affects composition of volatile compounds in coffee beans, which affect quality of the beverage. We distinguish between 3 different main types of coffee processing – wet (humid), dry (natural) and semi-dry (natural depulping) methods (Toledo et al. 2016). In 2012, Aruda et al. analysed impact of the processing method on aroma profiles of roasted Arabica coffee seeds. In his experiment, he discovered increased levels of phenols, ketones and esters in seeds, which were processed by wet or semi-dry method. Their common feature is the wet depulping process.

2.5 ROASTING

Nothing affects the flavour of coffee as significantly as roasting. During roasting, the beans are subjected to high temperatures, varying typically between 180 °C to 240 °C. By this short-term process, lasting usually from 8 to 15 minutes, an added value of 100 – 300 % can be achieved (Yeretzian et al. 2002; Sunarharum et al. 2014). Sometimes, the roasting time can be shortened to as little as 90 seconds as in the case of high-yield coffees, or on the contrary extended to 90 minutes, which is typical of Brazil (Buffo & Cardelli-Freire 2004). In the initial phase of roasting moisture content is reduced from 8-12 % to below 5 % and simultaneously colour of the beans become yellowish. Second (actual) roasting stage drastically modifies chemical composition by formation of hundreds of new substances and releasing carbon dioxide. It is an essential stage for flavour, aroma and colour development. During the last (third) stage coffee is rapidly cooled down to halt roasting. Depending on final colour of the beans, that has been accepted as an index of roasting, 3 basic roasting categories are distinguished – light, characterized by producing sweet, cocoa and nutty aromas, medium and dark, typical for sour, burnt/acrid, pungent and ashy/sooty notes (Buffo & Cardelli-Freire 2004; Hernandez et al. 2007; Franca et al. 2009; Bhumiratana et al. 2011; Gloess et al. 2014). The most critical parameters of roasting are the applied temperature profile and the mode of heat transfer (Schenker et al. 2002). According to Yang et al. (2016), higher roast intensity is associated with loss of organic acids (butanoic acid, hexanoic acid, acetic acid) and greater formation of products of Maillard reaction and lipid breakdown.

Because of the complexity of the reactions, the chemical processes occurring during roasting have not been fully elucidated yet. A large number of precursors, from which not all have been identified so far, react together and produce intermediates that are subjected to further reactions. In addition, the reaction varies considerably depending on temperature, pressure or moisture content. In terms of volatile compounds formation, the most prominent known reactions occurring during roasting are Maillard reaction and Strecker degradation of polysaccharides, sugar, proteins and other components (Flament

2002; Schenker et al. 2002; Gloess 2014). New substances are also produced by amino acids breakdown, degradation of lipids, trigonelline, quinic acid and pigments or by thermal decomposition of ferulic acid leading to the formation of 4-vinylguaiacol, one of the key aroma substances (Buffo & CardelliFreire 2004; Gloess et al. 2014). Roasting dramatically increases the levels of acetaldehyde, pyridine, pyrazine, methylbutanal, furans and acetic acid in the bean (Yeretzian et al. 2002; Somporn et al. 2011). Figure 4 shows simplified scheme of roasting reactions that lead to volatile compounds formation from non-volatile precursors present in green beans. Non-volatile compounds do not act only as precursors for volatile compounds, several of them also directly affect sweetness, bitterness and astringency of flavour to some level (Toledo et al. 2016).

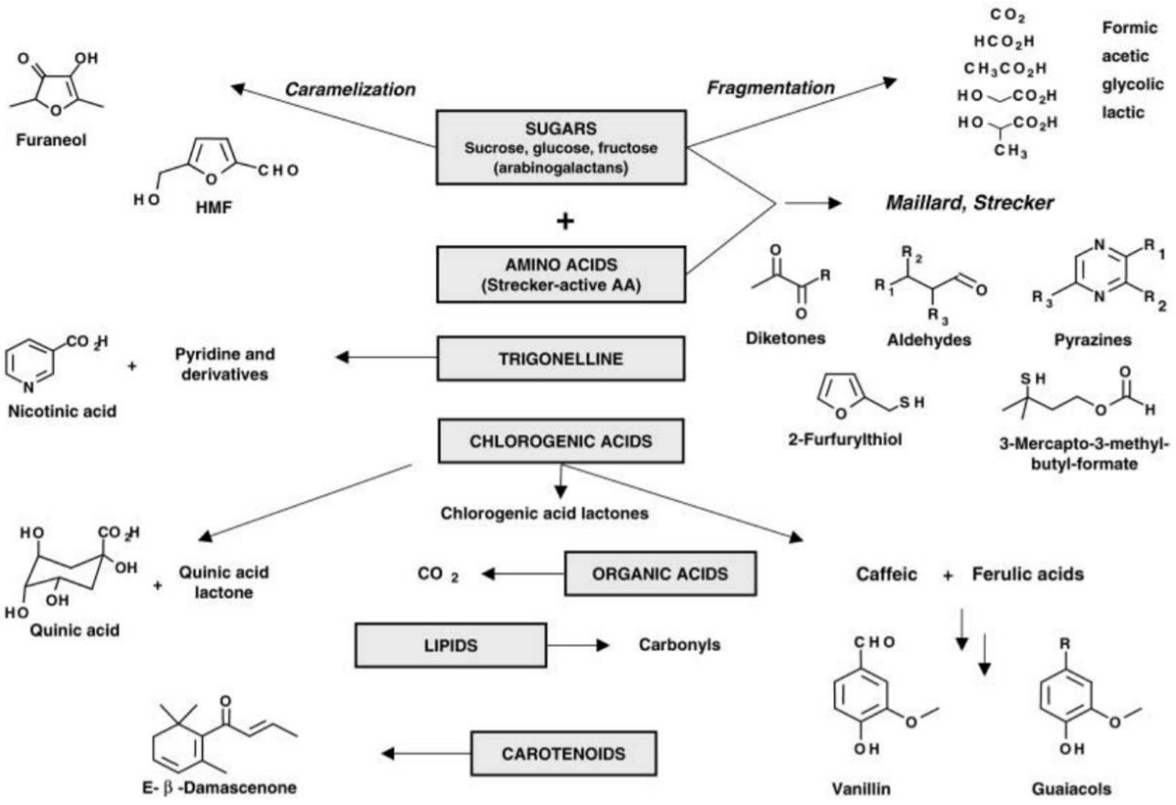


Figure 4. Simplified scheme of roasting reactions that lead to volatile compounds formation from non-volatile precursors present in green beans
(Source: Yeretzian et al. 2002)

2.5.1 MAILLARD REACTION

Maillard reaction, or so-called browning, is a complex non-enzymatic chemical reaction between reactive carbonyl group of reducing sugars and nucleophilic amino group of amino acids, proteins or peptides. During this reaction, hundreds of compounds of different molecular weights responsible for developing the flavour, aroma and colour of products are formed. That ranks the reaction among the indispensable parts of many food industry sectors (Wang et al. 2011).

2.6 STORAGE CONDITIONS, STORAGE LIFE AND SPOILAGE

Exposure of the green coffee to the high temperature during roasting makes roasted coffee a shelf-stable product with a very low water activity (Makri et al. 2011). Roasting is also associated with the emergence of Maillard reaction products, that are characterized by antimicrobial properties and thus contribute to the preservation of coffee (Daglia et al. 1994). Although, there is almost zero risk of spoilage due to microbial and enzymatic processes, coffee undergoes several chemical and physical changes, which can cause a rapid decline in quality (Nicoli et al. 1993; Illy & Viani 2005; Anese et al. 2006, Marin et al. 2008). The most critical quality factor of coffee is its freshness (Poltronieri & Rossi 2016). Loss of freshness is known as staling and depends on moisture, temperature conditions and, especially, on oxygen availability (Nicoli et al. 1993; Smith et al. 2004; Illy & Viani 2005). In general, the major changes in chemical composition reflecting the sensory properties of the coffee are associated with the oxidation and loss of volatile compounds, and with oxidative reactions to which the coffee oil is subjected (Marin et al. 2008). Rancid coffee flavour is one of the consequences of lipid degradation. Although, lipids account for only about 15 % of the dry weight, their impact on the coffee flavour after undergoing oxidation is more than significant (Illy & Viani 2005). Other reaction, Strecker degradation, reduces amino acids and sugars during roasting, while forming eminent amounts of CO_2 (Flament 2002). Physical properties are particularly important in terms of porosity and surface area. Greater porosity of the bean facilitates leakage of volatile substances into outer environment (Labuza et al. 2001), while higher surface area accelerates lipid

degradation (Vila et al. 2005). Among the volatile compounds perceived as being responsible for the coffee aging and alteration are aldehydes (hexanal, propanal, furfural, 2-methylbutanal, 5-methylfurfural), ketones (2,3-pentanedione, 2-butanone, 2,3-butanedione), carboxylic acids (acetic acid, formic acid, lactic acid, and glycolic acid), furans (2-methylfuran), thiols (dimethyl sulfide, methanothiol, 2-furynmethanethiol, and methional), and 2,6-diethylpyrazine (Marin et al. 2008; Toledo et al. 2016). Hexanal is a product of autooxidation of polyunsaturated fatty acids (linoleic acid) and confers to the rancid flavour of coffee beverage (Amstalden et al. 2001; Marin et al. 2008; Toci et al. 2013).

To prevent oxidation, irreversible loss of volatile substances and thus degradation of quality roasted coffee must be packed as soon as possible. Most of the CO_2 contained in the bean is released during roasting, but part of the CO_2 remains entrapped inside until the coffee is grinded. This results in an extended product shelf life (Wang & Lin 2015). Therefore coffee beans should be ground just before consumption. In the case of ground coffee, it is only a matter of minutes before the majority of volatile substances are released into the surrounded environment and coffee loses its unique freshness. Nevertheless, a small amount of CO_2 is released from the whole bean even during storage (Wang & Lin 2014). For this reason, semi-rigid gas-impervious containers capable of suppressing the pressure that is generated by the CO_2 should be used. Partial degasification by systems specially designed to minimize aroma loss is applied in order to avoid package bursting, leaking or swelling. An essential equipment of so-called active packaging system is a one-way vent valve. While CO_2 can escape out through the valve, O_2 should not be able to get inside the package (Glöss et al. 2014). Resealable mechanism is matter of course. The properties of packaging and the packaging material should preserve the sensorial quality of the coffee during the entire, but limited, storage time, even after opening and proper resealing of the product (Poltronieri & Rossi 2016). Coffee is commonly stored in a variety of packaging types, amongst the usual methods include, for example, folded-over paper bags, glass jars, plastic bags and airtight containers, metal cans, ceramic containers, multi-layer bags and others. To date, debates about the most

suitable packaging material and storage temperature are being held. Despite the fact that manufacturers' recommendations on the storage of their coffee are often different in some details, they usually agree on one - the greatest threat to coffee are moisture, air, light and heat. National Coffee Association of U.S.A. (2018b) recommends storing roasted coffee in an opaque, air-tight container placed in the cool and dark spot. Warm locations, such as places exposed to direct sunlight or places near the oven, should be avoided as well as clear canisters. National Coffee Association of U.S.A. (2018b) considers the room temperature to be the most appropriate for coffee storage. F. Gaviña & Sons (2018), a constantly growing well-known coffee importer and roaster located in California, on the contrary, supports, based on their independent studies, the storage of coffee in an airtight container in a refrigerator or a freezer. In 2012, the company unveiled a study comparing 3 different storage environments - room temperature (72 ° F), refrigerator (36 ° F) and freezer (0 ° F). Samples were tasted for 12 weeks. Results revealed that cup of coffee prepared from the material stored in the freezer (for maximum of 6 weeks in case of whole beans or 4 weeks for grounded coffee) evinced the best quality (Business Wire 2012). The main problem with storing coffee in the freezer or refrigerator is that coffee is hygroscopic and thus absorbs moisture, tasted and odours from its environment. An important parameter for the coffee placed in the refrigerator or freezer is therefore an absolute airtightness of its packaging. Once thawed coffee should not be frozen again. The recommended maximum storage time varies from a few days to two weeks after roasting (National Coffee Association of U.S.A. 2018b). The well-known coffee brand, Lavazza, recommends keeping coffee in the original packaging by the manufacturer, while National Coffee Association of U.S.A. (2018b) insists that the original packaging is not a suitable solution. Illycaffè promises that coffee produced in their company will last aromatic in the original, unopened package for more than a year (illycaffé 2017). Starbucks guarantees the quality of its coffee in undamaged well-stocked packages for several months (Starbucks 2016). Design and environmental impact of the production and subsequent disposal of packaging is also increasingly influencing form of packaging.

2.7 EXTRACTION METHODS

There are a number of extraction methods that differ from one another in many parameters, such as cost, efficiency, simplicity, total time consumption, length of extraction time, necessity of solvent, selectivity, compatibility with different instruments/analytical methods and applications and many others (Mottaleb 2014). A detailed scheme of some common existing extraction techniques is shown in Figure 5.

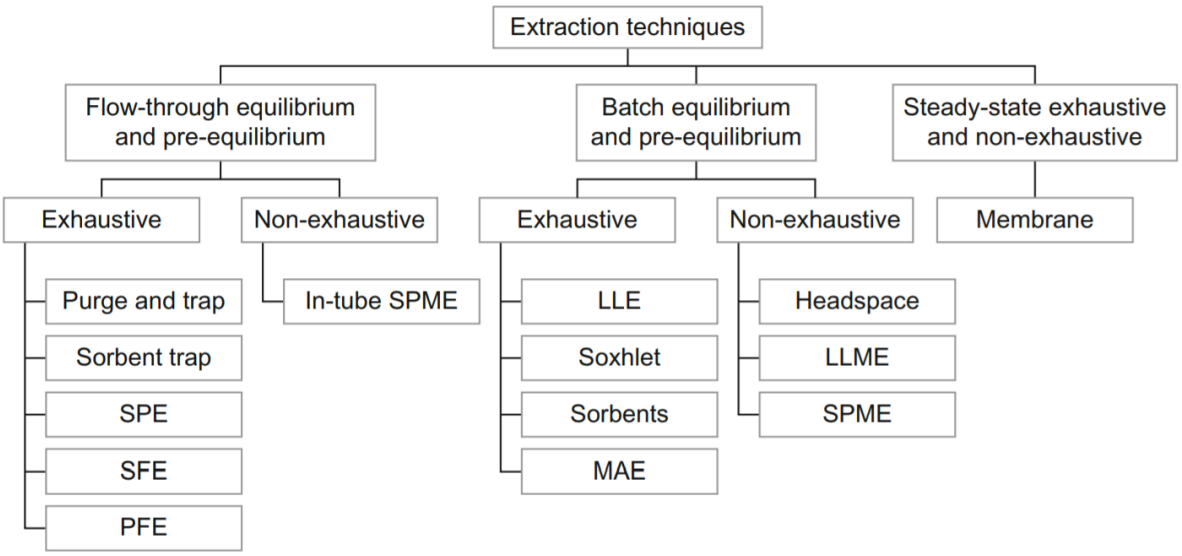


Figure 5. Classification of extraction methods
(Source: Pawliszyn 2012a)

The SPME method probably best meets the user's requirements, making it the best proximate to ideal sample preparation technique. Some benefits offered by SPME are apparent from Table 4, in which SPME performance is compared to performances of some other conventional extraction methods (Mottaleb et al. 2014).

Table 4. Comparison of performances of SPME and other conventional techniques

Detection limit (MS)	Precision (RSD)	Expense	Time	Solvent used	Simplicity
Purge and Trap (ppb)	1 – 30	High	30 min	None	No
Stripping (ppt)	3 – 20	High	2 h	None	No
Headspace (ppm)	5 – 50	Low	30 min	None	Yes
Liquid-liquid extraction	–	High	1 h	1000 ml	Yes
Solid-phase extraction	7 – 15	Medium	30 min	To 100 ml	Yes
SPME (ppt)	<1 – 12	Low	5 min	None	Yes

(Source: Mottaleb et al. 2014)

Additional great advantage of SPME is that sampling, extraction, concentration, and sample introduction are assimilated into one single step (Mottaleb et al. 2014). The process steps of the three selected extraction methods, including SPME, are shown in Table 5.

Table 5. Sample preparation steps in LLE, SPE, and SPME

LLE	SPE	SPME
Addition of organic solvents to the sample	Conditioning of cartridges or membranes	Exposing SPME fiber to the sample
Agitation in a separatory funnel	Sample elution	Desorption of analytes in the analytical instrument
Separation of aqueous organic phases	Solvent elution to remove interferences an analyte desorption	–
Removal of organic phase	Evaporation/concentration of the organic phase	–
Evaporation/concentration of the organic phase	Injection in the analytical instrument	–
Evaporation/concentration of the organic phase	–	–

(Source: Mottaleb et al. 2014)

In 2016, Figueroa & Vargas, looked into suitability of three extraction methods for isolation of aromatic volatile compounds from roasted ground Vilcabamba – Ecuadorian coffee. They compared usefulness of simultaneous distillation and extraction (SDE), supercritical fluid extraction with carbon dioxide (SCF) and SPME with four different coatings used (PDMS, PDMS/DVB, DVB/CAR/PDMS and PA). Identification of compounds was accomplished by using gas chromatography/mass spectrometry (GC-MS). After

evaluating the results, Figueroa & Vargas have suggested the SPME-DVB/CAR/PDMS method as the most appropriate method for representative identification of coffee aroma compounds. Another study, published in 2008 by Viegas et al., identifies soluble coffee volatile compounds comparing even five extraction methods: dynamic headspace (purge-and-trap), static headspace, solvent extraction, simultaneous distillation-extraction (SDE) and headspace solid-phase microextraction (HS-SPME) using four different types of coatings (PDMS with 100 µm thickness, DVB/CAR/PDMS with 50/30 µm thickness, PDMS/DVB with 65 µm thickness, CW/DVB with 70 µm thickness). As with the previous study, after analyzing the substances by using chromatography/mass spectrometry (GC-MS), it became clear that the SPME-DVB/CAR/PDMS method provides the most representative aroma profile.

2.7.1 SOLID-PHASE MICROEXTRACTION

Solid-phase microextraction (SPME) is a relatively recent simple, solvent-free, rapid, easy to automate, precise and extremely sensitive solid phase extraction sampling technique suitable for identification of volatile and non-volatile compounds of solid, liquid and gaseous analytes (Shirey 2012; Merkle et al. 2015). Thanks to countless advantages over other extraction methods, SPME technique gains in popularity and finds its application in an increasing number of disciplines (Merkle et al. 2015). The principle of the method is based on absorption of analytes onto an absorbent-coated fused-silica optical fibre (Mottaleb et al. 2014; Sgorbini et al. 2014). Currently, the most commonly used fibre length is 1 cm. For efficiency reasons, fibres longer than 2 cm are never used. Different fibre coating durability affects the maximum number of extractions that can be done using one single fibre. Overuse of fibre leads to its “bleeding”, which becomes evident due to the siloxane contamination. Coated fibre is attached to an inner needle or tubing that is hidden inside outer piercing needle. An essential part of this easy to use device is the sealing septum covering outer needle. It prevents leaking during insertion of needle into a pressurized injection port of gas chromatograph (GC). A colour of the hub on the top of the tubing (plunger) indicates what kind of coating is applied to the fibre. For a detailed

list of commercially available fibres together with a colour description of their hubs and technical properties, see Table 6.

Table 6. List of commercially available SPME fibers and their properties

Fibre coating	Film thickness	Hub description	Polarity	Maximum operating	Compatible with	Class of compounds
Polydimethylsiloxane (PDMS)	100	Red (plain)	Nonpolar	280	GC/HPLC	Volatiles
PDMS	30	Yellow (plain)	Nonpolar	280	GC/HPLC	Nonpolar semivolatiles
PDMS	7	Green (plain)	Nonpolar	280	GC/HPLC	Medium to nonpolar semivolatiles
PDMS-divinylbenzene (DVB)	65	Blue (plain)	Bipolar	270	GC	Polar volatiles
PDMS-DVB	60	Brown (notched)	Bipolar	270	HPLC	General purposes
PDMS-DVB[a]	65	Pink (plain)	Bipolar	270	GC	Polar volatiles
Polypyrrole coated	50	—	Polar	250	HPLC–MS	Catechins and caffeine
Polyacrylate (PA)	85	White (plain)	Polar	320	GC/HPLC	Polar semivolatiles (phenols)
Carboxen–PDMS (CAR/PDMS)	75	Black (plain)	Bipolar	320	GC	Gases and volatiles
CAR/PDMS[a]	85	Light blue (plain)	Bipolar	320	GC	Gases and volatiles
Carbowax–DVB (CW/DVB)	65	Orange (plain)	Polar	265	GC	Polar analytes (alcohols)
CW/DVB[a]	70	Yellow-green (plain)	Polar	265	GC	Polar analytes (alcohols)
Carbowax-templated resin (CW/TPR)	50	Purple (notched)	Polar	240	HPLC	Surfactants
DVB-Carboxen-PDMS[a]	50/30	Grey	Bipolar	270	GC	Odours and flavours
Supel-Q-PLOT	50	—	Polar	240	HPLC-UV	Isoflavones and phenols

[a] Stableflex type is on a 2-cm fibre

(Sources: Sigma-Aldrich 1999; Mottaleb 2014)

A spring, which is a part of the manual assembly and is missing in the assembly used with autosamplers, has the task of retracting the fibre after exposure for extraction and desorption (Shirey 2012). The schematic diagram of the SPME manual fiber assembly is shown in Figure 6.

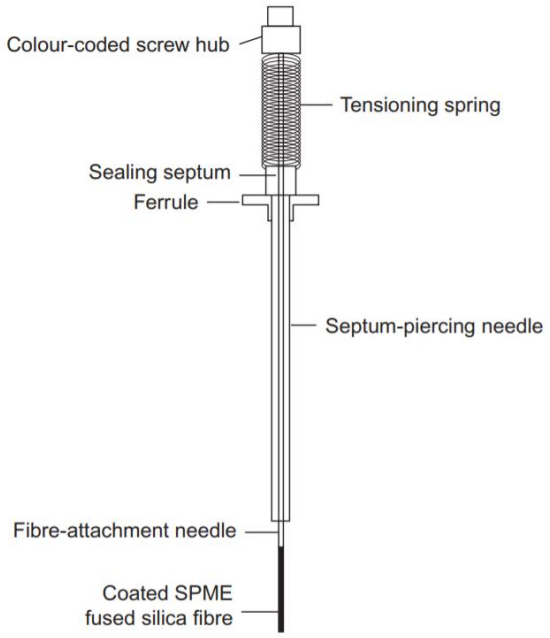


Figure 6. Schematic diagram of the SPME manual fiber assembly
(Source: Shirey 2012)

For better manipulation, the assembly is inserted into the manual holder (Figure 7). Because the needle is relatively brittle and can be quite easily damaged, the holder is provided with needle guide depth gauge that determines how far the needle goes either into the vial or in the injection port by being screwed down or up. Fibre can be locked in the exposed position by a z-slot attached to the manual holder (Shirey 2012).

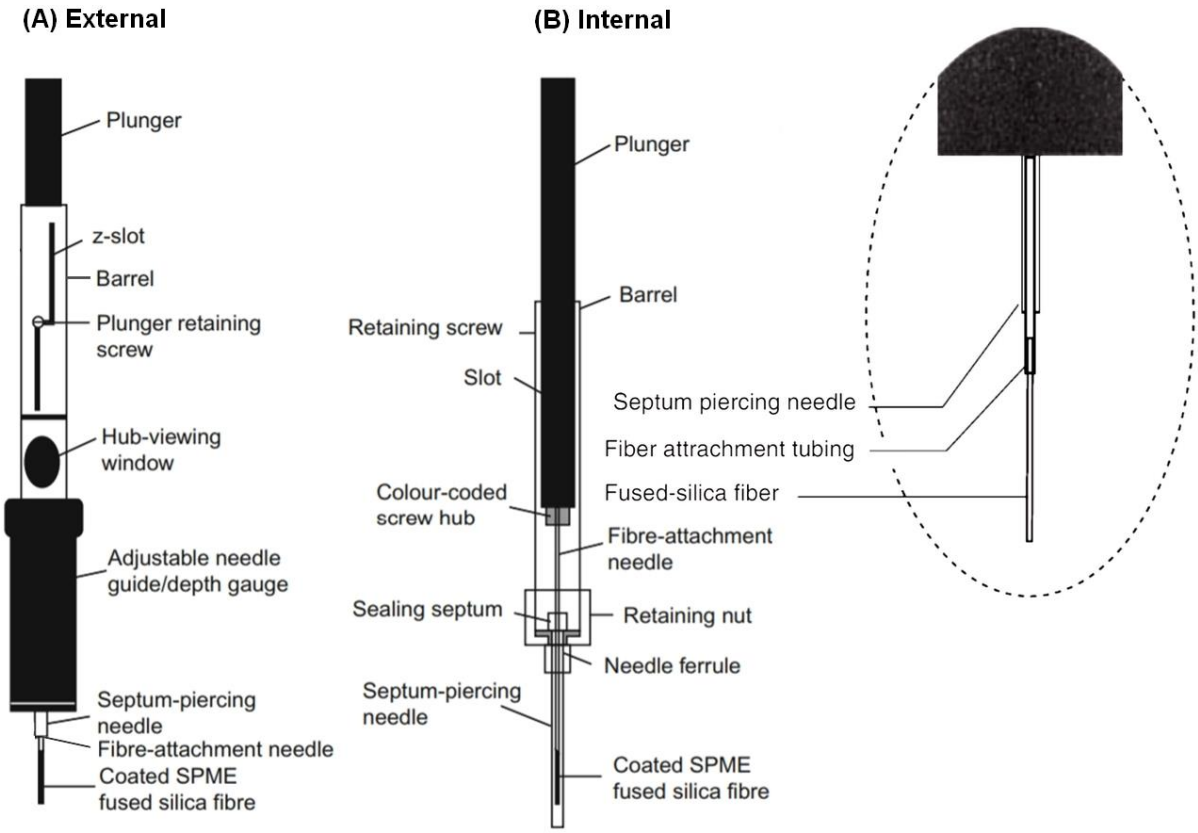


Figure 7. Schematic diagram of external (A) and internal (B) view of SPME manual fibre assembly holder

(Source: Zhang & Yang 1994; Shirey 2012)

During the transfer of the device, the plunger is located in the uppermost position of the z-slot, which indicates that the fibre is securely hidden in the hollow needle. The downward movement of the plunger along the z-slot drives the fused-silica fibre out of a hollow needle during extraction and subsequent injection into the chromatograph (GC). The whole process is illustrated in detail in Figure 8.

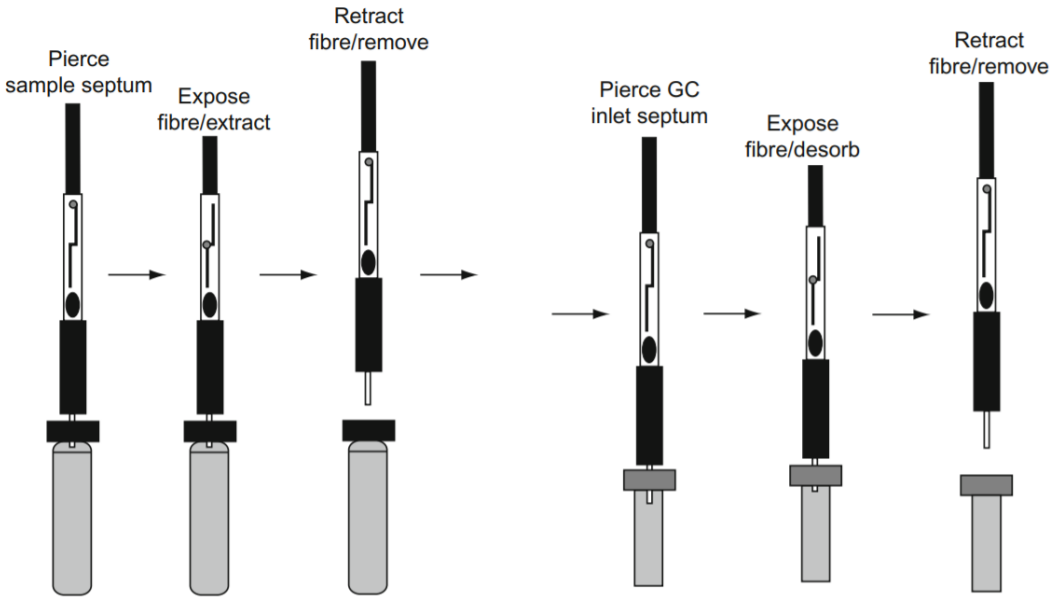


Figure 8. Typical steps of SPME extraction (direct immersion) and subsequent thermal desorption *(Source: Shirey 2012)*

Three basic modes of SPME operation are distinguished. First of them is a direct extraction (illustrated in Figure 9.A), during which the needle is directly immersed into the sample. In case that the fibre does not come into contact with the sample at all and is inserted only into the headspace sligtly above the sample, we talk about so called headspace SPME (Figure 9.B). In practice, this method is widespread in the analysis of samples with high-molecular-weight interferences. Heating the vial facilitate release of volatile substances and thus reduce extraction time. Enhance sensitivity of extraction can be achieved by minimazing the headspace volume. The last method, membrane-protected SPME (Figure 9.C), is suitable for accurate identification of sample including both, high-molecular weight interfering compounds and non-volatile target analytes at the same time. The extraction time is dependent on the length of the time that is required for the analyte concentration to achieve equilibrium between the fibre coating and the sample matrix (Pawliszyn 2012b). This time is, among other parameters, for example significantly influenced by the thickness of the coating. For the measurement of volatile substances,

a thick coating is used, whereas semivolatile compounds require thin coatings to be absorbed/desorbed with the highest effectiveness possible (Mottaleb et al. 2014).

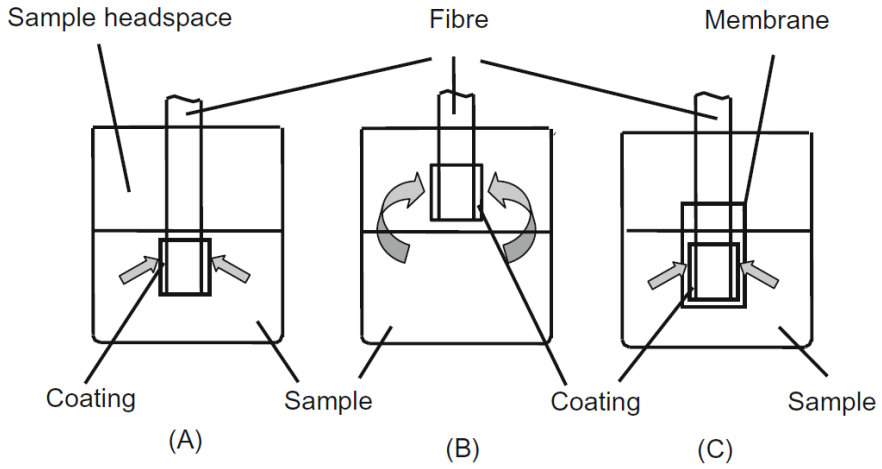


Figure 9. Three basic modes of SPME operation: (A) direct extraction; (B) headspace SPME; and (C) membrane-protected SPME
(Source: Pawliszyn 2012b)

2.8 GAS CHROMATROGRAPHY AND MASS SPECTROMETRY

Gas chromatography-mass spectrometry (GC-MS) is an analytical method combining the features of two highly compatible techniques to quantify and identify volatile and semi-volatile organic compounds in complex mixtures with a great resolution (Hites 1997). The gas chromatograph, developed in the mid-1950s, is used to precisely physically separate the compounds in the sample, but unfortunately, the device is not able to further reliably selectively detect separated particles. For this reason, shortly after its development, it has been connected with the mass spectrometer that has the opposite problem – it provides detailed information about the structure of the compounds, directly leading to their exact identification, but the device is not able to readily separate the mixture (Hites 1997; Sneddon et al. 2007).

The common gas chromatograph consist of the separation column, injector port, high-pressure cylinder with a supply of a carrier gas, flow control meters, attendant pressure regulators, detector, electrometer, and data-processing unit (Aniszewski 2007).

The sample, in the form of a liquid solution or a collection of molecules absorbed on the surface of the fiber (SPME method), is introduced via an injection port into the inlet (called injector). Injectors can be divided into two major categories: vaporization and on-column injectors (Forgács & Cserháti 2003). Vaporization injectors use rapid exposure of the sample to the high temperatures (200-300°C). The sample volatilizes immediately and mixes with a continuous flow of a carrier gas. The carrier gas acts only as a background gas facilitating the detection, and thus must be inert or non-reactive. The most commonly used gases are helium, hydrogen, nitrogen, and argon (Stauffer et al. 2008; Stashenko & Martinéz 2014). The second category, represented by on-column injectors, omits vaporization. Without using any heat, sample is deposit directly into the column (Forgács & Cserháti 2003).

The separation is based on the selective distribution of the compounds between the two phases, the mobile phase (gas) and the stationary phase (solid in the case of gas–solid chromatography (GSC) or liquid in the case or gas–liquid chromatography (GLC)). The gaseous mobile phase enters the separation section, where the long capillary tubing, called column, is located (Forgács & Cserháti 2003; Stashenko & Martinéz 2014). The column is enclosed in a temperature-controlled oven (Stauffer et al. 2008). The columns can be generally classified into two distinct groups: packed and capillary columns, also known as open tubular columns. Packed columns, made of a rigid metal or glass tubing, are, as their name indicates, densely packed with a solid support (like fluorocarbons, diatomaceous earth, graphitized carbon black or glass beads) coated with a stationary phase, represented by a thin layer of high molecular weight polymer (Forgács & Cserháti 2003). The second group, capillary columns, is further divided into two types. The first type is a wall-coated open tubular (WCOT) column with a microscopic film of the stationary phase coated directly along the inner column walls. Second type, support-coated open tubular (SCOT) column, has the inner walls of the capillary coated with a thin layer or adsorbant solid that is treated with the liquid stationary phase (Poole 2002).

As the sample mixed with the stream of the carrier gas is swept through the column, the gaseous compounds interact with the stationary phase. The molecules are retained by

the column and gradually elute from the column at different times, known as the retention times. Individual components of the gasified sample are separated by the column according to their physical characteristics, such as boiling point, polarity differences, or molecular size (molecular sieve columns). The most volatile compounds leave the column first. The quantity (concentration) of each compound exiting the column is measured by detectors (Emerson Process Management 2012). A plot of the detector signal as a function of time generated by the GC system is referred to as a chromatogram and it is the main output of the technique. Each peak on a chromatogram corresponds to a different compound of the mixture. By integrating the chromatogram, peak areas are obtained and so the mole fraction of each compound can be quantified. Example of chromatogram obtained from a sample of roasted coffee powder is shown in Figure 10.

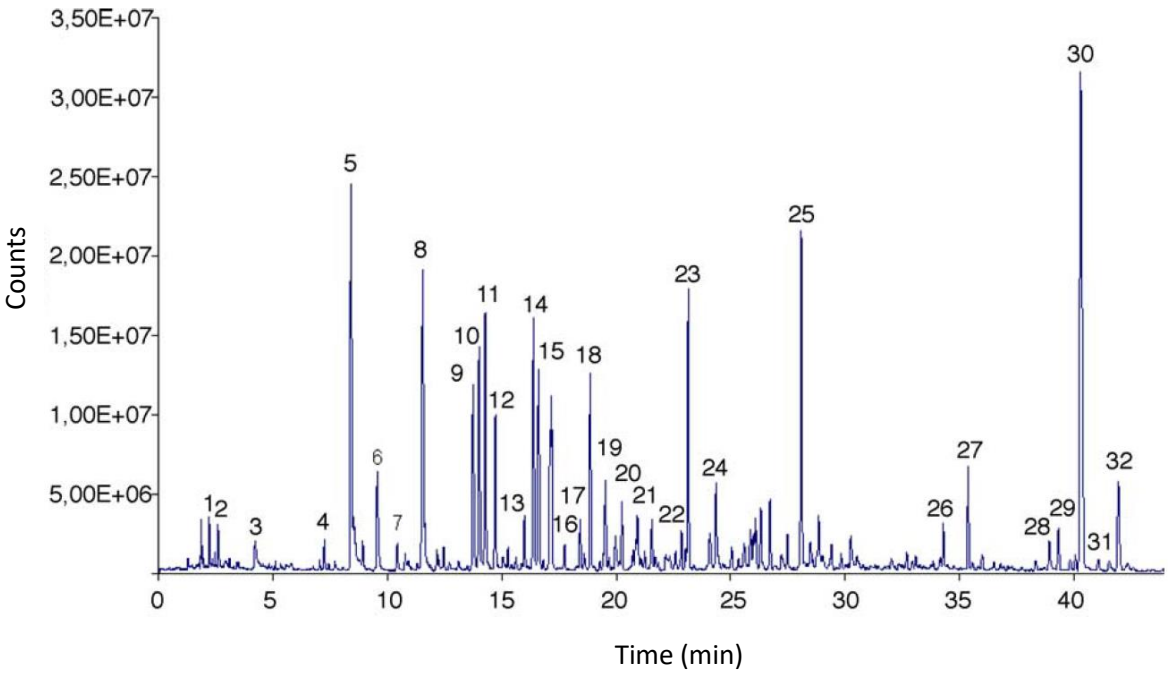


Figure 10. Chromatogram obtained from a sample of roasted coffee powder by using HS-SPME–GC–MS method of compound identification
(Source: Zambonin et al. 2005)

Degradation products eluted from the stationary phase may cause the normal background signal recognized as a column bleed. Column bleeding occurs to some extent in all cases, regardless of the quality or source, and does not necessarily mean damage of the column. With increasing column length, column diameter, and film thickness, the bleed levels will also increase. Slightly higher rate of bleeding is exhibited by polar phases comparing to nonpolar ones (MSP Kofel 2005).

Discrete peaks appeared during the blank runs always indicate contamination of the front portion or inlet. As, a peak generation, a one-time, isolated event, can be never caused by a continuous process, such as stationary phase degradation (MSP Kofel 2005).

Mass spectrometry is a highly sensitive and accurate tool for identification of molecular structure. The major components of the mass spectrometer are inlet, such as for example a gas chromatograph, an ionization source, one or more mass analysers, a detector, and a data processing system. The first step after introduction of the sample into the mass spectrometer is its ionization. Most commonly used ionization methods are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The ionized sample continues into the mass analyser where the ions are separated according to their mass-to-charge ratio (m/z). Several types of mass analysers are available, including quadrupole, ion trap, time-of-flight (TOF), and orbitrap (Pan et al. 2014; Vandell & Limbach 2017). Eventually, selected ions are fragmented and the fragments further analysed in the second mass analyser. After the ions emerge the last analyser, they are detected, measured, in terms of their abundance, and converted into electrical signals. The electrical signals are processed, transmitted into the computer and displayed as a mass spectrum (Hoffmann & Stroobant 2007). Mass spectrum represents a molecular fingerprint presented usually as a vertical bar graph. Each bar stands for an ion with a specific mass-to-charge ratio (m/z). The relative abundance of individual ions is indicated by the length of the bars. In case that the abundance is expressed in absolute form, the most intense ion (the highest bar) is assigned an abundance of 100 % and the others are normalized to this value (Chudoba 2016). For illustration, Figure 11 shows the mass spectrum of (-)-limonene.

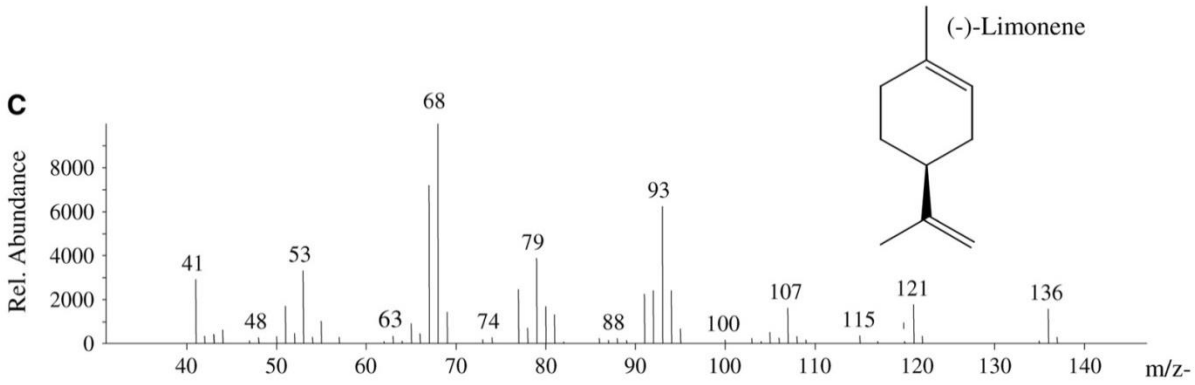


Figure 11. Mass spectrum of (-)-limonene library standard
(Source: Byun-McKay et al. 2006)

For the final identification of sample compounds, the mass spectrum together with the retention time of the molecule is the most important because these two attributes are compared and matched to the standard reference compounds analyzed under the same conditions.

In a modern spectrometry, one of the most commonly used mass analysers is a quadrupole mass filter (QMF) (Almazov & Konenkov 2017). QMF is formed by a parallel array of four metal electrodes with a hyperbolic or circular profile and length of between 15 and 25 cm. Electrodes are arranged in the shape of the block, forming a space between them. Opposing pairs of electrodes are electrically connected together. Alternate current (AC) and direct current (DC) voltages are applied using the rule that opposite electrodes have the same voltage (Tanna & Lawson 2016). The oscillating electrical fields created around the rods are able to selectively destabilize or stabilize the trajectories of ions passing through a radio frequency (RF) quadrupole field between the rods and thus filter the ions according to their mass-to-charge ratio values (m/z) (Clarke 2017). Main disadvantages of this relatively fast and simple operation, with no requirement for very high vacuum (> 10^{-7} Torr), are especially low (generally unit) resolution, a low *m/z* cutoff and low transmittance (Somogyi 2008)**.** Simplified schematic diagram of GC-MS system with quadrupole mass analyser is shown in Figure 12.

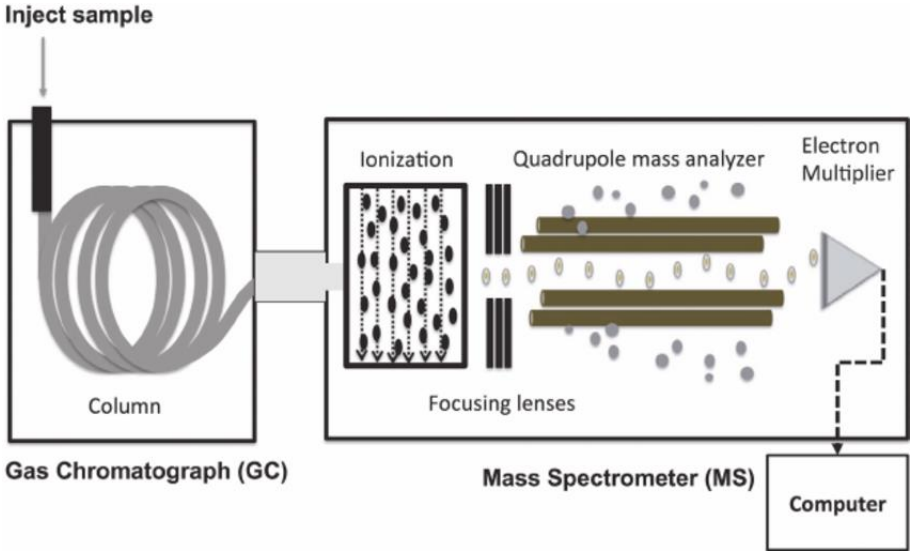


Figure 12. Simplified schematic diagram of GC-MS system with quadrupole mass analyser *(Source: Il-Young et al. 2016)*

3 AIMS OF THE THESIS

The diploma thesis focused on the analysis of the chemical composition of coffee beans, with a special emphasis on the description of the volatile compounds found in roasted coffee beans. The main objective of the diploma thesis was to investigate the influence of the method and length of storage on the content of volatile substances in roasted *Coffea arabica* L. coffee beans.

The secondary subject of interest of this research was to compare, whether coffee originating from different geographical locations respond dissimilarly to the method and length of storage.

Specific objectives were to continuously monitor, analyse and compare changes in the presence of volatile substances in roasted *Coffea arabica* L. coffee beans, originated from various geographical locations, stored in two different conditions (room temperature, fridge) during the period of 15 weeks. Overall changes were considered to include changes in the volume quantity of individual volatile substances present in the sample, as well as changes related to the formation of new or complete degradation of the initially identified volatile substances.

4 MATERIALS AND METHODS

4.1 PLANT MATERIAL

Four samples of *Coffea arabica* L. were subjected to the research. Sampling was based on the division of the world into four geographical locations typical for coffee growing, namely Asia, Africa, Central and South America, to obtain a wide geographical distribution of samples. From each geographical location, the country of origin of one of the samples was then randomly selected. Special effort has been put to achieve the greatest possible elimination of the influence of different processing method and roasting degree on a final volatile compound profile of the coffee bean.

All freshly roasted coffee samples were purchased from MANU JTC, s.r.o., located in Ludgeřovice, Moravian-Silesian Region, Czech Republic. Detailed information on the samples is summarized in Table 7.

Table 7. Detailed information on the samples

	Sample 1	Sample 2	Sample 3	Sample 4
Country	**Guatemala**	**Indonesia**	**Ethiopia**	**Ecuador**
Region	Antigua Valley, San Sebastian	Eastern Java	Caffa	Galapagos, San Cristóbal Island
Altitude (m.a.s.l.)	1,830	1,550	1,500	1,350 – 2,700
Variety	Bourbon	Bourbon	Forest coffee	Bourbon
Commercial name	Antiqua San Juan	Java Jampit	Ethiopia Djimmah	Galapagos SC 18 San Cristobal
BIO	No	No	Yes	No
Processing	Wet	Wet	Wet	Wet
Roasting degree	Medium intensity	Medium intensity	Medium intensity	Medium intensity
Sensorial Attribute	Peach, apricot	Chocolate	Grapevine, flowers	Caramel, lemon
Intensity	●●●●●	●●●○○	●●●●○	●●●●○
Acidity	●●○○○	●●○○○	●●●●○	●●○○○
Bean quality	●●●●○	●●●●●	●●●●●	●●●●●
Processing	●●●●●	●●●●●	●●●●●	●●●●●

(Source: MANU JTC 2018)

4.2 SAMPLE PREPARATION

All four samples were divided in half. The one half of each sample was stored at room temperature throughout the study. The other half of the samples was stored in the refrigerator at 4°C until the time of analysis. In both storage conditions, coffee beans were packed in 129µm thick PET/AL/PE bags with a one-way vent valve. The bags were provided with a zipper to guarantee their resealability. The hermetic sealing and dark opaque design of the packaging kept coffee beans protected against the light, oxygen and moisture.

Volatile substance measurements were performed at the following time intervals after roasting: 0 week, 2 weeks, 6 weeks, 10 weeks, and 15 weeks. Each measurement was repeated 3 times and the mean value was calculated. Total number of samples analyzed was 108.

4.3 EXTRACTION

Immediately before analysis, coffee was ground in RETSCH Knife Mill Grindomix GM 100 with operating speed 10,000 rpm for approximately 30 seconds. 1 g of ground coffee was weighed and placed into 4 ml clear vial and sealed with hole cap and PTFE-faced silicone septa. Prior to extraction, each sample was equilibrated for 2 hours in a thermostatic bath at 60 °C. After removing from the bath, the vial was manually shaken and subjected to HS-SPME. A silica fibre coated with 100 µm thick polydimethylsiloxane (PDMS) film set in the manual SPME device was exposed to the sample for 5 minutes. Throughout all the experimental work only one fibre was used. The extraction conditions were carried out according to the method of Zambonin et al. (2005).

4.4 GC-MS ANALYSIS

Before using the fibre for analyses, it was reconditioned at 250 °C each day, and the blank measurement was performed for 1 hour totally. The GC-MS analysis was performed on an Agilent 7890B/5977A GC/MSD System (Agilent Technologies, USA) equipped with a

HP-5 column ((5 %-phenyl)-methylpolysiloxane, 30 m length, 250 µm internal diameter, 0.25 µm film thickness) was employed.

Needle was inserted into the injection port immediately after extraction. Thermal desorption was performed directly into the GC injection port at 250°C and maintained during the whole chromatography run, which was set to 32 minutes. The GC injector port was operated in the splitless mode with a 0.75 mm i.d. liner. The optimized GC oven temperature program was 40 °C (1 min) to 160 °C at 4 °C/min (final temperature held for 5 min). As the carrier gas helium, with a flow of 1 ml/min was used. Kovats Index (KI) values for the volatile substances were calculated by running *n*-Alkanes (Sigma R-8769) under the same conditions.

The MSD transfer line temperature was maintained at 270 °C with the electron energy of 70 eV. Mass spectra were acquired in the mass range from *m/z* 30 to 600, using a scan time of 1 s.

Data were elaborated through MassHunter Workstation Software Qualitative Analysis Version B.07.00. Software also enabled obtaining of the peak areas by integration. Identification of the volatile compounds was done by comparison of their mass spectra against mass spectra covered by the NIST/EPA/NIH library version 2.2. Confirmation of the accuracy of identification was done by comparison of KI. Not all substances could be verified by comparison of KI, because some retention indexes were not available.

4.5 DATA ANALYSIS

All data were sorted and saved in Microsoft Excel 2010 and further analysed in SPSS Win 19 software. Student paired *t*-test analysis was applied to the results of the two storage temperatures in each analysis time.

5 RESULTS

In total, 65 compounds were identified. Of these, 58 compounds were detected in coffee samples of all four different geographical origins, stored at both conditions (room temperature, refrigerator). Only coffee samples originating in Ecuador contained α-pinene and β-pinene. Different storage temperatures had no effect on the content of these two substances. 2,3-butanediol was detected only in the samples originating in Ethiopia. The volume of 3-ethyl-1,2-cyclopentanedione, that was found during the first measurement (week 0) in all four samples, dropped during storage in samples stored at room temperature. Samples stored in the refrigerator showed complete loss of this volatile substance as early as during the second measurement (week 2). Over the 15-week storage period, only two newly emerging volatile substances were identified. In the second measurement (week 2), p-cymene appeared in all samples stored in the refrigerator. In samples stored at room temperature, p-cymene was not detected. Dodecane was firstly identified in samples stored at room temperature during week 6 in case of samples from Guatemala and Ethiopia and during week 10 in samples from Indonesia and Ecuador. None of the samples stored in the refrigerator evince evidence of dodecane.

Five most abundant volatile substances present in freshly roasted coffee samples from Ecuador and Ethiopia were, listed in the descendent order, as followed: pyridine, 2,6-dimethylpyrazine, 2-methylpyrazine, 2-furanmethanol and 5-methylfurfural. The same profile of five most prominent substances was also applicable for Indonesian coffee, with inverted order of 2-methylpyrazine, 2-furanmethanol. In fresh Guatemalan coffee, top 5 volatile substances according to volume were 2,6-dimethylpyrazine, pyridine, 2-methylpyrazine, 2-furanmethanol, and 2-ethylpyrazine. At the end of the research, the composition of the most plentiful compounds in the samples remained the same or very similar, despite the sharp decline in some of these substances.

The amount of 3-methylbutanal progressively declined with time in samples of all geographic origins and in both storage conditions. The temperature had a significant effect on the descent rate. For samples stored in the refrigerator, the loss of 3-

methylbutanal was much slower. The volume of 2,6-dimethylpyrazine also decreased in all samples. After 15 weeks of storage, amount of 2,6-dimethylpyrazine was reduced to roughly half, but in this case, with no significant impact of storage temperature on the final volume. Of volatile substances following a visibly gradual descending trend in volume in samples of all geographical origins and both storage conditions can be further mentioned 2-methylbutanal, 2,3-pentanedione, 2-methylpyrazine, 2-ethylpyrazine, and 2-methylcyclohexanone. The amount of substances affected by different storage temperatures has increased over time. During the first two weeks of storage, the difference in temperature influenced only up to 10 substances in each sample. Based on the results of the last measurement, the most responsive to the various conditions of storage was the Ethiopian coffee, with 24 volatile substances affected by the storage temperature. In other coffee samples no more than 15 volatiles were affected at the time of the last measurement.

Volumetric increase of acetic acid was observed for Guatemalan samples stored at room temperature. On the other hand, Ethiopian coffee recorded a significant drop in acetic acid in samples stored at room temperature. Apart from mentioned cases, volume of acetic acid seemed to more or less flucate randomly.

The total volume of all identified substances has decreased significantly over time. Compared to the last measurement, 1.5 to 2.2 times higher volumes of volatile substances were identified in fresh coffee samples. After 15 weeks of storage, samples of Ethiopian coffee stored in the refrigerator showed a higher total volatile volume compared to the samples stored at room temperature. In the case of Guatemalan coffee, it was exactly the opposite. Coffee samples from Ecuador and Indonesia, showed no significant differences in the total volume of volatile substances between the two storage conditions. It needs to be noted that numbers do not distinguish the positive or negative effect of the individual substances on the coffee aroma.

The following series of tables illustrate the evolution of the volume of selected volatile substances in individual coffee samples. The full versions of the tables are attached as the appendices.

Table 8. Evolution of the area (× 10^3) of selected volatile compounds identified in the roasted coffee beans of samples originating in **Guatemala** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		2,3-Butanedione				
	RT	126 ±14	86 ±17	61 ±42	94 ±19	127 ±73
	4°C	126 ±14	119 ±73	147 ±112	209 ±18	143 ±63
	SL		ns	ns	*	ns
MS		Acetic acid				
	RT	38 ±2	41 ±14	347 ±149	289 ±32	594 ±128
	4°C	38 ±2	867 ±224	676 ±221	279 ±101	72 ±22
	SL		*	*	ns	*
MS		3-Methylbutanal				
	RT	709 ±57	492 ±44	403 ±23	261 ±57	190 ±5
	4°C	709 ±57	505 ±10	519 ±24	394 ±56	335 ±33
	SL		ns	***	ns	*
MS		2-Methylbutanal				
	RT	1900 ±156	1546 ±152	1195 ±68	898 ±101	808 ±91
	4°C	1900 ±156	1991 ±8	1563 ±22	1333 ±103	1014 ±99
	SL		*	**	ns	ns
MS		2,3-Pentanedione				
	RT	729 ±51	608 ±73	487 ±61	330 ±53	241 ±21
	4°C	729 ±51	639 ±103	541 ±37	431 ±38	362 ±37
	SL		ns	ns	ns	*
MS, KI		Pyrazine				
	RT	310 ±26	294 ±22	163 ±16	155 ±20	184 ±21
	4°C	310 ±26	341 ±51	244 ±24	240 ±7	160 ±28
	SL		ns	ns	*	ns
MS, KI		Pyridine				
	RT	5661 ±300	5487 ±461	2983 ±145	3227 ±109	3383 ±618
	4°C	5661 ±300	10012 ±675	5929 ±829	6190 ±199	2879 ±240
	SL		**	*	**	ns
MS, KI		2-Methylpyrazine				
	RT	4611 ±276	4529 ±484	2391 ±110	2177 ±227	2273 ±67
	4°C	4611 ±276	4568 ±288	3134 ±186	3070 ±109	2137 ±111
	SL		ns	*	**	ns
MS, KI		2-Furanmethanol				
	RT	2522 ±87	3087 ±353	2287 ±268	2297 ±99	2488 ±316
	4°C	2522 ±87	4785 ±1283	3604 ±652	2652 ±338	1726 ±169
	SL		ns	ns	ns	ns
MS, KI		2,6-Dimethylpyrazine				
	RT	6308 ±414	6291 ±863	3452 ±297	3185 ±369	3159 ±119
	4°C	6308 ±414	6453 ±497	4355 ±237	4026 ±101	3005 ±65
	SL		ns	*	*	ns
MS, KI		2-Ethylpyrazine				
	RT	1937 ±162	1794 ±459	1083 ±199	1197 ±94	1145 ±88
	4°C	1937 ±162	2222 ±276	1657 ±53	1407 ±117	989 ±118
	SL		ns	*	*	ns

MS, KI		5-Methylfurfural												
	RT	1695	±64	1951	±238		1622	±67		1434	±47		1396	±37
	4°C	1695	±64	2453	±504		1864	±125		1559	±115		1115	±75
	SL			ns			ns			ns			*	
MS		3-Ethyl-1,2-cyclopentanedione												
	RT	74	±8	77	±10		31	±7		38	±9		39	±12
	4°C	74	±8	0			0			0			0	
	SL			**			*			*			*	
MS, KI		p-Cymene												
	RT	0		0			0			0			0	
	4°C	0		339	±11		128	±23		118	±31		113	±5
	SL			***			*			*			***	
MS, KI		2-Ethyl-3,5-dimethylpyrazine												
	RT	311	±13	337	±95		225	±38		152	±74		194	±13
	4°C	311	±13	328	±27		233	±29		184	±17		180	±12
	SL			ns			ns			ns			*	
MS, KI		Dodecane												
	RT	0		0			49	±9		41	±8		38	±7
	4°C	0		0			0			0			0	
	SL						*			*			*	
MS, KI		4-Ethylguaiacol												
	RT	47	±21	55	±35		34	±11		43	±8		17	±6
	4°C	47	±21	56	±13		40	±9		33	±16		23	±3
	SL			ns			ns			ns			ns	
MS, KI		2-Methoxy-4-vinylphenol												
	RT	150	±32	185	±71		39	±18		78	±32		39	±6
	4°C	150	±32	145	±33		56	±12		55	±13		43	±20
	SL			ns			ns			ns			ns	

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

Table 9. Evolution of the area (× 10^3) of selected volatile compounds identified in the roasted coffee beans of samples originating in **Indonesia** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		2,3-Butanedione				
	RT	42 ±6	112 ±80	108 ±75	140 ±22	86 ±1
	4°C	42 ±6	154 ±117	146 ±20	189 ±27	140 ±64
	SL		ns	ns	ns	ns
MS		Acetic acid				
	RT	1461 ±101	95 ±34	389 ±320	1093 ±1170	193 ±54
	4°C	1461 ±101	84 ±48	252 ±94	657 ±187	296 ±105
	SL		ns	ns	ns	ns
MS		3-Methylbutanal				
	RT	644 ±82	528 ±44	329 ±128	316 ±36	265 ±20
	4°C	644 ±82	543 ±32	524 ±20	412 ±29	301 ±17
	SL		ns	ns	*	*
MS		2-Methylbutanal				
	RT	2128 ±88	1581 ±85	992 ±459	866 ±233	891 ±131
	4°C	2128 ±88	1596 ±67	1312 ±134	1349 ±113	1274 ±13
	SL		ns	ns	ns	*
MS		2,3-Pentanedione				
	RT	787 ±133	594 ±23	385 ±121	381 ±21	345 ±18
	4°C	787 ±133	669 ±64	557 ±40	507 ±32	394 ±34
	SL		ns	ns	**	ns
MS, KI		Pyrazine				
	RT	262 ±32	316 ±39	130 ±52	144 ±15	125 ±20
	4°C	262 ±32	275 ±44	133 ±31	169 ±17	136 ±9
	SL		ns	ns	ns	ns
MS, KI		Pyridine				
	RT	6933 ±529	4446 ±327	2885 ±1322	3106 ±759	2134 ±441
	4°C	6933 ±529	4076 ±177	2552 ±642	3891 ±693	4746 ±241
	SL		ns	ns	*	*
MS, KI		2-Methylpyrazine				
	RT	4048 ±401	4214 ±317	2321 ±576	2080 ±94	1687 ±140
	4°C	4048 ±401	3599 ±179	2180 ±83	2634 ±460	1918 ±89
	SL		*	ns	ns	*
MS, KI		2-Furanmethanol				
	RT	4718 ±458	2899 ±185	2397 ±771	2484 ±619	1913 ±246
	4°C	4718 ±458	3302 ±92	1991 ±135	3167 ±480	2065 ±211
	SL		ns	ns	ns	ns
MS, KI		2,6-Dimethylpyrazine				
	RT	5654 ±707	5331 ±548	3301 ±497	3017 ±256	2423 ±10
	4°C	5654 ±707	4876 ±81	3159 ±194	3681 ±533	2495 ±105
	SL		ns	ns	ns	ns
MS, KI		2-Ethylpyrazine				
	RT	1932 ±316	1848 ±187	1245 ±280	1209 ±205	789 ±110
	4°C	1932 ±316	1726 ±4	1110 ±161	1391 ±198	953 ±85
	SL		ns	ns	***	*
MS, KI		5-Methylfurfural				
	RT	2548 ±383	1817 ±122	1576 ±238	1574 ±218	1206 ±56
	4°C	2548 ±383	2163 ±68	1525 ±89	1841 ±150	1179 ±64
	SL		*	ns	*	ns
MS		3-Ethyl-1,2-cyclopentanedione				
	RT	83 ±18	62 ±4	34 ±11	39 ±20	32 ±5
	4°C	83 ±18	0	0	0	0
	SL		**	*	ns	**

MS, KI		p-Cymene										
	RT	0		0		0		0		0		
	4°C	0		378	±53	146	±26	148	±21	127	±3	
	SL			**		*		**		***		
MS, KI		2-Ethyl-3,5-dimethylpyrazine										
	RT	225	±33	255	±131	139	±75	186	±36	143	±23	
	4°C	225	±33	331	±83	144	±52	195	±20	91	±33	
	SL			ns		ns		ns		*		
MS, KI		Dodecane										
	RT	0		0		0		33	±11	31	±11	
	4°C	0		0		0		0		0		
	SL							*		*		
MS, KI		4-Ethylguaiacol										
	RT	38	±8	54	±10	32	±24	29	±14	22	±6	
	4°C	38	±8	56	±8	24	±10	55	±20	19	±7	
	SL			ns		ns		ns		ns		
MS, KI		2-Methoxy-4-vinylphenol										
	RT	80	±20	149	±26	67	±32	68	±23	44	±10	
	4°C	80	±20	184	±51	38	±25	71	±14	27	±8	
	SL			ns		ns		ns		ns		

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

Table 10. Evolution of the area (× 10^3) of selected volatile compounds identified in the roasted coffee beans of samples originating in **Ecuador** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		2,3-Butanedione				
	RT	193 ±28	259 ±61	142 ±51	130 ±67	72 ±49
	4°C	193 ±28	269 ±151	216 ±60	305 ±20	200 ±12
	SL		ns	ns	*	ns
MS		Acetic acid				
	RT	50 ±5	828 ±268	366 ±177	281 ±7	320 ±79
	4°C	50 ±5	50 ±18	593 ±202	81 ±31	174 ±69
	SL		*	ns	**	ns
MS		3-Methylbutanal				
	RT	606 ±55	498 ±36	327 ±33	299 ±85	239 ±24
	4°C	606 ±55	536 ±36	446 ±25	408 ±28	325 ±19
	SL		**	**	ns	**
MS		2-Methylbutanal				
	RT	1640 ±127	1479 ±232	894 ±167	840 ±125	753 ±54
	4°C	1640 ±127	1568 ±101	1178 ±78	1298 ±102	886 ±34
	SL		ns	ns	*	ns
MS		2,3-Pentanedione				
	RT	756 ±60	681 ±54	367 ±64	383 ±97	303 ±9
	4°C	756 ±60	658 ±64	541 ±37	504 ±30	410 ±53
	SL		ns	*	ns	ns
MS, KI		Pyrazine				
	RT	275 ±15	252 ±23	167 ±24	126 ±28	118 ±9
	4°C	275 ±15	288 ±20	165 ±39	189 ±9	170 ±10
	SL		ns	ns	*	*
MS, KI		Pyridine				
	RT	4495 ±332	4685 ±1311	2148 ±1381	2465 ±19	1957 ±287
	4°C	4495 ±332	4946 ±322	3219 ±378	3374 ±165	2450 ±342
	SL		ns	ns	*	ns
MS, KI		2-Methylpyrazine				
	RT	3659 ±342	3467 ±347	2381 ±89	1847 ±53	1534 ±33
	4°C	3659 ±342	3803 ±192	2195 ±328	2392 ±38	1854 ±67
	SL		ns	ns	**	*
MS, KI		2-Furanmethanol				
	RT	3075 ±317	4539 ±713	2697 ±93	2635 ±143	2261 ±157
	4°C	3075 ±317	3712 ±265	2998 ±444	2786 ±165	2055 ±191
	SL		ns	ns	ns	ns
MS, KI		2,6-Dimethylpyrazine				
	RT	4280 ±506	4564 ±382	3287 ±307	2621 ±8	2302 ±62
	4°C	4280 ±506	4571 ±189	2974 ±337	3085 ±190	2433 ±100
	SL		ns	ns	ns	ns
MS, KI		2-Ethylpyrazine				
	RT	1421 ±106	1651 ±101	1092 ±58	883 ±40	714 ±23
	4°C	1421 ±106	1664 ±87	1071 ±169	1103 ±42	766 ±28
	SL		ns	ns	*	ns
MS, KI		5-Methylfurfural				
	RT	1897 ±180	2708 ±207	1866 ±243	1779 ±120	1453 ±58
	4°C	1897 ±180	2118 ±122	1886 ±225	1686 ±79	1340 ±54
	SL		ns	ns	ns	ns
MS		3-Ethyl-1,2-cyclopentanedione				
	RT	65 ±15	64 ±18	21 ±11	27 ±19	22 ±8
	4°C	65 ±15	0	0	0	0
	SL		*	ns	ns	*

MS, KI		p-Cymene								
	RT	0		0		0		0		
	4°C	0		274	±79	117	±22	157	±13	115 ±11
	SL			*		*		**		**
MS, KI		2-Ethyl-3,5-dimethylpyrazine								
	RT	140	±38	251	±24	208	±59	160	±18	124 ±10
	4°C	140	±38	243	±64	120	±9	156	±51	103 ±26
	SL			ns		ns		ns		ns
MS, KI		Dodecane								
	RT	0		0		0		33	±4	35 ±10
	4°C	0		0		0		0		0
	SL							**		*
MS, KI		4-Ethylguaiacol								
	RT	30	±11	43	±4	37	±12	21	±14	31 ±17
	4°C	30	±11	31	±25	29	±11	34	±28	23 ±14
	SL			ns		ns		ns		ns
MS, KI		2-Methoxy-4-vinylphenol								
	RT	111	±47	208	±19	145	±37	114	±23	98 ±6
	4°C	111	±47	225	±88	76	±36	177	±67	83 ±28
	SL			ns		ns		ns		ns

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

Table 11. Evolution of the area (× 10^3) of selected volatile compounds identified in the roasted coffee beans of samples originating in **Ethiopia** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		2,3-Butanedione				
	RT	74 ±27	118 ±23	159 ±6	154 ±8	127 ±16
	4°C	74 ±27	217 ±161	228 ±19	148 ±110	233 ±9
	SL		ns	*	ns	**
MS		Acetic acid				
	RT	745 ±418	824 ±248	342 ±251	303 ±189	242 ±75
	4°C	745 ±418	45 ±14	426 ±164	76 ±41	44 ±25
	SL		*	ns	ns	*
MS		3-Methylbutanal				
	RT	446 ±19	458 ±27	369 ±35	292 ±25	204 ±9
	4°C	446 ±19	490 ±19	434 ±33	319 ±10	289 ±8
	SL		**	ns	ns	**
MS		2-Methylbutanal				
	RT	1832 ±104	1373 ±124	1085 ±128	986 ±114	823 ±41
	4°C	1832 ±104	1557 ±105	1329 ±152	1087 ±50	1034 ±38
	SL		ns	*	ns	*
MS		2,3-Pentanedione				
	RT	570 ±31	602 ±16	423 ±59	392 ±74	264 ±39
	4°C	570 ±31	597 ±41	559 ±33	400 ±42	400 ±24
	SL		ns	*	ns	**
MS, KI		Pyrazine				
	RT	282 ±22	255 ±24	192 ±27	146 ±31	140 ±14
	4°C	282 ±22	284 ±30	223 ±4	197 ±2	190 ±24
	SL		ns	ns	ns	*
MS, KI		Pyridine				
	RT	7098 ±422	3731 ±295	2881 ±495	2154 ±528	2472 ±216
	4°C	7098 ±422	4459 ±377	3394 ±51	3217 ±113	2915 ±125
	SL		ns	ns	ns	ns
MS, KI		2-Methylpyrazine				
	RT	3957 ±289	3245 ±252	2511 ±119	1885 ±154	1843 ±57
	4°C	3957 ±289	3682 ±418	2559 ±112	2597 ±146	2234 ±146
	SL		ns	ns	*	*
MS, KI		2-Furanmethanol				
	RT	3765 ±413	3393 ±280	2588 ±654	2285 ±293	1965 ±215
	4°C	3765 ±413	3044 ±283	2746 ±330	1960 ±173	1907 ±123
	SL		ns	ns	ns	ns
MS, KI		2,6-Dimethylpyrazine				
	RT	4591 ±441	4020 ±49	2960 ±198	2586 ±43	2513 ±114
	4°C	4591 ±441	4514 ±404	3065 ±186	2880 ±63	2683 ±205
	SL		ns	*	*	ns
MS, KI		2-Ethylpyrazine				
	RT	1773 ±173	1721 ±65	1287 ±117	1001 ±88	870 ±83
	4°C	1773 ±173	1749 ±185	1276 ±115	1065 ±53	1112 ±93
	SL		ns	ns	ns	**
MS, KI		5-Methylfurfural				
	RT	1875 ±226	2134 ±145	1596 ±176	1531 ±85	1276 ±58
	4°C	1875 ±226	1895 ±206	1816 ±113	1195 ±71	1177 ±51
	SL		ns	*	ns	*
MS		3-Ethyl-1,2-cyclopentanedione				
	RT	34 ±22	57 ±1	26 ±10	27 ±4	27 ±0
	4°C	34 ±22	0	0	0	0
	SL		***	*	**	***

MS, KI		p-Cymene							
	RT	0		0		0		0	
	4 °C	0		362	±68	148	±22	108	±25	182	±15
	SL			*		**		*		**	
MS, KI		2-Ethyl-3,5-dimethylpyrazine							
	RT	175	±16	244	±20	153	±10	162	±28	133	±15
	4 °C	175	±16	249	±71	165	±20	154	±1	135	±15
	SL			ns		ns		ns		ns	
MS, KI		Dodecane							
	RT	0		0		26	±2	28	±3	29	±7
	4 °C	0		0		0		0		0	
	SL					**		**		*	
MS, KI		4-Ethylguaiacol							
	RT	16	±5	38	±5	12	±6	28	±7	21	±3
	4 °C	16	±5	33	±35	14	±5	13	±6	13	±7
	SL			ns		ns		ns		ns	
MS, KI		2-Methoxy-4-vinylphenol							
	RT	61	±14	94	±11	50	±5	75	±19	51	±16
	4 °C	61	±14	141	±86	37	±12	63	±9	65	±11
	SL			ns		ns		ns		ns	

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

6 DISCUSSION

Effect of different storage temperatures and time on the volatile profile of *Coffea arabica* L. roasted coffee beans was investigated in this research. For greater generalization, research was performed on samples from different geographic locations and environments. The results revealed a very heterogeneous influence of storage conditions on individual volatile substances.

Marin et al. (2008) studied staling of ground roasted blends of Arabica and Robusta coffee throughout the 12 months of storage at room temperature. He evaluated loss of aroma freshness according to packaging technique and oxygen presence. For obtaining volatile compounds HS-SPME with 20 minutes preconditioning at 70°C and 30-minute extraction was used. By employing GC-MS analysis study identified 40 volatile compounds, of which 9 (acetaldehyde, methanethiol, propanal, 2,3-butanedione, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, guaiacol and 2-furfurylthiol) were important coffee odourants, according to Mayer et al. (2000). Bresanello et al. (2017) described 72 volatile compounds identified in roasted coffee beans by HS-SPME sampling approach combined with GC-MS. In our research, we identified 65 volatiles in roasted Arabica coffee, but including only 7 potent odourants (2-methylbutanal, 3-methylbutanal, 2,3-pentanedione, 2,3-butanedione, 2-ethyl-3,5-dimethylpyrazine, 4-ethylguaiacol, and 2-methoxy-4-vinylphenol) provably contributing to the characteristic ground coffee flavour. Acetaldehyde also appeared in our samples, but due to the problematic merging of several peaks, it was not possible to precisely distinguish its amount in the individual samples and therefore it was omitted from the research.

For all study samples, Marin et al. (2008) found loss of most volatile substances over time, including a refrigerated reference sample. Also, our samples showed the decreasing pattern of most of volatile substances, the evolution of which in many cases followed a visibly gradual trend in volume change with a great precision. In 2001, Czerny & Schieberle confirmed a previous study of Holscher et al. (1990) disproving significant role of products formed by oxidative degradation of unsaturated fatty acids in coffee aroma. Even after a

year of storage of coffee at room temperature, Marin et al. (2008), did not identify any other product of lipid oxidation, except hexanal. Makri et al. (2011) suggested hexanal, that confer to the rancid coffee flavour, as a marker compound for storage. Exponential growth of this volatile substance usually occurs as a reaction to exposure to oxidizing atmosphere. During our research, the maximum possible amount of air was removed through vent valves with every bag reclosure. The effective removing of oxygen from the storage environment in combination with relatively short storage period probably caused, that hexanal was not detected in any of our samples. No off-flavour compounds were generated during the 15-week-period in any of our samples. The only new substances emerged were p-cymene and dodecane, which is associated with the attractiveness for *Prorops nasuta*, a parasitoid of the coffee berry borer (Román-Ruíz et al. 2012). According to our results, lower storage temperature fully prevents formation of dodecane.

Very similar conditions to our research were applied by Pérez-Martínez et al. (2008) in the study investigating influence of temperature on the volatile fraction of Arabica coffee brews stored at 4°C and 25°C for the period of one month. Interesting conclusions were made on acetic acid. Research demonstrated an increase in the volume of acetic acid in coffee samples, with a faster rate at those stored at 25°C. The volume of this volatile compound, believed for being responsible for the alteration of coffee, has doubled after 15 days. Similar behaviour was partially recorded with a coffee sample from Guatemala in which the amount of acetic acid in the beans also gradually rose. But, increasing character stood only for the sample stored at room temperature and besides, over the first 14 days the volume growth was very slight. However, the resulting amount exceeded the volume of acetic acid in the fresh Guatemalan beans about 15 times. In a Guatemalan coffee samples stored in refrigerator volume of acetic acid fluctuate randomly throughout the time. The same fluctuations occurred with samples from Ecuador and Indonesia, and so for the both storage conditions. The volume of acetic acid in Ethiopia samples stored at room temperature even declined remarkably. Therefore, no generally valid trend regarding the formation of acetic acid in roasted coffee beans can be inferred from our

research. Kwon et al. (2015) found a positive correlation between the levels of acetic acid and fatty acids in green coffee beans.

Our findings confirmed decreasing trend of pyrazine (earthy/musty flavour) in both storage temperatures, previously reported by Pérez-Martínez et al. (2008). 4-Ethylguaiacol (burnt flavour), that was identified in all our samples in low amounts, is associated with low cup quality (Farah, 2006), being considered by Czerny & Grosch (2000) as an impact compound. From our research, it seems that the temperature does not affect occurance of 4-ethylguaiacol in coffee beans in any way. Later confirmation of negative effect of 4-ethylguaiacol was proved by detection of this volatile exclusively in defective roasted beans (Toci & Farah 2008). The same negative properties are attributed to 2-phenyl-1-ethanol, which did not occur in any of our samples.

Undesired fermentation can lead to formation of 2,3-butanediol, that is characteristic for its slightly ammoniacal, sweet, and pungent odour. Its detection is associated with stinker coffee beans known for their normal appearance, but very unpleasant flavour (Bee et al. 2005). 2,3-butanediol was identified only in our Ethiopian samples. While all other samples were Bourbon varieties, coffee from Ethiopia was the only Forest coffee variety. In addition, it was the only sample of BIO coffee. However, the link between these aspects and the occurrence of 2,3-butanediol would have to be explored more thoroughly. According to the results of our research, this volatile substance reacts quite sensitively to the storage temperature.

7 CONCLUSIONS

SPME is a clean, sensitive and effective method for extraction of volatile compounds from headspace of roasted ground coffee samples. Combining SPME with GC-MS provided detailed and precise information on the chemical composition of the volatile profile of *Coffea arabica* L. coffee samples. The analysis of the results has shown that the volume of volatile substances decreased rapidly during storage. The influence of temperature on the preservation or loss of individual volatile substances differed significantly. Of the 65 volatile compounds identified, only 9 of them remained completely inert to different storage environments in the samples of all four geographical origins and during the all measurements. If the substances responded unequally to different storage temperatures, in most cases, research demonstrated decreasing rate of loss of the volatile substance when storing the samples in the refrigerator. Minor differences in response to the interconnection of the volatile profile with storage conditions also occurred among samples of different geographical origins, but this may also be the result of a different handling method of coffee before roasting. No off-flavour compounds emerged during 15-week period of research. According to our results and literature review, it is clear that for the decreasing quality of coffee during storage are not as much responsible newly generated substances, but disturbance of the balance of potent volatile compounds.

The obtained chemical data would be a good basis for future sensory evaluation of the investigated samples. It could reveal the correlation of individual volatile substances with sensory perception of the beverage and precisely identify which substances are crucial to preserving coffee quality. Those would become the focus of attention. Significantly different prices of various coffee beans bring more and more attention to research on the influence of diverse factors on the coffee quality. Increasingly, the matter of interest is also the ability to identify coffee's origin and other characteristics from its chemical profile, and thus prevent frauds associated with the delivery of unwanted mixtures or coffee of different origin or quality.

REFERENCES

Agresti PDCM, Franca AS, Oliveira LS, Augusti R. 2008. Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. Food Chemistry **106**:787-796.

Akiyama M, Murakami K, Ikeda M, Iwatsuki K, Kokubo S, Wada A, Tanaka K. 2005. Characterization of flavor compounds released during grinding of roasted Robusta coffee seeds. Food Science and Technology Research **11(3)**:298-307.

Al-Abdulkader AM, Al-Namazi AA, AlTurki TA, Al-Khuraish MM, Al-Dakhil AI. 2017. Optimizing coffee cultivation and its impact on economic growth and export earnings of the producing countries: The case of Saudi Arabia. Saudi Journal of Biological Sciences:1-7.

Almazov VB, Konenkov NV. 2017. Acceptance and transmission of a quadrupole mass filter with a prefilter. Technical Physics **62(10)**:1569-1577.

Amamo AA. 2014. Coffee production and marketing in Ethiopia. European Journal of Business and Management **6(37)**:109-121.

Amstalden LC, Leite F, Menezes HC. 2001. Identification and quantification of coffee volatile components through high resolution gas chromatoghaph/mass spectrometer using a headspace automatic sampler. Food Science and Technology (Campinas) **21(1)**:123-128.

Anaya AL, Cruz-Ortega R, Waller GR. 2006. Metabolism and ecology of purine alkaloids. Frontiers in Bioscience **11(1)**:2354–2370.

Anese M, Manzocco L, Nicoli MC. 2006. Modeling the secondary shelf life of ground roasted coffee. Journal of Agricultural and Food Chemistry **54(15)**:5571-5576.

Aniszewski T. 2007. Alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam.

Arruda NP, Hovell AM, Rezende CM, Freitas SP, Couri S, Bizzo HR. 2012. Correlation between precursors and volatiles in Brazilian Arabica coffee processed by dry, semi-dry and wet methods and discrimination by principal component analysis. Quím Nova **35(10)**:2044-2051.

Ashihara H. 2015. Plant Biochemistry**.** Trigonelline biosynthesis in *Coffea arabica* and *Coffea canephora*. Pages 19-28 in Preedy V, editor. Coffee in Health and Disease Prevention. Academic Press, USA.

Ashihara H, Sano H, Crozier A. 2008. Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. Phytochemistry **69(4)**:841-856.

Ashihara H. 2006. Metabolism of alkaloids in coffee plants. Brazilian Journal of Plant Physiology **18(1)**:1-8.

Avelino J, Cristancho M, Georgiou S, Imbach P, Aguilar L, Bornemann G, Läderach P, Anzueto F, Hruska AJ, Morales C. 2015. The coffee rust crises in Colombia and Central America (2008-2013): Impacts, plausible causes and proposed solutions. Food Security **7(2):**303-321.

Batista LR, Chalfoun de Souza SM, Silva e Batista CF, Schwan RF. 2016. Coffee: Types and production. Pages 244-251 in Caballero B, Finglas P, Toldra F, editors. Encyclopedia of food and health. Academic Press, Waltham.

Barter SJ. 2016. Coffee: An Indian Ocean perspective. International Journal of Area Studies **11(2)**:61-81.

Bee S, Brando CHJ, Brumen G, Carvalhaes N, Kolling-Speer I, Speer K. 2005. The raw bean. Pages 87-178 in Illy A, Viani R, editors. Espresso coffee, the science of quality. Elsevier Academic Press, Italy.

Belitz HD, Grosch W, Schieberle P. 2009. Food Chemistry. Springer-Verlag Berlin Heidelberg, Berlin.

Bertrand B, Boulanger R, Dussert S, Ribeyre F, Berthiot L, Descroix F, Jöet T. 2012. Climatic factors directly impact the volatile organic compound fingerprint in green Arabica coffee seed as well as coffee beverage quality. Food Chemistry **135(4)**:2575-2583.

Bhumiratana N, Adhikari K, Chambers E. 2011. Evolution of sensory aroma attributes from coffee beans to brewed coffee. LWT - Food Science and Technology **44(10)**:2185-2192.

Blank I, Sen A, Grosch W. 1991. Aroma impact compounds of Arabica and Robusta coffee. Qualitative and quantitative investigations. Pages 117-129. 14th International Scientific Colloquium on Coffee. ASIC, San Francisco.

Brata AG. 2007. Social impact of coffee crisis on the Pasemah coffee farmers in South Sumatera. MPRA Paper, Yogyakarta.

Bresanello D, Liberto E, Cordero Ch, Rubiolo P, Pellegrino G, Ruosi MR, Bicchi C. 2017. Coffee aroma: Chemometric comparison of the chemical information provided by three different samplings combined with GC–MS to describe the sensory properties in cup. Food Chemistry **214**:218-226.

Buffo RA, Cardelli-Freire C. 2004. Coffee flavour: and overview. Flavour & Fragrance Journal **19(2)**:99-104.

Bunn Ch, Läderach P, Rivera OO, Kirschke D. 2014. A bitter cup: climate change profile of global production of Arabica and Robusta coffee. Climatic Change **129(1)**:89-101.

Business Wire. 2012. Don Francisco's coffee unveils new study that answers the age old question: to freeze or not to freezek. Business Wire Inc., Los Angeles. Available from https://www.businesswire.com/news/home/20120529006452/en/Don-Francisco%E2%80%99s-Coffee-Unveils-New-Study-Answers (accessed February 2018).

Butler R. 1999. Solubles into the millennium. Tea coffee trade J **171**:72-76.

Campa C, Ballester JF, Doulbeau S, Dussert S, Hamon S, Noirot M. 2004. Trigonelline and sucrose diversity in wild *Coffea* species. Food Chemistry **88(1)**:39-43.

Caprioli G, Cortese M, Sagratini G, Vittori S. 2015. The influence of different types of preparation (espresso and brew) on coffee aroma and main bioactive constituents. International Journal of Food Sciences and Nutrition **66(5)**:505-513.

Cheong MW, Tong KH, Ong JJM, Liu SQ, Curran P, Yu B. 2013. Volatile composition and antioxidant capacity of Arabica coffee. Food Research International **51(1)**:388-96.

Chudoba J. 2016. Mass Spectrometry Laboratory. UCT Prague, Prague. Available from https://clab.vscht.cz/ms-en/interpretation-ms (accessed March 2018).

Clarke RJ. 2003. Coffee: green coffee/roast and ground. Pages 1487-1493 in Caballero B, Trugo LC, Finglas P editors. Encyclopedia of Food Science and Nutrition. Academic Press, Oxford.

Clarke RJ, Macrae R. 1985. Coffee chemistry. Elsevier Science, Essex.

Clarke W. 2017. Mass spectrometry in the clinical laboratory: determining the need and avoiding pitfalls. Pages 1-15 in Nair H, Clarke W editors. Mass Spectrometry for the Clinical Laboratory. Elsevier, Baltimore.

Czerny M, Mayer F, Grosch W. 1999. Sensory study on the character impact odorants of roasted Arabica coffee. Journal of Agricultural and Food Chemistry **47(2)**:695-699.

Czerny M, Grosch W. 2000. Potent odorants of Raw Arabica coffee: Their changes during roasting. Journal of Agricultural and Food Chemistry **48**:868-872.

Czerny M, Schieberle P. 2001. Changes in roasted coffee aroma during storage-influence of the packaging. Proceedings of the 19th International Conference on Coffee Science. Trieste, Italy.

Daglia M, Cuzzoni MT, Dacarro C. 1994. Antibacterial activity of coffee. Journal of Agricultural and Food Chemistry **42**:2270-2272.

Davis AP, Tosh J, Ruch N, Fay MF, Museum NH, Road C, Sw L. 2011. Growing coffee: Psilanthus (Rubiaceae) subsumed on the basis of molecular and morphological data; implications for the size, morphology, distribution and evolutionary history of *Coffea*. Botanical Journal of the Linnean Society **167(4)**:357-377.

Deibler KD, Acree TE, Lavin EH. 1998. Formation, analysis and packaging influences. Pages 69-78 in Contis ET, Ho C-T, Mussinan CJ editors. Food Flavors. Elsevier, Amsterdam.

Dutra ER, Oliveira LS, Franca AS, Ferraz VP, Afonso RJCF. 2001. A preliminary study on the feasibility of using the composition of coffee roasting exhaust gas for the determination of the degree of roast. Journal of Food Engineering **47**:241-246.

Emerson Process Management. 2012. Fundamentals of Gas Chromatography. Rosemount Analytical, Houston.

Fairtrade Foundation. 2017. Coffee Farmers. Fairtrade Foundation. Available from http://www.fairtrade.org.uk/Farmers-and-Workers/Coffee (accessed July 2017).

FAO. 2018. Crops. FAOSTAT. Available from http://www.fao.org/faostat/en/#data/QC (accessed April 2018).

FAO. 2017. Statistics. FAOSTAT. Available from http://www.fao.org/statistics/en (accessed July 2017).

FAO. 2015. FAO Coffee Pocketbook. Food and Agriculture Organization of the United Nations, Rome.

Farah A, Donangelo CM. 2006. Phenolic compounds in coffee. Brazilian Journal of Plant Physiology **18(1)**:23-36.

Farah A, Monteiro MC, Calado V, Franca AS, Trugo LC. 2006. Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chemistry **98**:373-380.

Farah A. 2012. Coffee Constituents. Pages 21-58 in Yi-Fang Ch editor. Coffee**:** Emerging health effects and disease prevention. John Wiley & Sons, Inc. and the Institute of Food Technologists.

Feleke ST, Walters LM. 2005. Global coffee import demand in a new era: Implications for developing countries. Review of Applied Economics **1(2)**:223-237.

F. Gaviña & Sons. 2018. Coffee facts. F. Gaviña & Sons Inc. Available from https://www.gavina.com/coffee-facts (accessed February 2018).

Figueroa JG, Vargas LF. 2016. Evaluation of SDE, SFE and SPME/GC-MS for extraction and determination of aroma compounds from Vilcabamba - Ecuadorian roasted coffee. Quimica Nova **39(6)**:712-719.

Flament I, Gautschi F, Winter M, Willhalm B, Stoll M. 1968. Les composants furanniques de l'arome de café: quelques aspects chimiques et spectroscopiques. Pages 197-215. Proceedings of the 3rd International Conference on Coffee Science. ASIC, Paris.

Flament I. 2002. Coffee flavor chemistry. John Wiley & Sons, Chichester.

Franca AS, Mendonça JCF, Oliveira SD. 2005. Composition of green and roasted coffees of different cup qualities. LWT - Food Science and Technology **38(7)**:709-715.

Franca AS, Oliveira LS, Oliveira RC, Agresti PCM, Augusti R. 2009. A preliminary evaluation of the effect of processing temperature on coffee roasting degree assessment. Journal of Food Engineering **92(3)**:345-352.

Freitas AMC, Parreira C, Vilas-Boas L. 2001. The use of an electronic aroma-sensing device to assess coffee differentiation-comparison with SPME gas chromatography–mass spectrometry aroma patterns. Journal of Food Composition and Analysis **14(5)**:513-522.

Garg RC. 2016. Fenugreek: Multiple health benefits. Pages 599-617 in Gupta RC editor. Nutraceuticals: Efficacy, Safety and Toxicity. Academic Press, Chenai.

George SE, Ramalakshmi K, Rao LJM. 2008. A Perception on Health Benefits of Coffee. Critical Reviews in Food Science and Nutrition **48**:464-486.

Ginz M, Balzer HH, Bradbury AGW, Maier HG. 2000. Formation of aliphatic acids by carbohydrate degradation during roasting of coffee. European Food Research & Technology **211(6)**:404-410.

Global Washington. 2017. The Role and Impact of Coffee in Global Development. Global Washington. Available from http://globalwa.org/issues/coffee/ (accessed July 2017).

Gloess AN, Vietri A, Wieland F, Smrke S, Schönbächler B, López JAS, Petrozzi S, Bongers S, Koziorowski T, Yeretzian Ch. 2014. Evidence of different flavour formation dynamics by roasting coffee from different origins: On-line analysis with PTR-ToF-MS. International Journal of Mass Spectrometry **365-366**:324-337.

Glöss, AN, Schönbächler B, Rast M, Deuber L, Yeretzian C. 2014. Freshness indices of roasted coffee: Monitoring the loss of freshness for single serve capsules and roasted whole beans in different packaging. Chimia (Aarau) **68(3)**:179-182.

Grigg D. 2002. The worlds of tea and coffee: Patterns of consumption. GeoJournal **57**:283-294.

Grosch W. 2001. Chemistry III: Volatile compounds. Pages 68-89 in Clarke RJ, Vitzthum OG editors. Coffee: recent developments. Blackwell Science, London.

Haskell CF, Kennedy DO, Wesnes KA, Scholey AB. Cognitive and mood improvements of caffeine in habitual consumers and habitual non-consumers of caffeine. Psychopharmacology **179(4)**:813-825.

Henry-Vitrac C, Ibarra A, Roller M, Mérillon JM, Vitrac X. 2010. Contribution of chlorogenic acids to the inhibition of human hepatic glucose-6-phosphatase activity *in vitro* by svetol, a standardized decaffeinated green coffee extract. Journal of agricultural and food chemistry **58(7)**:4141-4144.

Hernandez JA, Heyd B, Irles C, Valdovinos B, Trystram G. 2007. Analysis of the heat and mass transfer during coffee batch roasting. Journal of Food Engineering **78**:1141-1148.

Herrera JC, Lambot Ch. 2017. The coffee tree - genetic diversity and origin. Pages 1-16 in Folmer B editor. The Craft and Science of Coffee. Academic Press, Lausanne.

Hites RA. 1997. Gas chromatography mass spectrometry. Pages 609-626 in Settle FA editor. Handbook of instrumental techniques for analytical chemistry. Prentice Hall PTR, Upper Saddle River.

Hoffmann E, Stroobant V. 2007. Mass spectrometry: Principles and applications. John Willey & Sons, Chichester.

Holscher W, Vitzthum OG, Steinhart H. 1990. Indentification and sensory evaluation of aroma-impact-compounds in roasted Colombian coffee. Café Cacao Thé **34**:205-212.

ICO. 2017. Trade Statistics Tables. ICO. Available from http://www.ico.org/trade_statistics.asp?section=Statistics (accessed July 2017).

Illy A, Viani R. 2005. Espresso coffee: the chemistry of quality. Academic Press, London.

illycafé. 2017. Pressurized packaging. illycafé S.p.A., Trieste. Available from https://www.illy.com/en-us/company/coffee/packaging (accessed February 2018).

IndexMundi. 2017. Coffee, other mild Arabicas vs cofee, Robusta – price rate of change comparison. IndexMundi. Available from http://www.indexmundi.com/commodities/?commodity=other-mild-arabicas-coffee&months=60&commodity=robusta-coffee (accessed August 2017).

Iwasa K, Setoyama D, Shimizu H, Seta H, Fujimura Y, Miura D, Wariishi H, Nagai C, Nakahara K. 2015. Identification of 3-methylbutanoyl glycosides in green *Coffea arabica* beans as causative determinants for the quality of coffee flavors. Journal of Agricultural & Food Chemistry **63(14)**:3742-3751.

Joët T, Salmona J, Laffargue A, Descroix F, Dussert S. 2010. Use of the € growing environment as a source of variation to identify the quantitative trait transcripts and modules of co-expressed genes that determine chlorogenic acid accumulation. Plant, Cell & Environment **33(7)**:1220-1233.

Knysak D. 2017. Volatile compounds profiles in unroasted *Coffea arabica* and *Coffea canephora* beans from different countries. Food Science and Technology (Campinas) **37(3)**:444-448.

Kochko AD, Akaffou S, Andrade A, Campa C, Crouzillat D, Guyot R, Hamon P, Ming R, Mueller LA, Poncet V, Tranchant-Dubreuil Ch, Hamon S. 2010. Advances in *Coffea* genomics. Advances in Botanical Research **53**:23-63.

Korhoňová M, Hron Klimčíková D, Müller L, Bednář P, Barták P. 2009. Coffee aroma-statistical analysis of compositional data. Talanta **80(2)**:710-715.

Kwon D-J, Jeong HJ, Moon H, Kim H-N, Cho J-H, Lee J-E, Hong KS, Hong Y-S. 2015. Assessment of green coffee bean metabolites dependent on coffee quality using a 1H NMR-based metabolomics approach. Food Research International **67**:175-182.

Ky CL, Louarn J, Dussert S, Guyot B, Hamon S, Noirot M. 2001. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *C. arabica* L. and *Coffea canephora* P. accessions. Food Chemistry **75(2)**:223-230.

Labuza TP, Cardelli C, Anderson B, Shimoni E. 2001. Physical chemistry of roasted and ground coffee: shelf life improvement for flexible packaging. Pages 106-144. Colloque Scientifique International sur le Cafe, 19th. ASIC, Trieste.

Lieberman HR, Tharion WJ, Shukitt-Hale B, Speckman KL, Tulley R. 2002. Effects of caffeine, sleep loss, and stress on cognitive performance and mood during U.S. Navy SEAL training. Sea-Air-Land. Psychopharmacology **164(3)**:250-261.

Lou ZX, Wang HX, Zhu S, Ma CY, Wang ZP. 2011. Antibacterial activity and mechanism of action of chlorogenic acid. Journal of Food Science **76(6)**:398-403.

Maeztu L, Sanz C, Andueza S, Paz de Pena M, Bello J, Cid C. 2001. Characterization of espresso coffee aroma by static headspace GC-MS and sensory flavor profile. Journal of Agricultural and Food Chemistry **49(11)**:5437-5444.

Maeztu L, Andueza S, Iban C, Paz MPD. 2001. Multivariate methods for characterization and classification of espresso coffees from different botanical varieties and types of roast by foam, taste, and mouthfeel. Journal of Agricultural and Food Chemistry **49(10)**:4743-4747.

Maga JA. 1978. Simple phenol and phenolic compounds in food flavor. Food Technology **10**:323–372.

MANU JTC. 2018. Zrnková káva – 100% arabica. MANU JTC s.r.o., Ludgeřovice. Available from https://www.manucafe.cz/zrnkova-kava-100-arabica-x2v10039 (accessed April 2018).

Marin K, Zlatić E, Plestenjak A, Požrl T. 2008. A new aroma index to determine the aroma quality of roasted and ground coffee during storage. Food Technology and Biotechnology **46(4)**:442-447.

Masarirambi MT, Chingwara V, Shongwe VD. 2009. The effect of irrigation on synchronization of coffee (*Coffea arabica* L.) flowering and berry ripening at Chipinge, Zimbabwe. Physics & Chemistry of the Earth - Parts A/B/C **34(13-16)**:786-789.

Mathieu F, Malosse C, Cain AH, Frérot B. 1996. Comparative headspace analysis of fresh red coffee berries from different cultivated varieties of coffee trees. Journal of High Resolution Chromatography **19(5)**:298-300.

Mayer F, Czerny M, Grosch W. 2000. Sensory study of the character impact aroma compounds of a coffee beverage. European Food Research and Technology **211**:272-276.

Mondello L, Costa R, Tranchida PQ, Dugo P, Lo Presti M, Festa S, Dugo G. 2005. Reliable characterization of coffee bean aroma profiles by automated headspace solid phase microextraction-gas chromatography-mass spectrometry with the support of a dual-filter mass spectra library. Journal of Separation Science **28(9-10)**:1101-1109.

Mottaleb MA, Meziani MJ, Islam MR. 2014. Solid-phase microextraction (SPME) and its application to natural products. Pages 105-127 in Mayers RA editor. Encyclopedia of Analytical Chemistry. John Wiley and Sons, USA.

MSP Kofel. 2005. Gas chromatography troubleshooting and reference guide. MSP Kofel, Zollikofen.

Nair KPP. 2010. Coffee. Pages 181-208 in Nair KPP editor. Elsevier, New Delhi.

National Coffee Association of U.S.A. 2018a. What is coffee? National Coffee Association of U.S.A. Inc. Available from http://www.ncausa.org/About-Coffee/How-to-Store-Coffee (accessed April 2018).

National Coffee Association of U.S.A. 2018b. How to store coffee. National Coffee Association of U.S.A. Inc. Available from http://www.ncausa.org/About-Coffee/How-to-Store-Coffee (accessed February 2018).

Netherlands Ministry of Foreign Affairs. 2016. CBI Trade statistics: coffee. CBI Market Intelligence, Hague.

Nicoli M, Calligaris S, Manzocco L. 2009. Shelf-life testing of coffee and related products: uncertainties, pitfalls, and perspectives. Food Engineering Reviews **1(2)**:159-168.

Nijssen LM, Visscher CA, Maarse H, Willemsense LC, Boelens MH. 1996. Volatile compounds in food. TNO Nutrition and Food Research Institute, Zeist.

Noor AAM, Fareez EAM, Noor DAM. 2015. A review of quality coffee roasting degree evaluation. Journal of Applied Science and Agriculture **10(7)**:18-23.

Oestreich-Janzen S. 2010. Chemistry of coffee. Pages 1085-1117 in Mander L, Liu HW editors. Comprehensive Natural Products II. Elsevier, Kidlington.

Paes de Camargo MB. 2010. The impact of climatic variability and climate change on arabic coffee crop in Brazil. Bragantia **69(1)**:239-247.

Pan S, Chen R, Brentnall TA. 2014. Proteomics in pancreatic cancer translational research. Pages 197-219 in Azmi AS editor. Molecular Diagnostics and Treatment of Pancreatic Cancer. Elsevier, Seattle.

Patay ÉB, Bencsik T, Papp N. 2016. Phytochemical overview and medicinal importance of *Coffea* species from the past until now. Asian Pacific Journal of Tropical Medicine **9(12)**:1127-1135.

Pawliszyn J. 2012b. Theory of solid-phase microextraction. Pages 13-59 in Pawliszyn J editor. Handbook of Solid Phase Microextraction. Elsevier, Bellefonte.

Perrone D, Neves YP, Brandao JM, Martinez HEP, Farah A. 2009. Influence of zinc fertilization on chlorogenic acids and antioxidant activity of coffee seeds. Pages 220-223. 22nd International Conference on Coffee Science: Coffee Chemistry and Human Physiology. ASIC, Campinas.

Petriková V, Patočka J. 2006. Káva očima toxikologa. Vojenské zdravotnické listy **3-4**:120-125.

Poltronieri P, Rossi F. 2016. Challenges in specialty coffee processing and quality assurance. Challenges **7(19)**:1-22.

Poole CF. 2002. The Essence of Chromatography. Elsevier, Detroit.

Pypker J, Brouwer H. 1969. Headspace analysis of less volatile constituents of coffee. Pages 122-130. 4th International Scientific Colloquium on on Green and Roasted Coffee Chemistry. ASIC, Amsterdam.

Qiao Y, Bi JX, Zhang Y, Zhang Y, Fan G, Xiao LY, Si YP. 2008. Characterization of aroma active compounds in fruit juice and peel oil of jinchen sweet orange fruit (*Citrus sinensis* (L.) Osbeck) by GC-MS and GC-O. Molecules **13(6)**:1333-1344.

Riedel A, Hochkogler CM, Lang R, Bytof G, Lantz I, Hofmann T, Somoza V. 2014. *N*-Methylpyridinium, a degradation product of trigonelline upon coffee roasting, stimulates respiratory activity and promotes glucose utilization in HepG2 cells. Food & Function **5(3)**:454-462.

Rohwer JG. 2002. A trópusok növényei. Magyar Könyvklub, Budapest.

Román-Ruíz AK, Malo EA, Huerta G, Castillo A, Barrera JF, Rojas JC. 2012. Identification and origin of host-associated volatiles attractive to *Prorops nasuta*, a parasitoid of the coffee berry borer. Arthropod-Plant Interactions **6(4)**:611-620.

Sanz C, Czerny M, Cid C, Schieberle P. 2002. Comparison of potent odorants in a filtered coffee brew and in an instant coffee beverage by aroma extract dilution analysis. European Food Research and Technology **214(4)**:299-302.

Schenker S, Heinemann C, Huber R, Pompizzi R, Ferren R, Escher F. 2002. Impact of roasting temperature profiles on chemical reaction conditions in coffee beans. Journal of Food Science **67(1)**:60-66.

Schievano E, Finotello C, De Angelis E, Mammi S, Navarini L. 2014. Rapid authentication of coffee blends and quantification of 16-O-methylcafestol in roasted coffee beans by nuclear magnetic resonance. Journal of Agricultural and Food Chemistry **62(51)**:12309-12314.

Schivelbusch W. 1992. Tastes of paradise: a social history of spices, stimulants, and intoxicants. Pantheon Books, New York.

Selmar D, Bytof G, Knopp SE, Breitenstein B. 2006. Germination of coffee seeds and its significance for coffee quality. Plant Biology **8(2)**:260-264.

Sgorbini B, Cagliero C, Cordero Ch, Liberto E, Rubiolo P, Bicchi C. 2014. Headspace sampling and gas chromatography: a successful combination to study the composition of a plant volatile fraction. Pages 254-277 in Hostettmann K, Chen S, Marston A, Stuppner H editors. Handbook of Chemical and Biological Plant Analytical Methods. Wiley, Hoboken.

Shirey RE. 2012. SPME Commercial devices and fibre coatings. Pages 99-133 in Pawliszyn J editor. Handbook of Solid Phase Microextraction. Elsevier, Bellefonte.

Sigma-Aldrich. 1999. Solid phase microextraction fiber assemblies. Sigma-Aldrich, Bellefonte.

Smělíková E, Zdráhal I. 2011. Participation of developing countries producers in global networks – the case of coffee. The scientific journal for economics and informatics in agriculture **14(1)**:8-12.

Smith JP, Daifas DP, El-Khoury W, Koukoutsis J, El-Khoury A. 2004. Shelf life and safety concerns of bakery products: A review. Critical Reviews in Food Science and Nutrition **44(1)**:19-55.

Sneddon J, Masuram S, Richert JC. 2007. Gas chromatography-mass spectrometry-Basic principles, instrumentation and selected applications for detection of Organic compounds. Analytical Letters **40(6)**:1003-1012.

Somogyi Á. 2008. Mass spectrometry instrumentation and techniques. Pages 93-140 in Vékey K, Telekes A, Vertes A editors. Medical Applications of Mass Spectrometry. Elsevier, Tucson.

Somporn Ch, Kamtuo A, Theerakulpisut P, Siriamornpun S. 2011. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica L. cv.* Catimor). International Journal of Food Science & Technology **46(11)**:2287-2296.

Spence Ch. 2015. Just how much of what we taste derives from the sense of smell? Flavour **4**:1-10.

Starbucks. 2016. Příprava kávy – Často kladené otázky. Starbucks Corporation. Available from http://www.starbuckscoffee.cz/coffee/learn/coffee-faqs (accessed February 2018).

Statista. 2017. Largest coffee producing countries 2016. Statista. Available from https://www.statista.com/statistics/277137/world-coffee-production-by-leading-countries/ (accessed July 2017).

Stauffer E, Dolan JA, Newman R. 2008. Gas chromatography and gas chromatography-mass spectrometry. Pages 235-293 in Soucy J editor. Fire Debris Analysis. Elsevier, Burlington.

Sunarharum WB, Williams DJ, Smyth HE. 2014. Complexity of coffee flavor: A compositional and sensory perspective. Food Research International **62**:315-325.

Tanna S, Lawson G. 2016. Analytical chemistry methods for the assessment of medication adherence. Pages 51-86 in Tanna S, Lawson G editors. Analytical Chemistry for Assessing Medication Adherence. Elsevier in cooperation with RTI Press, Cambridge.

Tesfaye K, Govers K, Bekele E, Borsch B. 2013. ISSR fingerprinting of *Coffea arabica* throughout Ethiopia reveals high variability in wild populations and distinguishes them from landraces. Plant Systematics & Evolution **300(5)**:881-897.

Toci AT, Farah A. 2008. Volatile compounds as potential defective coffee beans' markers. Food chemistry **108(3)**:1133-1141.

Toledo PRAB, Pezza L, Pezza HR, Toci AT. 2016. Relationship between the different aspects related to coffee quality and their volatile compounds. Comprehensive Reviews in Food Science & Food Safety **15(4)**:705-719.

Topik SC. 2000. Coffee. Pages 641-653 in Kiple KF, Ornelas KC editors. The Cambridge World History of Food. Cambridge University Press, Cambridge.

Trugo L, Macrae RJ. 1984. A study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. Food Chemistry **15(3)**:219-227.

US Department of Agriculture. 2017. Coffee: World Markets and Trade. Foreign Agricultural Service/USDA, Office of Global Analysis, Washington, DC.

Vaast P, Bertrand B, Perriot JJ, Guyot B, Genard M. 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. Journal of the Science of Food and Agriculture **86(2)**:197-204.

Vandel VE, Limbach PA. 2017. Overview of biochemical applications of mass spectrometry. Pages 514-516 in Lindon JC, Tranter GE, Koppenaal DW editors. Encyclopedia of Spectroscopy and Spectrometry. Elsevier, Baton Rouge.

Viegas MC, Bassoli DG, Vignoli JA, Silva RSSF. 2008. Pages 321-325. Comparison of different extraction methods to the identification of soluble coffee volatile compounds. 22nd International Conference on Coffee Science, Campinas.

Vila MA, Andueza S, de Peña MP, Concepción C. 2005. Fatty acid evolution during the storage of ground, roasted coffees. Journal of the American Oil Chemists' Society **82(9)**:639.

Wang X, Lim L-T. 2014. Effect of roasting conditions on carbon dioxide degassing behavior in coffee. Food Research International **61**:144-151.

Wang X, Lim L-T. 2015. Corrigendum to "Effect of roasting conditions on carbon dioxide degassing behavior in coffee". Food Research International **69**:434.

Wang HY, Qian H, Yao WR. 2011. Melanoidins produced by the Maillard reaction: Structure and biological activity. Food Chemistry **128(3)**:573-584.

Wei F, Tanokura M. 2015. Chemical changes in the components of coffee beans during roasting. Pages 83-91 in Preedy E editors. Coffee in Health and Disease Prevention. Academic Press, USA.

Wintgens JN. 2009. Coffee: growing, processing, sustainable production - a guidebook for growers, processors, traders, and researchers. Wiley-VCH, Weinheim.

WITS. 2017. Trade Summary Data. World Integrated Trade Solution. Available from http://wits.worldbank.org/CountryProfile/en/Country/HND/Year/2015/Summary (accessed July 2017).

Wolf A, Bray GA, Popkin BM. 2008. A short history of beverages and how our body treats them. Obesity Reviews **9(2)**:151-164.

Xu JG, Hu QP, Liu Y. 2012. Antioxidant and DNA-protective activities of chlorogenic acid isomers. Journal of Agricultural & Food Chemistry **60(46)**:11625-11630.

Yang N, Liu Ch, Liu X, Degn TK, Munchow M, Fisk I. 2016. Determination of volatile marker compounds of common coffee roast defects. Food Chemistry **211**:206-214.

Yeretzian C, Badoud R, Jordan A, Lindinger W. 2002. From the green bean to the cup of coffee: investigating coffee roasting by on-line monitoring of volatiles. European Food Research and Technology **214(2)**:92-104.

Yeretzian C, Jordan A, Lindinger W. 2003. Analysing the headspace of coffee by proton-transfer-reaction mass-spectrometry. International Journal of Mass Spectrometry **223**:115-139.

Zambonin CG, Balest L, De Benedetto GE, Palmisano F. 2005. Solid-phase microextraction–gas chromatography mass spektrometry and multivariate analysis for the characterization of roasted coffees. Talanta **66(1)**:261-265.

Zheng XQ, Ashihara H. 2004. Distribution, biosynthesis and function of purine and pyridine alkaloids in *Coffea arabica* seedlings. Plant Science **166(3)**:807-813.

REFERENCES (FIGURES)

Byun-McKay A, Godard K-A, Toudefallah M, Martin DM, Alfaro R, King J, Bohlmann J, Aine L. 2006. Wound-induced terpene synthase gene expression in sitka spruce that exhibit resistance or susceptibility to attack by the white pine weevil. Plant Physiology. **140(3)**:1009-1021.

Farah A. 2012. Coffee Constituents. Pages 21-58 in Yi-Fang Ch editor. Coffee**:** Emerging Health Effects and Disease Prevention. John Wiley & Sons, Inc. and the Institute of Food Technologists.

Il-Young K In-kyu L, Sang-Hoon S, Robert RW. 2016. Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research. Experimental and Molecular Medicine **48(1)**:1-10.

National Coffee Association of U.S.A. 2018a. What is coffee? National Coffee Association of U.S.A. Inc. Available from http://www.ncausa.org/About-Coffee/How-to-Store-Coffee (accessed April 2018).

Oestreich-Janzen S. 2010. Chemistry of coffee. Pages 1085-1117 in Mander L, Liu HW editors. Comprehensive Natural Products II. Elsevier, Kidlington.

Pawliszyn J. 2012a. Solid-phase microextraction in perspective. Pages 1-12 in Pawliszyn J editor. Handbook of Solid Phase Microextraction. Elsevier, Bellefonte.

Pawliszyn J. 2012b. Theory of solid-phase microextraction. Pages 13-59 in Pawliszyn J editor. Handbook of Solid Phase Microextraction. Elsevier, Bellefonte.

Shirey RE. 2012. SPME commercial devices and fibre coatings. Pages 99-133 in Pawliszyn J editor. Handbook of Solid Phase Microextraction. Elsevier, Bellefonte.

Yeretzian C, Badoud R, Jordan A, Lindinger W. 2002. From the green bean to the cup of coffee: investigating coffee roasting by on-line monitoring of volatiles. European Food Research and Technology **214(2)**:92-104.

Zambonin CG, Balest L, De Benedetto GE, Palmisano F. 2005. Solid-phase microextraction–gas chromatography mass spektrometry and multivariate analysis for the characterization of roasted coffees. Talanta **66(1)**:261-265.

Zhang Z & Yang MJ. 1994. Solid-phase microextraction. Analytical Chemistry **66(17)**:844-854.

LIST OF THE APPENDICES

APPENDICES

Appendix I. An overview of the volatile substances identified in the coffees of four different geographical origins and two different storage environments

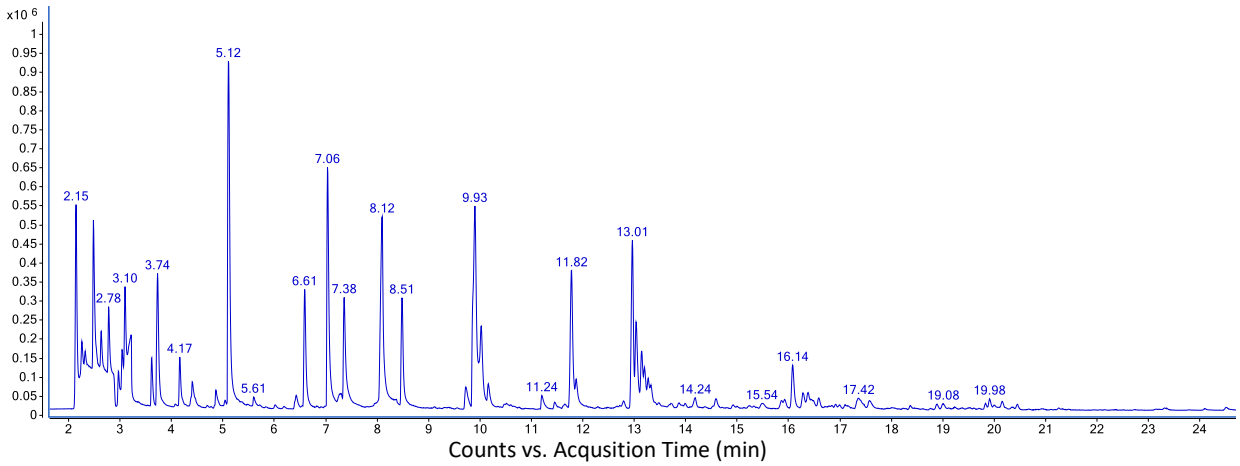
KI (M)	KI (L)	Volatile compound	GU[a]		IN[b]		EC[c]		ET[d]	
			RT[e]	4 °C	RT[e]	4 °C	RT[e]	4 °C	RT[e]	4 °C
–	–	Acetone	x	x	x	x	x	x	x	x
–	–	Butanal	x	x	x	x	x	x	x	x
–	–	2,3-Butanedione	x	x	x	x	x	x	x	x
–	–	2-Butanone	x	x	x	x	x	x	x	x
–	–	2-Methylfuran	x	x	x	x	x	x	x	x
–	–	Acetic acid	x	x	x	x	x	x	x	x
–	–	3-Methylbutanal	x	x	x	x	x	x	x	x
–	–	2-Methylbutanal	x	x	x	x	x	x	x	x
–	–	2,3-Pentanedione	x	x	x	x	x	x	x	x
–	–	2,5-Dimethylfuran	x	x	x	x	x	x	x	x
–	–	Acetoin	x	x	x	x	x	x	x	x
712	712	Pyrazine	x	x	x	x	x	x	x	x
722	717	1-Methylpyrrole	x	x	x	x	x	x	x	x
725	727	Pyridine	x	x	x	x	x	x	x	x
751	748	1-Hydroxy-2-butanone	x	x	x	x	x	x	x	x
768	769	2,3-Butanediol							x	x
772	763	4-Methyl-2,3-pentanedione	x	x	x	x	x	x	x	x
–	–	2,3-Butanediol (another isomer)							x	x
801	810	2-Methyltetrahydrofuran-3-one	x	x	x	x	x	x	x	x
816	826	2-Methylpyrazine	x	x	x	x	x	x	x	x
827	830	Furfural	x	x	x	x	x	x	x	x
852	866	2-Furanmethanol	x	x	x	x	x	x	x	x
865	870	Acetoxyacetone	x	x	x	x	x	x	x	x
906	902	Furfuryl formate	x	x	x	x	x	x	x	x
910	912	2,6-Dimethylpyrazine	x	x	x	x	x	x	x	x
914	906	2-Ethylpyrazine	x	x	x	x	x	x	x	x
918	918	2,3-Dimethylpyrazine	x	x	x	x	x	x	x	x
928	n/a	2,3-Dimethoxy-1,3-butadiene	x	x	x	x	x	x	x	x
934	939	α-Pinene					x	x		
947	953	2-Methylcyclohexanone	x	x	x	x	x	x	x	x
954	n/a	2-Butylfuran	x	x	x	x	x	x	x	x
959	959	3-Ethylpyridine	x	x	x	x	x	x	x	x
963	962	5-Methylfurfural	x	x	x	x	x	x	x	x
966	n/a	1-Acetoxy-2-butanone	x	x	x	x	x	x	x	x
978	980	β-Pinene					x	x		
997	995	2-Furanmethyl acetate	x	x	x	x	x	x	x	x
999	1003	2-Ethyl-6-methylpyrazine	x	x	x	x	x	x	x	x
1002	1000	2-Ethyl-5-methylpyrazine	x	x	x	x	x	x	x	x
1003	999	2,3,5-Trimethylpyrazine	x	x	x	x	x	x	x	x
1005	1001	2-Ethyl-3-methylpyrazine	x	x	x	x	x	x	x	x
1007	1010	2-Formyl-1-methylpyrrole	x	x	x	x	x	x	x	x
1021	n/a	1-Acetyl-1,4-dihydropyridine	x	x	x	x	x	x	x	x
1025	n/a	3-Ethyl-1,2-cyclopentanedione	x		x		x		x	
1027	1026	p-Cymene		x		x		x		x
1030	1031	Limonene	x	x	x	x	x	x	x	x
1064	1060	2-Acetylpyrrole	x	x	x	x	x	x	x	x
1075	1074	cis-Linalool oxide	x	x	x	x	x	x	x	x

1077	n/a	2-Acetyl-1-methylpyrrole	x	x	x	x	x	x	x	x
1081	1080	2-Ethyl-3,6-dimethylpyrazine	x	x	x	x	x	x	x	x
1087	1089	2-Furfurylfuran	x	x	x	x	x	x	x	x
1087	1085	3-Ethyl-2,5-dimethylpyrazine	x	x	x	x	x	x	x	x
1089	1085	2-Ethyl-3,5-dimethylpyrazine	x	x	x	x	x	x	x	x
1091	n/a	3,5-Dimethyl-4-allylpyrazole	x	x	x	x	x	x	x	x
1095	1082	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	x	x	x	x	x	x	x	x
1116	1108	Maltol	x	x	x	x	x	x	x	x
1121	n/a	2-Methyl-6-acetylpyrazine	x	x	x	x	x	x	x	x
1143	n/a	5-Methyl-6,7-dihydrocyclopentapyrazine	x	x	x	x	x	x	x	x
1161	1160	3,5-Diethyl-2-methylpyrazine	x	x	x	x	x	x	x	x
1184	1190	2-(2-Furylmethyl)-5-methylfuran	x	x	x	x	x	x	x	x
1186	1182	1-Furfurylpyrrole	x	x	x	x	x	x	x	x
1193	n/a	Flamenol	x	x	x	x	x	x	x	x
1202	1199	Dodecane	x		x		x		x	
1283	1281	4-Ethylguaiacol	x	x	x	x	x	x	x	x
1306	1292	Difurfuryl ether	x	x	x	x	x	x	x	x
1318	1313	2-Methoxy-4-vinylphenol	x	x	x	x	x	x	x	x
		TOTAL COMPOUNDS IDENTIFIED	60	59	60	59	62	61	62	61

[a]GU = Guatemala, [b]IN = Indonesia, [c]EC = Ecuador, [d]ET = Ethiopia. [e]RT = room temperature.

KI(M) = Kovats index (measured), KI(L) = Kovats index (library). n/a = not available.

Appendix II. An example of one of the chromatograms



Appendix III.

a. Evolution of the area (× 10^3) of the volatile compounds identified in the roasted coffee beans of samples originating in **Guatemala** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		Acetone				
	RT	487 ±87	1443 ±7	952 ±324	879 ±78	1096 ±433
	4°C	487 ±87	693 ±703	1393 ±70	1280 ±74	1121 ±29
	SL		ns	ns	*	ns
MS		Butanal				
	RT	303 ±29	522 ±233	713 ±285	408 ±45	524 ±311
	4°C	303 ±29	348 ±319	669 ±36	545 ±24	449 ±25
	SL		ns	ns	ns	ns
MS		2,3-Butanedione				
	RT	126 ±14	86 ±17	61 ±42	94 ±19	127 ±73
	4°C	126 ±14	119 ±73	147 ±112	209 ±18	143 ±63
	SL		ns	ns	*	ns
MS		2-Butanone				
	RT	228 ±30	51 ±42	88 ±21	112 ±27	165 ±140
	4°C	228 ±30	241 ±114	210 ±67	201 ±16	148 ±4
	SL		ns	*	*	ns
MS		2-Methylfuran				
	RT	384 ±49	450 ±89	758 ±76	315 ±82	431 ±251
	4°C	384 ±49	336 ±196	624 ±33	499 ±37	383 ±60
	SL		ns	*	ns	ns
MS		Acetic acid				
	RT	38 ±2	41 ±14	347 ±149	289 ±32	594 ±128
	4°C	38 ±2	867 ±224	676 ±221	279 ±101	72 ±22
	SL		*	*	ns	*
MS		3-Methylbutanal				
	RT	709 ±57	492 ±44	403 ±23	261 ±57	190 ±5
	4°C	709 ±57	505 ±10	519 ±24	394 ±56	335 ±33
	SL		ns	***	ns	*
MS		2-Methylbutanal				
	RT	1900 ±156	1546 ±152	1195 ±68	898 ±101	808 ±91
	4°C	1900 ±156	1991 ±8	1563 ±22	1333 ±103	1014 ±99
	SL		*	**	ns	ns
MS		2,3-Pentanedione				
	RT	729 ±51	608 ±73	487 ±61	330 ±53	241 ±21
	4°C	729 ±51	639 ±103	541 ±37	431 ±38	362 ±37
	SL		ns	ns	ns	*
MS		2,5-Dimethylfuran				
	RT	77 ±9	75 ±12	98 ±16	46 ±11	19 ±6
	4°C	77 ±9	164 ±92	71 ±10	48 ±15	39 ±5
	SL		ns	*	ns	**
MS		Acetoin				
	RT	347 ±42	11774 ±1980 3	180 ±35	190 ±10	178 ±35
	4°C	347 ±42	363 ±54	237 ±18	262 ±16	226 ±23
	SL		ns	ns	*	ns
MS, KI		Pyrazine				
	RT	310 ±26	294 ±22	163 ±16	155 ±20	184 ±21
	4°C	310 ±26	341 ±51	244 ±24	240 ±7	160 ±28
	SL		ns	ns	*	ns

MS, KI		1-Methylpyrrole													
	RT	61	±12	105	±56	80	±28	66	±35	52	±22				
	4°C	61	±12	132	±76	112	±45	117	±9	47	±26				
	SL			ns		ns		ns		ns					
MS, KI		Pyridine													
	RT	5661	±300	5487	±461	2983	±145	3227	±109	3383	±618				
	4°C	5661	±300	10012	±675	5929	±829	6190	±199	2879	±240				
	SL			**		*		**		ns					
MS, KI		1-Hydroxy-2-butanone													
	RT	68	±4	145	±98	36	±1	49	±6	92	±42				
	4°C	68	±4	134	±15	151	±131	114	±69	54	±7				
	SL			ns		ns		ns		ns					
MS, KI		2,3-Butanediol													
	RT	0		0		0		0		0					
	4°C	0		0		0		0		0					
	SL														
MS, KI		4-Methyl-2,3-pentanedione													
	RT	61	±9	62	±16	41	±3	30	±5	23	±1				
	4°C	61	±9	62	±14	46	±3	29	±3	29	±5				
	SL			ns		*		ns		ns					
MS, KI		2-Methyltetrahydrofuran-3-one													
	RT	1255	±95	1149	±215	1025	±6	870	±60	892	±1				
	4°C	1255	±95	1593	±339	1285	±27	1087	±109	809	±109				
	SL			ns		**		*		ns					
MS, KI		2-Methylpyrazine													
	RT	4611	±276	4529	±484	2391	±110	2177	±227	2273	±67				
	4°C	4611	±276	4568	±288	3134	±186	3070	±109	2137	±111				
	SL			ns		*		**		ns					
MS, KI		Furfural													
	RT	989	±132	1151	±41	970	±103	876	±94	825	±117				
	4°C	989	±132	1462	±237	1002	±154	878	±107	784	±65				
	SL			ns		ns		ns		ns					
MS, KI		2-Furanmethanol													
	RT	2522	±87	3087	±353	2287	±268	2297	±99	2488	±316				
	4°C	2522	±87	4785	±1283	3604	±652	2652	±338	1726	±169				
	SL			ns		ns		ns		ns					
MS, KI		Acetoxyacetone													
	RT	1242	±110	1328	±69	877	±97	830	±51	1032	±116				
	4°C	1242	±110	2188	±461	1442	±290	1316	±69	890	±67				
	SL			ns		ns		**		ns					
MS, KI		Furfuryl formate													
	RT	347	±16	393	±86	205	±11	179	±25	257	±20				
	4°C	347	±16	446	±100	199	±8	172	±31	231	±10				
	SL			*		ns		ns		ns					
MS, KI		2,6-Dimethylpyrazine													
	RT	6308	±414	6291	±863	3452	±297	3185	±369	3159	±119				
	4°C	6308	±414	6453	±497	4355	±237	4026	±101	3005	±65				
	SL			ns		*		*		ns					
MS, KI		2-Ethylpyrazine													
	RT	1937	±162	1794	±459	1083	±199	1197	±94	1145	±88				
	4°C	1937	±162	2222	±276	1657	±53	1407	±117	989	±118				
	SL			ns		*		*		ns					
MS, KI		2,3-Dimethylpyrazine													
	RT	654	±107	499	±159	265	±92	315	±20	305	±56				
	4°C	654	±107	587	±64	415	±60	359	±41	292	±20				
	SL			ns		ns		ns		ns					

MS		2,3-Dimethoxy-1,3-butadiene								
	RT	128	±39	67	±49	66	±29	46	±30	59	±48
	4°C	128	±39	163	±41	89	±35	65	±15	50	±4
	SL			*		ns		ns		ns	
MS, KI		α-Pinene								
	RT	0		0		0		0		0	
	4°C	0		0		0		0		0	
	SL										
MS, KI		2-Methylcyclohexanone								
	RT	339	±8	346	±45	181	±27	174	±18	153	±18
	4°C	339	±8	448	±59	277	±36	226	±18	176	±6
	SL			*		*		***		ns	
MS		2-Butylfuran								
	RT	104	±17	122	±19	101	±23	75	±6	53	±5
	4°C	104	±17	146	±48	107	±3	89	±13	60	±5
	SL			ns		ns		ns		ns	
MS, KI		3-Ethylpyridine								
	RT	154	±8	162	±38	75	±11	95	±11	46	±2
	4°C	154	±8	271	±34	132	±24	113	±14	78	±10
	SL			*		*		*		*	
MS, KI		5-Methylfurfural								
	RT	1695	±64	1951	±238	1622	±67	1434	±47	1396	±37
	4°C	1695	±64	2453	±504	1864	±125	1559	±115	1115	±75
	SL			ns		ns		ns		*	
MS		1-Acetoxy-2-butanone								
	RT	434	±18	404	±19	326	±55	340	±58	346	±43
	4°C	434	±18	633	±129	437	±62	418	±95	239	±66
	SL			ns		ns		ns		*	
MS, KI		β-Pinene								
	RT	0		0		0		0		0	
	4°C	0		0		0		0		0	
	SL										
MS, KI		2-Furanmethyl acetate								
	RT	1661	±70	1953	±324	1746	±63	1491	±117	1446	±120
	4°C	1661	±70	2229	±382	1800	±46	1480	±139	1181	±55
	SL			*		ns		ns		ns	
MS, KI		2-Ethyl-6-methylpyrazine								
	RT	1714	±84	1832	±269	967	±59	949	±69	951	±9
	4°C	1714	±84	1850	±175	1144	±114	1091	±64	831	±9
	SL			ns		ns		ns		***	
MS, KI		2-Ethyl-5-methylpyrazine								
	RT	1060	±52	1077	±156	553	±28	564	±38	596	±11
	4°C	1060	±52	1138	±87	667	±54	645	±60	483	±24
	SL			ns		ns		ns		**	
MS, KI		2,3,5-Trimethylpyrazine								
	RT	925	±72	889	±134	344	±20	406	±85	442	±30
	4°C	925	±72	869	±56	513	±34	477	±17	435	±18
	SL			ns		*		ns		ns	
MS, KI		2-Ethyl-3-methylpyrazine								
	RT	591	±8	543	±95	270	±35	264	±33	290	±16
	4°C	591	±8	543	±43	297	±23	329	±11	263	±4
	SL			ns		ns		ns		ns	
MS, KI		2-Formyl-1-methylpyrrole								
	RT	375	±28	379	±64	210	±95	128	±90	260	±16
	4°C	375	±28	456	±59	279	±104	289	±42	234	±11
	SL			ns		ns		*		ns	
MS		1-Acetyl-1,4-dihydropyridine								
	RT	133	±9	228	±34	64	±24	87	±16	87	±14
	4°C	133	±9	222	±27	86	±7	66	±17	57	±4
	SL			ns		ns		ns		ns	

MS		3-Ethyl-1,2-cyclopentanedione									
	RT	74	±8	77	±10	31	±7	38	±9	39	±12
	4°C	74	±8	0		0		0		0	
	SL			**		*		*		*	
MS, KI		p-Cymene									
	RT	0		0		0		0		0	
	4°C	0		339	±11	128	±23	118	±31	113	±5
	SL			***		*		*		***	
MS, KI		Limonene									
	RT	188	±21	90	±21	112	±11	109	±20	105	±11
	4°C	188	±21	189	±51	114	±20	108	±19	152	±7
	SL			ns		ns		ns		*	
MS, KI		2-Acetylpyrrole									
	RT	193	±33	263	±54	143	±29	131	±16	90	±15
	4°C	193	±33	257	±87	157	±14	118	±25	85	±9
	SL			ns		ns		ns		ns	
MS, KI		cis-Linalool oxide									
	RT	100	±10	159	±19	91	±21	76	±3	64	±5
	4°C	100	±10	135	±47	103	±3	85	±22	46	±10
	SL			ns		ns		ns		ns	
MS		2-Acetyl-1-methylpyrrole									
	RT	156	±3	134	±14	105	±14	92	±2	93	±5
	4°C	156	±3	125	±31	118	±11	101	±2	116	±18
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3,6-dimethylpyrazine									
	RT	1022	±40	1027	±170	583	±119	594	±113	523	±18
	4°C	1022	±40	1088	±74	616	±80	551	±41	416	±29
	SL			ns		ns		ns		*	
MS, KI		2-Furfurylfuran									
	RT	197	±29	132	±40	118	±7	78	±14	117	±25
	4°C	197	±29	146	±18	127	±11	92	±7	110	±11
	SL			ns		ns		ns		ns	
MS, KI		3-Ethyl-2,5-dimethylpyrazine									
	RT	76	±18	138	±28	85	±23	73	±23	29	±10
	4°C	76	±18	142	±20	83	±18	81	±10	44	±9
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3,5-dimethylpyrazine									
	RT	311	±13	337	±95	225	±38	152	±74	194	±13
	4°C	311	±13	328	±27	233	±29	184	±17	180	±12
	SL			ns		ns		ns		*	
MS		3,5-Dimethyl-4-allylpyrazole									
	RT	150	±10	140	±36	114	±42	69	±34	90	±24
	4°C	150	±10	159	±32	135	±23	95	±1	92	±5
	SL			ns		ns		ns		ns	
MS, KI		3-Ethyl-2-hydroxy-2-cyclopenten-1-one									
	RT	139	±20	125	±29	124	±16	74	±34	98	±7
	4°C	139	±20	139	±28	128	±27	83	±12	105	±16
	SL			ns		ns		ns		ns	
MS, KI		Maltol									
	RT	388	±10	583	±134	232	±50	283	±88	190	±24
	4°C	388	±10	493	±47	254	±28	202	±8	214	±40
	SL			ns		ns		ns		ns	
MS		2-Methyl-6-acetylpyrazine									
	RT	220	±9	258	±36	148	±26	160	±34	114	±22
	4°C	220	±9	298	±35	195	±24	130	±9	118	±8
	SL			ns		ns		ns		ns	
MS		5-Methyl-6,7-dihydrocyclopentapyrazine									
	RT	126	±21	130	±57	39	±29	37	±8	32	±7
	4°C	126	±21	96	±14	59	±25	32	±3	31	±7
	SL			ns		ns		ns		ns	

MS, KI		3,5-Diethyl-2-methylpyrazine							
	RT	134	±2	174	±43	85	±14	87	±26	76	±8
	4°C	134	±2	156	±24	88	±11	75	±7	60	±20
	SL			ns		ns		ns		ns	
MS, KI		2-(2-Furylmethyl)-5-methylfuran							
	RT	43	±7	58	±13	57	±20	58	±8	44	±8
	4°C	43	±7	75	±26	53	±19	30	±7	40	±13
	SL			ns		ns		ns		ns	
MS, KI		1-Furfurylpyrrole							
	RT	76	±1	96	±8	90	±29	99	±8	75	±6
	4°C	76	±1	115	±28	95	±17	54	±10	61	±26
	SL			ns		ns		*		ns	
MS		Flamenol							
	RT	72	±12	77	±10	79	±31	100	±14	65	±17
	4°C	72	±12	98	±42	83	±30	57	±8	64	±27
	SL			ns		ns		ns		ns	
MS, KI		Dodecane							
	RT	0		0		49	±9	41	±8	38	±7
	4°C	0		0		0		0		0	
	SL					*		*		*	
MS, KI		4-Ethylguaiacol							
	RT	47	±21	55	±35	34	±11	43	±8	17	±6
	4°C	47	±21	56	±13	40	±9	33	±16	23	±3
	SL			ns		ns		ns		ns	
MS, KI		Difurfuryl ether							
	RT	35	±7	51	±14	32	±16	47	±12	12	±5
	4°C	35	±7	54	±13	27	±2	18	±1	12	±4
	SL			ns		ns		ns		ns	
MS, KI		2-Methoxy-4-vinylphenol							
	RT	150	±32	185	±71	39	±18	78	±32	39	±6
	4°C	150	±32	145	±33	56	±12	55	±13	43	±20
	SL			ns		ns		ns		ns	

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

b. Evolution of the area (× 10^3) of the volatile compounds identified in the roasted coffee beans of samples originating in **Indonesia** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		Acetone				
	RT	1062 ±695	1498 ±54	862 ±506	964 ±77	843 ±20
	4°C	1062 ±695	1513 ±71	1023 ±139	1089 ±10	1017 ±33
	SL		ns	ns	ns	**
MS		Butanal				
	RT	156 ±22	513 ±265	669 ±652	428 ±28	527 ±398
	4°C	156 ±22	637 ±57	583 ±30	536 ±29	451 ±21
	SL		ns	ns	ns	ns
MS		2,3-Butanedione				
	RT	42 ±6	112 ±80	108 ±75	140 ±22	86 ±1
	4°C	42 ±6	154 ±117	146 ±20	189 ±27	140 ±64
	SL		ns	ns	ns	ns
MS		2-Butanone				
	RT	154 ±62	118 ±52	100 ±69	117 ±17	76 ±22
	4°C	154 ±62	100 ±52	141 ±62	167 ±4	153 ±45
	SL		ns	ns	*	ns
MS		2-Methylfuran				
	RT	290 ±42	705 ±471	344 ±190	418 ±38	430 ±89
	4°C	290 ±42	481 ±153	588 ±46	533 ±34	420 ±32
	SL		ns	ns	ns	ns
MS		Acetic acid				
	RT	1461 ±101	95 ±34	389 ±320	1093 ±1170	193 ±54
	4°C	1461 ±101	84 ±48	252 ±94	657 ±187	296 ±105
	SL		ns	ns	ns	ns
MS		3-Methylbutanal				
	RT	644 ±82	528 ±44	329 ±128	316 ±36	265 ±20
	4°C	644 ±82	543 ±32	524 ±20	412 ±29	301 ±17
	SL		ns	ns	*	*
MS		2-Methylbutanal				
	RT	2128 ±88	1581 ±85	992 ±459	866 ±233	891 ±131
	4°C	2128 ±88	1596 ±67	1312 ±134	1349 ±113	1274 ±13
	SL		ns	ns	ns	*
MS		2,3-Pentanedione				
	RT	787 ±133	594 ±23	385 ±121	381 ±21	345 ±18
	4°C	787 ±133	669 ±64	557 ±40	507 ±32	394 ±34
	SL		ns	ns	**	ns
MS		2,5-Dimethylfuran				
	RT	61 ±40	90 ±31	52 ±21	54 ±38	45 ±8
	4°C	61 ±40	78 ±11	94 ±17	47 ±26	48 ±22
	SL		ns	ns	ns	ns
MS		Acetoin				
	RT	274 ±180	326 ±32	194 ±128	168 ±113	175 ±58
	4°C	274 ±180	381 ±78	181 ±56	270 ±52	262 ±25
	SL		ns	ns	ns	ns
MS, KI		Pyrazine				
	RT	262 ±32	316 ±39	130 ±52	144 ±15	125 ±20
	4°C	262 ±32	275 ±44	133 ±31	169 ±17	136 ±9
	SL		ns	ns	ns	ns
MS, KI		1-Methylpyrrole				
	RT	34 ±28	106 ±53	27 ±12	47 ±17	27 ±10
	4°C	34 ±28	108 ±56	38 ±8	40 ±7	38 ±8
	SL		ns	*	ns	ns

MS, KI		Pyridine												
	RT	6933	±529		4446	±327		2885	±1322		3106	±759		2134	±441
	4°C	6933	±529		4076	±177		2552	±642		3891	±693		4746	±241
	SL				ns			ns			*			*	
MS, KI		1-Hydroxy-2-butanone												
	RT	169	±7		97	±14		65	±29		79	±29		57	±8
	4°C	169	±7		111	±17		58	±16		81	±28		118	±44
	SL				ns			ns			ns			ns	
MS, KI		2,3-Butanediol												
	RT	0			0			0			0			0	
	4°C	0			0			0			0			0	
	SL														
MS, KI		4-Methyl-2,3-pentanedione												
	RT	69	±26		68	±7		30	±11		34	±4		25	±2
	4°C	69	±26		70	±15		40	±9		43	±10		30	±4
	SL				ns			ns			ns			ns	
MS, KI		2-Methyltetrahydrofuran-3-one												
	RT	1815	±337		1122	±125		1039	±290		1006	±155		833	±72
	4°C	1815	±337		1180	±104		1070	±174		1220	±110		969	±93
	SL				ns			ns			ns			*	
MS, KI		2-Methylpyrazine												
	RT	4048	±401		4214	±317		2321	±576		2080	±94		1687	±140
	4°C	4048	±401		3599	±179		2180	±83		2634	±460		1918	±89
	SL				*			ns			ns			*	
MS, KI		Furfural												
	RT	1761	±289		1209	±92		1145	±382		1003	±109		751	±81
	4°C	1761	±289		1652	±117		971	±176		1289	±226		888	±120
	SL				ns			ns			ns			ns	
MS, KI		2-Furanmethanol												
	RT	4718	±458		2899	±185		2397	±771		2484	±619		1913	±246
	4°C	4718	±458		3302	±92		1991	±135		3167	±480		2065	±211
	SL				ns			ns			ns			ns	
MS, KI		Acetoxyacetone												
	RT	1985	±115		1243	±148		896	±391		881	±219		715	±133
	4°C	1985	±115		1352	±34		754	±122		1186	±313		1149	±21
	SL				ns			ns			ns			*	
MS, KI		Furfuryl formate												
	RT	449	±77		348	±36		180	±25		167	±17		219	±39
	4°C	449	±77		388	±24		213	±30		205	±14		203	±58
	SL				*			**			ns			ns	
MS, KI		2,6-Dimethylpyrazine												
	RT	5654	±707		5331	±548		3301	±497		3017	±256		2423	±10
	4°C	5654	±707		4876	±81		3159	±194		3681	±533		2495	±105
	SL				ns			ns			ns			ns	
MS, KI		2-Ethylpyrazine												
	RT	1932	±316		1848	±187		1245	±280		1209	±205		789	±110
	4°C	1932	±316		1726	±4		1110	±161		1391	±198		953	±85
	SL				ns			ns			***			*	
MS, KI		2,3-Dimethylpyrazine												
	RT	465	±147		511	±84		352	±82		310	±14		170	±47
	4°C	465	±147		467	±14		296	±74		351	±46		197	±53
	SL				ns			*			ns			ns	
MS		2,3-Dimethoxy-1,3-butadiene												
	RT	180	±78		97	±27		80	±57		72	±8		38	±8
	4°C	180	±78		124	±23		101	±45		101	±18		57	±25
	SL				ns			ns			ns			ns	
MS, KI		α-Pinene												
	RT	0			0			0			0			0	
	4°C	0			0			0			0			0	
	SL														

MS, KI		2-Methylcyclohexanone									
	RT	436	±37	299	±19	213	±48	194	±28	135	±6
	4°C	436	±37	360	±6	186	±4	234	±44	195	±1
	SL			*		ns		ns		**	
MS		2-Butylfuran									
	RT	131	±44	103	±7	91	±11	81	±2	58	±7
	4°C	131	±44	134	±15	85	±19	101	±2	50	±19
	SL			ns		ns		**		ns	
MS, KI		3-Ethylpyridine									
	RT	146	±38	106	±7	57	±11	67	±10	39	±10
	4°C	146	±38	123	±4	30	±13	71	±21	66	±21
	SL			*		ns		ns		ns	
MS, KI		5-Methylfurfural									
	RT	2548	±383	1817	±122	1576	±238	1574	±218	1206	±56
	4°C	2548	±383	2163	±68	1525	±89	1841	±150	1179	±64
	SL			*		ns		*		ns	
MS		1-Acetoxy-2-butanone									
	RT	592	±85	380	±32	301	±9	300	±27	281	±37
	4°C	592	±85	377	±58	375	±57	345	±44	286	±45
	SL			ns		ns		ns		ns	
MS, KI		β-Pinene									
	RT	0		0		0		0		0	
	4°C	0		0		0		0		0	
	SL										
MS, KI		2-Furanmethyl acetate									
	RT	1920	±396	1606	±134	1498	±133	1417	±208	1250	±123
	4°C	1920	±396	1813	±55	1618	±182	1593	±46	1104	±18
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-6-methylpyrazine									
	RT	1360	±236	1485	±142	987	±46	936	±143	735	±56
	4°C	1360	±236	1426	±64	944	±109	1094	±149	665	±27
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-5-methylpyrazine									
	RT	803	±137	832	±97	555	±22	523	±90	422	±32
	4°C	803	±137	825	±49	529	±60	618	±83	375	±9
	SL			ns		ns		ns		ns	
MS, KI		2,3,5-Trimethylpyrazine									
	RT	644	±96	623	±71	366	±10	350	±70	289	±29
	4°C	644	±96	575	±52	304	±16	394	±76	304	±9
	SL			ns		*		ns		ns	
MS, KI		2-Ethyl-3-methylpyrazine									
	RT	381	±80	410	±63	290	±6	388	±232	208	±18
	4°C	381	±80	402	±60	253	±38	284	±51	211	±16
	SL			ns		ns		ns		ns	
MS, KI		2-Formyl-1-methylpyrrole									
	RT	378	±54	365	±109	286	±8	291	±36	225	±44
	4°C	378	±54	392	±21	307	±28	334	±43	220	±8
	SL			ns		ns		ns		ns	
MS		1-Acetyl-1,4-dihydropyridine									
	RT	123	±24	160	±8	71	±18	72	±43	58	±7
	4°C	123	±24	155	±38	36	±10	62	±20	46	±7
	SL			ns		*		ns		ns	
MS		3-Ethyl-1,2-cyclopentanedione									
	RT	83	±18	62	±4	34	±11	39	±20	32	±5
	4°C	83	±18	0		0		0		0	
	SL			**		*		ns		**	
MS, KI		p-Cymene									
	RT	0		0		0		0		0	
	4°C	0		378	±53	146	±26	148	±21	127	±3
	SL			**		*		**		***	

MS, KI		Limonene								
	RT	184	±18	225	±11	107	±18	112	±30	104	±22
	4°C	184	±18	260	±61	134	±34	105	±21	125	±20
	SL			ns		ns		ns		ns	
MS, KI		2-Acetylpyrrole								
	RT	165	±35	228	±14	128	±12	133	±34	70	±7
	4°C	165	±35	248	±11	92	±6	142	±17	0	
	SL			ns		ns		ns		**	
MS, KI		cis-Linalool oxide								
	RT	130	±29	132	±32	107	±5	103	±29	72	±15
	4°C	130	±29	143	±56	114	±11	121	±22	52	±1
	SL			ns		ns		ns		ns	
MS		2-Acetyl-1-methylpyrrole								
	RT	136	±34	117	±12	90	±4	93	±20	85	±13
	4°C	136	±34	113	±46	90	±11	103	±7	97	±7
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3,6-dimethylpyrazine								
	RT	633	±119	763	±103	476	±14	460	±105	366	±37
	4°C	633	±119	749	±182	384	±71	526	±48	275	±24
	SL			ns		ns		ns		ns	
MS, KI		2-Furfurylfuran								
	RT	161	±33	111	±65	90	±26	103	±11	100	±9
	4°C	161	±33	165	±40	87	±24	99	±20	80	±13
	SL			ns		ns		ns		ns	
MS, KI		3-Ethyl-2,5-dimethylpyrazine								
	RT	60	±16	97	±68	44	±24	55	±13	31	±1
	4°C	60	±16	100	±64	35	±23	80	±11	24	±3
	SL			ns		ns		*		ns	
MS, KI		2-Ethyl-3,5-dimethylpyrazine								
	RT	225	±33	255	±131	139	±75	186	±36	143	±23
	4°C	225	±33	331	±83	144	±52	195	±20	91	±33
	SL			ns		ns		ns		*	
MS		3,5-Dimethyl-4-allylpyrazole								
	RT	159	±35	126	±74	89	±42	128	±22	80	±38
	4°C	159	±35	183	±64	71	±60	126	±19	58	±34
	SL			ns		ns		ns		ns	
MS, KI		3-Ethyl-2-hydroxy-2-cyclopenten-1-one								
	RT	137	±31	139	±31	90	±39	113	±21	80	±10
	4°C	137	±31	163	±70	74	±13	103	±27	70	±8
	SL			ns		ns		ns		ns	
MS, KI		Maltol								
	RT	332	±48	497	±91	280	±48	273	±52	178	±35
	4°C	332	±48	597	±122	216	±101	258	±21	158	±17
	SL			ns		ns		ns		ns	
MS		2-Methyl-6-acetylpyrazine								
	RT	193	±23	256	±46	156	±4	155	±29	101	±14
	4°C	193	±23	154	±14	123	±32	164	±9	76	±20
	SL			ns		ns		ns		ns	
MS		5-Methyl-6,7-dihydrocyclopentapyrazine								
	RT	67	±26	48	±4	41	±9	34	±11	25	±8
	4°C	67	±26	72	±21	30	±4	42	±9	18	±3
	SL			ns		ns		ns		ns	
MS, KI		3,5-Diethyl-2-methylpyrazine								
	RT	91	±6	110	±14	79	±16	72	±16	58	±13
	4°C	91	±6	135	±29	68	±7	82	±1	28	±21
	SL			ns		ns		ns		ns	
MS, KI		2-(2-Furylmethyl)-5-methylfuran								
	RT	66	±52	47	±13	44	±7	31	±4	26	±2
	4°C	66	±52	57	±11	42	±9	38	±11	23	±10
	SL			ns		ns		ns		ns	

MS, KI		1-Furfurylpyrrole				
	RT	96 ±26	95 ±17	85 ±3	73 ±3	56 ±8
	4°C	96 ±26	112 ±19	68 ±16	79 ±8	52 ±17
	SL		ns	ns	ns	ns
MS		Flamenol				
	RT	85 ±29	93 ±29	74 ±18	61 ±11	43 ±11
	4°C	85 ±29	99 ±46	52 ±9	57 ±5	54 ±22
	SL		ns	ns	ns	ns
MS, KI		Dodecane				
	RT	0	0	0	33 ±11	31 ±11
	4°C	0	0	0	0	0
	SL				*	*
MS, KI		4-Ethylguaiacol				
	RT	38 ±8	54 ±10	32 ±24	29 ±14	22 ±6
	4°C	38 ±8	56 ±8	24 ±10	55 ±20	19 ±7
	SL		ns	ns	ns	ns
MS, KI		Difurfuryl ether				
	RT	30 ±7	40 ±8	34 ±19	23 ±10	14 ±3
	4°C	30 ±7	52 ±17	15 ±4	25 ±5	5 ±4
	SL		ns	ns	ns	ns
MS, KI		2-Methoxy-4-vinylphenol				
	RT	80 ±20	149 ±26	67 ±32	68 ±23	44 ±10
	4°C	80 ±20	184 ±51	38 ±25	71 ±14	27 ±8
	SL		ns	ns	ns	ns

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

c. Evolution of the area ($\times 10^3$) of the volatile compounds identified in the roasted coffee beans of samples originating in **Ecuador** throughout storage at room temperature (RT) and in the refrigerator (4°C)[a]

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		Acetone				
	RT	1334 ±114	1239 ±126	857 ±16	742 ±109	642 ±29
	4°C	1334 ±114	1064 ±584	940 ±238	1082 ±121	851 ±21
	SL		ns	ns	ns	*
MS		Butanal				
	RT	231 ±8	573 ±201	318 ±39	325 ±34	247 ±14
	4°C	231 ±8	396 ±206	458 ±31	424 ±5	346 ±9
	SL		ns	*	*	**
MS		2,3-Butanedione				
	RT	193 ±28	259 ±61	142 ±51	130 ±67	72 ±49
	4°C	193 ±28	269 ±151	216 ±60	305 ±20	200 ±12
	SL		ns	ns	*	ns
MS		2-Butanone				
	RT	63 ±4	148 ±30	63 ±38	67 ±62	81 ±17
	4°C	63 ±4	214 ±61	225 ±40	162 ±12	126 ±4
	SL		ns	*	ns	*
MS		2-Methylfuran				
	RT	350 ±74	561 ±59	383 ±28	238 ±153	217 ±18
	4°C	350 ±74	464 ±157	491 ±26	439 ±37	305 ±14
	SL		ns	ns	ns	**

MS		Acetic acid											
	RT	50	±5	828	±268	366	±177	281	±7	320	±79		
	4°C	50	±5	50	±18	593	±202	81	±31	174	±69		
	SL			*		ns		**		ns			
MS		3-Methylbutanal											
	RT	606	±55	498	±36	327	±33	299	±85	239	±24		
	4°C	606	±55	536	±36	446	±25	408	±28	325	±19		
	SL			**		**		ns		**			
MS		2-Methylbutanal											
	RT	1640	±127	1479	±232	894	±167	840	±125	753	±54		
	4°C	1640	±127	1568	±101	1178	±78	1298	±102	886	±34		
	SL			ns		ns		*		ns			
MS		2,3-Pentanedione											
	RT	756	±60	681	±54	367	±64	383	±97	303	±9		
	4°C	756	±60	658	±64	541	±37	504	±30	410	±53		
	SL			ns		*		ns		ns			
MS		2,5-Dimethylfuran											
	RT	84	±6	66	±12	48	±10	56	±27	25	±17		
	4°C	84	±6	64	±26	70	±22	48	±7	40	±13		
	SL			ns		ns		ns		ns			
MS		Acetoin											
	RT	379	±29	335	±86	178	±76	173	±51	445	±507		
	4°C	379	±29	377	±40	289	±58	358	±45	194	±34		
	SL			ns		ns		ns		ns			
MS, KI		Pyrazine											
	RT	275	±15	252	±23	167	±24	126	±28	118	±9		
	4°C	275	±15	288	±20	165	±39	189	±9	170	±10		
	SL			ns		ns		*		*			
MS, KI		1-Methylpyrrole											
	RT	68	±8	114	±27	67	±9	52	±2	31	±2		
	4°C	68	±8	100	±39	78	±30	51	±7	64	±7		
	SL			ns		ns		ns		*			
MS, KI		Pyridine											
	RT	4495	±332	4685	±1311	2148	±1381	2465	±19	1957	±287		
	4°C	4495	±332	4946	±322	3219	±378	3374	±165	2450	±342		
	SL			ns		ns		*		ns			
MS, KI		1-Hydroxy-2-butanone											
	RT	131	±40	180	±105	104	±85	76	±29	75	±47		
	4°C	131	±40	175	±74	85	±20	110	±9	60	±13		
	SL			ns		ns		*		ns			
MS, KI		2,3-Butanediol											
	RT	0		0		0		0		0			
	4°C	0		0		0		0		0			
	SL												
MS, KI		4-Methyl-2,3-pentanedione											
	RT	58	±13	65	±7	31	±7	33	±5	27	±3		
	4°C	58	±13	64	±12	41	±6	42	±10	32	±2		
	SL			ns		*		ns		ns			
MS, KI		2-Methyltetrahydrofuran-3-one											
	RT	1178	±113	1344	±139	814	±96	850	±116	704	±18		
	4°C	1178	±113	1108	±100	1078	±157	794	±75	792	±23		
	SL			ns		ns		ns		ns			
MS, KI		2-Methylpyrazine											
	RT	3659	±342	3467	±347	2381	±89	1847	±53	1534	±33		
	4°C	3659	±342	3803	±192	2195	±328	2392	±38	1854	±67		
	SL			ns		ns		**		*			
MS, KI		Furfural											
	RT	1774	±277	2334	±247	1416	±110	1416	±124	1294	±31		
	4°C	1774	±277	1897	±276	1517	±185	1601	±190	1228	±116		
	SL			ns		ns		ns		ns			

MS, KI		2-Furanmethanol											
	RT	3075	±317	4539	±713	2697	±93	2635	±143	2261	±157		
	4°C	3075	±317	3712	±265	2998	±444	2786	±165	2055	±191		
	SL			ns		ns		ns		ns			
MS, KI		Acetoxyacetone											
	RT	1279	±93	1601	±412	777	±162	808	±32	768	±105		
	4°C	1279	±93	1370	±193	1052	±172	1019	±100	783	±40		
	SL			ns		ns		ns		ns			
MS, KI		Furfuryl formate											
	RT	349	±46	289	±6	197	±30	247	±20	250	±5		
	4°C	349	±46	244	±11	247	±25	181	±2	243	±10		
	SL			*		**		*		ns			
MS, KI		2,6-Dimethylpyrazine											
	RT	4280	±506	4564	±382	3287	±307	2621	±8	2302	±62		
	4°C	4280	±506	4571	±189	2974	±337	3085	±190	2433	±100		
	SL			ns		ns		ns		ns			
MS, KI		2-Ethylpyrazine											
	RT	1421	±106	1651	±101	1092	±58	883	±40	714	±23		
	4°C	1421	±106	1664	±87	1071	±169	1103	±42	766	±28		
	SL			ns		ns		*		ns			
MS, KI		2,3-Dimethylpyrazine											
	RT	368	±51	394	±41	297	±56	225	±32	200	±6		
	4°C	368	±51	442	±36	273	±38	271	±22	206	±19		
	SL			ns		ns		ns		ns			
MS		2,3-Dimethoxy-1,3-butadiene											
	RT	112	±26	170	±23	88	±31	95	±14	54	±8		
	4°C	112	±26	150	±32	108	±33	76	±1	70	±6		
	SL			ns		ns		ns		ns			
MS, KI		α-Pinene											
	RT	148	±46	206	±12	153	±43	179	±43	80	±4		
	4°C	148	±46	220	±38	114	±44	107	±17	111	±15		
	SL			ns		ns		ns		ns			
MS, KI		2-Methylcyclohexanone											
	RT	335	±51	395	±57	229	±25	196	±4	162	±10		
	4°C	335	±51	373	±10	267	±32	254	±11	184	±20		
	SL			ns		ns		*		ns			
MS		2-Butylfuran											
	RT	104	±22	133	±16	86	±13	68	±8	58	±2		
	4°C	104	±22	89	±37	93	±7	69	±5	61	±7		
	SL			ns		ns		ns		ns			
MS, KI		3-Ethylpyridine											
	RT	75	±20	120	±20	51	±18	46	±24	34	±7		
	4°C	75	±20	85	±29	56	±6	70	±17	42	±10		
	SL			ns		ns		ns		*			
MS, KI		5-Methylfurfural											
	RT	1897	±180	2708	±207	1866	±243	1779	±120	1453	±58		
	4°C	1897	±180	2118	±122	1886	±225	1686	±79	1340	±54		
	SL			ns		ns		ns		ns			
MS		1-Acetoxy-2-butanone											
	RT	451	±36	513	±60	314	±93	322	±39	277	±105		
	4°C	451	±36	304	±21	286	±45	330	±82	307	±44		
	SL			*		ns		ns		ns			
MS, KI		β-Pinene											
	RT	151	±22	129	±13	116	±10	113	±17	80	±6		
	4°C	151	±22	141	±14	69	±14	93	±7	84	±23		
	SL			ns		**		ns		ns			
MS, KI		2-Furanmethyl acetate											
	RT	1281	±140	1784	±36	1329	±226	1390	±148	1054	±29		
	4°C	1281	±140	1541	±88	1413	±103	1172	±68	914	±33		
	SL			ns		ns		ns		**			

MS, KI		2-Ethyl-6-methylpyrazine												
	RT	824	±223	1132	±38	692	±128	679	±19	522	±84
	4°C	824	±223	1074	±67	661	±76	725	±60	535	±58
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-5-methylpyrazine												
	RT	520	±139	735	±42	440	±88	445	±28	323	±46
	4°C	520	±139	683	±55	417	±45	454	±36	328	±43
	SL			ns		ns		ns		ns	
MS, KI		2,3,5-Trimethylpyrazine												
	RT	433	±125	497	±27	292	±20	256	±37	228	±44
	4°C	433	±125	501	±58	262	±43	367	±53	237	±48
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3-methylpyrazine												
	RT	195	±72	249	±31	137	±30	138	±26	104	±22
	4°C	195	±72	248	±66	127	±16	146	±36	123	±45
	SL			ns		ns		ns		ns	
MS, KI		2-Formyl-1-methylpyrrole												
	RT	265	±90	375	±14	228	±72	313	±10	185	±52
	4°C	265	±90	376	±33	261	±50	325	±30	157	±83
	SL			ns		ns		ns		ns	
MS		1-Acetyl-1,4-dihydropyridine												
	RT	114	±21	159	±30	92	±12	84	±19	52	±14
	4°C	114	±21	126	±56	59	±22	83	±8	32	±16
	SL			ns		ns		ns		ns	
MS		3-Ethyl-1,2-cyclopentanedione												
	RT	65	±15	64	±18	21	±11	27	±19	22	±8
	4°C	65	±15	0		0		0		0	
	SL			*		ns		ns		*	
MS, KI		p-Cymene												
	RT	0		0		0		0		0	
	4°C	0		274	±79	117	±22	157	±13	115	±11
	SL			*		*		**		**	
MS, KI		Limonene												
	RT	211	±46	189	±36	114	±27	124	±23	96	±13
	4°C	211	±46	188	±65	121	±14	171	±43	87	±24
	SL			ns		ns		ns		ns	
MS, KI		2-Acetylpyrrole												
	RT	96	±33	262	±11	164	±12	143	±22	91	±3
	4°C	96	±33	234	±16	128	±19	166	±6	76	±25
	SL			ns		ns		ns		ns	
MS, KI		cis-Linalool oxide												
	RT	89	±27	171	±11	114	±15	118	±14	87	±2
	4°C	89	±27	132	±7	104	±11	117	±7	78	±17
	SL			**		ns		ns		ns	
MS		2-Acetyl-1-methylpyrrole												
	RT	81	±49	119	±17	94	±25	89	±9	86	±9
	4°C	81	±49	137	±14	85	±24	102	±10	80	±8
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3,6-dimethylpyrazine												
	RT	309	±64	482	±28	308	±50	244	±36	222	±6
	4°C	309	±64	465	±92	230	±34	311	±56	209	±46
	SL			ns		ns		ns		ns	
MS, KI		2-Furfurylfuran												
	RT	92	±23	131	±14	78	±18	90	±12	82	±5
	4°C	92	±23	127	±19	76	±16	73	±37	81	±17
	SL			ns		ns		ns		ns	
MS, KI		3-Ethyl-2,5-dimethylpyrazine												
	RT	73	±31	96	±19	64	±9	38	±1	24	±2
	4°C	73	±31	121	±6	40	±13	77	±33	19	±3
	SL			ns		ns		ns		*	

MS, KI		2-Ethyl-3,5-dimethylpyrazine							
	RT	140	±38	251	±24	208	±59	160	±18	124	±10
	4 °C	140	±38	243	±64	120	±9	156	±51	103	±26
	SL			ns		ns		ns		ns	
MS		3,5-Dimethyl-4-allylpyrazole							
	RT	135	±41	181	±23	128	±34	141	±6	112	±10
	4 °C	135	±41	153	±44	95	±32	110	±52	118	±18
	SL			ns		*		ns		ns	
MS, KI		3-Ethyl-2-hydroxy-2-cyclopenten-1-one							
	RT	116	±37	161	±42	99	±44	104	±4	88	±13
	4 °C	116	±37	145	±44	90	±23	98	±38	82	±11
	SL			ns		ns		ns		ns	
MS, KI		Maltol							
	RT	238	±84	501	±21	275	±39	272	±27	225	±13
	4 °C	238	±84	499	±146	226	±57	362	±68	151	±35
	SL			ns		ns		ns		ns	
MS		2-Methyl-6-acetylpyrazine							
	RT	136	±48	237	±32	62	±42	151	±9	122	±8
	4 °C	136	±48	225	±39	43	±5	154	±29	94	±5
	SL			ns		ns		ns		**	
MS		5-Methyl-6,7-dihydrocyclopentapyrazine							
	RT	39	±16	51	±7	33	±6	22	±0	21	±8
	4 °C	39	±16	60	±25	26	±17	39	±10	23	±5
	SL			ns		ns		ns		ns	
MS, KI		3,5-Diethyl-2-methylpyrazine							
	RT	41	±10	102	±3	58	±21	53	±16	37	±6
	4 °C	41	±10	88	±30	37	±8	60	±27	30	±17
	SL			ns		ns		ns		ns	
MS, KI		2-(2-Furylmethyl)-5-methylfuran							
	RT	31	±10	49	±7	39	±10	34	±10	30	±10
	4 °C	31	±10	38	±10	35	±1	28	±5	20	±11
	SL			ns		ns		ns		ns	
MS, KI		1-Furfurylpyrrole							
	RT	69	±16	99	±7	98	±28	79	±17	64	±18
	4 °C	69	±16	88	±21	62	±3	68	±8	50	±29
	SL			ns		ns		ns		ns	
MS		Flamenol							
	RT	93	±24	88	±13	112	±43	81	±38	64	±26
	4 °C	93	±24	98	±38	56	±2	75	±28	60	±35
	SL			ns		ns		ns		ns	
MS, KI		Dodecane							
	RT	0		0		0		33	±4	35	±10
	4 °C	0		0		0		0		0	
	SL							**		*	
MS, KI		4-Ethylguaiacol							
	RT	30	±11	43	±4	37	±12	21	±14	31	±17
	4 °C	30	±11	31	±25	29	±11	34	±28	23	±14
	SL			ns		ns		ns		ns	
MS, KI		Difurfuryl ether							
	RT	13	±3	47	±10	27	±7	23	±7	15	±1
	4 °C	13	±3	35	±14	19	±6	28	±13	10	±4
	SL			ns		ns		ns		ns	
MS, KI		2-Methoxy-4-vinylphenol							
	RT	111	±47	208	±19	145	±37	114	±23	98	±6
	4 °C	111	±47	225	±88	76	±36	177	±67	83	±28
	SL			ns		ns		ns		ns	

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.

d. Evolution of the area (× 10^3) of the volatile compounds identified in the roasted coffee beans of samples originating in **Ethiopia** throughout storage at room temperature (RT) and in the refrigerator (4°C)a

		Storage time				
IM	t	week 0	week 2	week 6	week 10	week 15
MS		Acetone				
	RT	212 ±25	969 ±44	811 ±37	690 ±27	629 ±23
	4°C	212 ±25	1224 ±122	906 ±20	882 ±6	876 ±14
	SL		*	*	**	***
MS		Butanal				
	RT	130 ±14	572 ±20	462 ±26	381 ±27	507 ±188
	4°C	130 ±14	564 ±42	533 ±14	451 ±19	406 ±31
	SL		ns	*	ns	ns
MS		2,3-Butanedione				
	RT	74 ±27	118 ±23	159 ±6	154 ±8	127 ±16
	4°C	74 ±27	217 ±161	228 ±19	148 ±110	233 ±9
	SL		ns	*	ns	**
MS		2-Butanone				
	RT	114 ±20	44 ±20	97 ±9	82 ±5	66 ±6
	4°C	114 ±20	169 ±19	152 ±15	117 ±20	119 ±10
	SL		*	**	ns	*
MS		2-Methylfuran				
	RT	118 ±9	204 ±111	282 ±21	240 ±16	169 ±14
	4°C	118 ±9	339 ±46	357 ±25	208 ±35	245 ±53
	SL		ns	*	ns	ns
MS		Acetic acid				
	RT	745 ±418	824 ±248	342 ±251	303 ±189	242 ±75
	4°C	745 ±418	45 ±14	426 ±164	76 ±41	44 ±25
	SL		*	ns	ns	*
MS		3-Methylbutanal				
	RT	446 ±19	458 ±27	369 ±35	292 ±25	204 ±9
	4°C	446 ±19	490 ±19	434 ±33	319 ±10	289 ±8
	SL		**	ns	ns	**
MS		2-Methylbutanal				
	RT	1832 ±104	1373 ±124	1085 ±128	986 ±114	823 ±41
	4°C	1832 ±104	1557 ±105	1329 ±152	1087 ±50	1034 ±38
	SL		ns	*	ns	*
MS		2,3-Pentanedione				
	RT	570 ±31	602 ±16	423 ±59	392 ±74	264 ±39
	4°C	570 ±31	597 ±41	559 ±33	400 ±42	400 ±24
	SL		ns	*	ns	**
MS		2,5-Dimethylfuran				
	RT	73 ±10	66 ±12	38 ±24	33 ±27	21 ±5
	4°C	73 ±10	49 ±6	51 ±5	34 ±1	31 ±7
	SL		ns	ns	ns	*
MS		Acetoin				
	RT	622 ±537	226 ±35	136 ±94	141 ±87	129 ±11
	4°C	622 ±537	345 ±26	262 ±44	206 ±30	218 ±28
	SL		*	ns	ns	ns
MS, KI		Pyrazine				
	RT	282 ±22	255 ±24	192 ±27	146 ±31	140 ±14
	4°C	282 ±22	284 ±30	223 ±4	197 ±2	190 ±24
	SL		ns	ns	ns	*
MS, KI		1-Methylpyrrole				
	RT	47 ±19	82 ±56	93 ±12	52 ±40	29 ±8
	4°C	47 ±19	56 ±4	119 ±5	66 ±19	68 ±21
	SL		ns	ns	ns	*

MS, KI		Pyridine													
	RT	7098	±422		3731	±295		2881	±495		2154	±528		2472	±216
	4°C	7098	±422		4459	±377		3394	±51		3217	±113		2915	±125
	SL				ns			ns			ns			ns	
MS, KI		1-Hydroxy-2-butanone													
	RT	137	±35		90	±20		61	±5		64	±24		53	±16
	4°C	137	±35		114	±24		74	±12		91	±40		90	±8
	SL				ns			ns			ns			*	
MS, KI		2,3-Butanediol													
	RT	55	±17		27	±5		3	±1		2	±1		0	
	4°C	55	±17		129	±32		6	±1		40	±4		50	±12
	SL				*			ns			**			*	
MS, KI		4-Methyl-2,3-pentanedione													
	RT	34	±21		60	±2		38	±6		29	±3		17	±2
	4°C	34	±21		61	±1		39	±8		31	±3		33	±6
	SL				ns			ns			ns			ns	
MS, KI		2,3-Butanediol (another isomer)													
	RT	108	±0		61	±15		0			0			0	
	4°C	108	±0		167	±13		0			85	±18		67	±9
	SL				***						*			**	
MS, KI		2-Methyltetrahydrofuran-3-one													
	RT	1154	±133		1092	±85		848	±60		833	±55		683	±43
	4°C	1154	±133		959	±71		1070	±46		661	±60		720	±54
	SL				**			ns			ns			ns	
MS, KI		2-Methylpyrazine													
	RT	3957	±289		3245	±252		2511	±119		1885	±154		1843	±57
	4°C	3957	±289		3682	±418		2559	±112		2597	±146		2234	±146
	SL				ns			ns			*			*	
MS, KI		Furfural													
	RT	1537	±230		1758	±119		1434	±53		1202	±82		964	±125
	4°C	1537	±230		1446	±132		1710	±168		1037	±145		926	±30
	SL				ns			ns			ns			ns	
MS, KI		2-Furanmethanol													
	RT	3765	±413		3393	±280		2588	±654		2285	±293		1965	±215
	4°C	3765	±413		3044	±283		2746	±330		1960	±173		1907	±123
	SL				ns			ns			ns			ns	
MS, KI		Acetoxyacetone													
	RT	1596	±45		1105	±120		925	±270		751	±127		777	±56
	4°C	1596	±45		1303	±163		1120	±145		762	±44		836	±63
	SL				ns			ns			ns			ns	
MS, KI		Furfuryl formate													
	RT	272	±66		207	±20		155	±10		162	±11		180	±24
	4°C	272	±66		172	±5		197	±12		116	±4		193	±7
	SL				ns			**			*			ns	
MS, KI		2,6-Dimethylpyrazine													
	RT	4591	±441		4020	±49		2960	±198		2586	±43		2513	±114
	4°C	4591	±441		4514	±404		3065	±186		2880	±63		2683	±205
	SL				ns			*			*			ns	
MS, KI		2-Ethylpyrazine													
	RT	1773	±173		1721	±65		1287	±117		1001	±88		870	±83
	4°C	1773	±173		1749	±185		1276	±115		1065	±53		1112	±93
	SL				ns			ns			ns			**	
MS, KI		2,3-Dimethylpyrazine													
	RT	534	±51		447	±6		321	±54		282	±11		293	±30
	4°C	534	±51		475	±55		320	±41		302	±46		313	±57
	SL				ns			ns			ns			ns	
MS		2,3-Dimethoxy-1,3-butadiene													
	RT	157	±32		124	±6		66	±45		79	±		73	±22
	4°C	157	±32		108	±22		107	±21		67	±9		76	±28
	SL				ns			ns			ns			ns	

MS, KI		α-Pinene								
	RT	0		0		0		0		0	
	4 °C	0		0		0		0		0	
	SL									
MS, KI		2-Methylcyclohexanone								
	RT	314	±36	282	±22	183	±28	165	±16	135	±13
	4 °C	314	±36	306	±29	226	±9	166	±12	175	±10
	SL			ns		ns		ns		**	
MS		2-Butylfuran								
	RT	60	±20	79	±40	60	±10	43	±17	37	±12
	4 °C	60	±20	75	±11	71	±8	28	±14	35	±7
	SL			ns		ns		ns		ns	
MS, KI		3-Ethylpyridine								
	RT	121	±26	74	±41	52	±20	43	±3	27	±13
	4 °C	121	±26	80	±14	36	±13	33	±16	45	±16
	SL			ns		ns		ns		ns	
MS, KI		5-Methylfurfural								
	RT	1875	±226	2134	±145	1596	±176	1531	±85	1276	±58
	4 °C	1875	±226	1895	±206	1816	±113	1195	±71	1177	±51
	SL			ns		*		ns		*	
MS		1-Acetoxy-2-butanone								
	RT	450	±35	360	±114	247	±28	296	±80	301	±64
	4 °C	450	±35	291	±50	305	±74	211	±72	201	±21
	SL			ns		ns		ns		ns	
MS, KI		β-Pinene								
	RT	0		0		0		0		0	
	4 °C	0		0		0		0		0	
	SL									
MS, KI		2-Furanmethyl acetate								
	RT	1114	±137	1510	±125	1185	±99	1080	±96	905	±86
	4 °C	1114	±137	1349	±192	1361	±145	854	±52	877	±45
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-6-methylpyrazine								
	RT	1161	±124	1251	±28	825	±60	630	±55	755	±61
	4 °C	1161	±124	1243	±137	778	±183	541	±214	753	±81
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-5-methylpyrazine								
	RT	745	±78	848	±20	550	±38	469	±68	483	±40
	4 °C	745	±78	826	±89	543	±109	367	±144	481	±49
	SL			ns		ns		ns		ns	
MS, KI		2,3,5-Trimethylpyrazine								
	RT	635	±80	516	±13	351	±30	300	±64	364	±29
	4 °C	635	±80	651	±90	311	±72	287	±118	411	±58
	SL			ns		ns		ns		ns	
MS, KI		2-Ethyl-3-methylpyrazine								
	RT	386	±34	356	±18	243	±14	205	±52	248	±26
	4 °C	386	±34	400	±102	231	±48	162	±89	275	±32
	SL			ns		ns		ns		*	
MS, KI		2-Formyl-1-methylpyrrole								
	RT	273	±74	417	±44	231	±70	191	±61	226	±24
	4 °C	273	±74	312	±68	277	±99	138	±99	226	±26
	SL			ns		ns		ns		ns	
MS		1-Acetyl-1,4-dihydropyridine								
	RT	83	±46	169	±3	96	±17	73	±3	68	±5
	4 °C	83	±46	174	±30	91	±12	58	±20	80	±14
	SL			ns		ns		ns		ns	
MS		3-Ethyl-1,2-cyclopentanedione								
	RT	34	±22	57	±1	26	±10	27	±4	27	±0
	4 °C	34	±22	0		0		0		0	
	SL			***		*		**		***	

MS, KI		p-Cymene												
	RT	0		0			0			0			0	
	4 °C	0		362	±68		148	±22		108	±25		182	±15
	SL			*			**			*			**	
MS, KI		Limonene												
	RT	151	±13	187	±13		175	±13		162	±5		136	±11
	4 °C	151	±13	295	±58		206	±19		121	±17		180	±21
	SL			ns			ns			ns			*	
MS, KI		2-Acetylpyrrole												
	RT	67	±45	211	±25		124	±19		137	±12		74	±13
	4 °C	67	±45	208	±28		120	±31		116	±19		105	±41
	SL			ns			ns			ns			ns	
MS, KI		cis-Linalool oxide												
	RT	171	±16	221	±6		174	±7		153	±14		128	±20
	4 °C	171	±16	220	±50		176	±10		126	±6		107	±12
	SL			ns			ns			ns			ns	
MS		2-Acetyl-1-methylpyrrole												
	RT	85	±11	105	±6		68	±6		67	±8		58	±3
	4 °C	85	±11	89	±16		73	±14		67	±4		84	±19
	SL			ns			ns			ns			ns	
MS, KI		2-Ethyl-3,6-dimethylpyrazine												
	RT	661	±57	696	±20		474	±13		442	±41		435	±53
	4 °C	661	±57	709	±151		410	±35		413	±11		414	±75
	SL			ns			*			ns			ns	
MS, KI		2-Furfurylfuran												
	RT	134	±30	88	±9		49	±3		58	±18		87	±11
	4 °C	134	±30	88	±37		71	±16		65	±44		85	±14
	SL			ns			ns			ns			ns	
MS, KI		3-Ethyl-2,5-dimethylpyrazine												
	RT	33	±13	119	±9		87	±11		76	±15		24	±5
	4 °C	33	±13	123	±27		58	±1		59	±20		27	±6
	SL			ns			*			ns			ns	
MS, KI		2-Ethyl-3,5-dimethylpyrazine												
	RT	175	±16	244	±20		153	±10		162	±28		133	±15
	4 °C	175	±16	249	±71		165	±20		154	±1		135	±15
	SL			ns			ns			ns			ns	
MS		3,5-Dimethyl-4-allylpyrazole												
	RT	234	±46	245	±12		223	±25		183	±31		179	±14
	4 °C	234	±46	231	±78		195	±25		153	±27		139	±18
	SL			ns			**			ns			**	
MS, KI		3-Ethyl-2-hydroxy-2-cyclopenten-1-one												
	RT	89	±29	128	±5		78	±22		87	±21		77	±4
	4 °C	89	±29	99	±53		95	±14		61	±21		52	±4
	SL			ns			ns			**			*	
MS, KI		Maltol												
	RT	172	±39	310	±14		189	±2		224	±29		144	±22
	4 °C	172	±39	393	±129		154	±10		178	±14		162	±36
	SL			ns			*			ns			ns	
MS		2-Methyl-6-acetylpyrazine												
	RT	130	±24	184	±10		140	±12		130	±12		100	±14
	4 °C	130	±24	222	±50		115	±8		138	±4		108	±23
	SL			ns			**			ns			ns	
MS		5-Methyl-6,7-dihydrocyclopentapyrazine												
	RT	86	±13	89	±33		43	±4		44	±6		40	±9
	4 °C	86	±13	126	±92		44	±11		43	±2		52	±8
	SL			ns			ns			ns			ns	
MS, KI		3,5-Diethyl-2-methylpyrazine												
	RT	84	±11	115	±7		79	±10		66	±21		64	±12
	4 °C	84	±11	128	±33		63	±3		51	±14		67	±8
	SL			ns			ns			ns			ns	

MS, KI		2-(2-Furylmethyl)-5-methylfuran							
	RT	16	±3	21	±4	11	±1	16	±3	12	±3
	4 °C	16	±3	16	±5	15	±4	7	±3	10	±0
	SL			ns		ns		ns		ns	
MS, KI		1-Furfurylpyrrole							
	RT	70	±18	92	±12	57	±2	62	±14	67	±9
	4 °C	70	±18	82	±27	67	±12	50	±7	46	±9
	SL			ns		ns		ns		ns	
MS		Flamenol							
	RT	102	±36	91	±30	44	±2	60	±16	81	±10
	4 °C	102	±36	97	±46	79	±5	61	±18	51	±20
	SL			ns		**		ns		ns	
MS, KI		Dodecane							
	RT	0		0		26	±2	28	±3	29	±7
	4 °C	0		0		0		0		0	
	SL					**		**		*	
MS, KI		4-Ethylguaiacol							
	RT	16	±5	38	±5	12	±6	28	±7	21	±3
	4 °C	16	±5	33	±35	14	±5	13	±6	13	±7
	SL			ns		ns		ns		ns	
MS, KI		Difurfuryl ether							
	RT	10	±0	20	±2	10	±2	15	±2	6	±4
	4 °C	10	±0	24	±12	9	±3	8	±1	9	±3
	SL			ns		ns		*		ns	
MS, KI		2-Methoxy-4-vinylphenol							
	RT	61	±14	94	±11	50	±5	75	±19	51	±16
	4 °C	61	±14	141	±86	37	±12	63	±9	65	±11
	SL			ns		ns		ns		ns	

[a]All values are shown as means ± standard deviations. SL, significance level between the two storage temperatures in the same day: ns, nonsignificant ($p > 0.05$); *, significant ($p < 0.05$); **, very significant ($p < 0.01$); ***, highly significant ($p < 0.001$). IM, identification method used: MS, mass spectrum, KI, Kovats index.