**Universidade de Aveiro
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Departamento de Química

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THE EFFECT OF MICROWAVE EXTRACTION ON THE CHEMICAL COMPOSITION AND THE ANTIOXIDANT PROPERTIES OF THE COFFEE RESIDUE

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos
requisitos necessários à obtenção do grau de Mestre em Biotecnologia
Alimentar, realizada sob a orientação científica do Doutor Manuel António
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resumo   O presente trabalho, pretendeu estudar o efeito da extracção em microondas na composição química do resíduo de café. As analises ao resíduo de café incluíram: conteúdo em açucares e aminoácidos (GC-FID), conteúdo em gordura (extracção em Soxhlet), composição volátil do óleo do resíduo de café (GCxGC), e capacidade antioxidante (espectrofotometria) do óleo, supernadantes, e resíduos após as extracções com microondas.

As extracções com microondas foram efectuadas em ciclos de 8 minutos atingindo uma temperatura de 200℃. Ambos os supern adantes e resíduos for examinados pelo seu rendimento em massa, conteúdo e composição em polissacarídeos e aminoácidos, conteúdo em óleo e capacidade antioxidante. Os rendimentos obtidos por extracção com microondas atingiram 20-25% em massa, da qual cerca de 41-63% são açúcares solúveis em água. A composição em polissacarídeos alterou-se do primeiro para o segundo ciclo de extracção, sendo que as arabinogalactanas são preferencialmente extraídas durante o primeiro ciclo, enquanto que no segundo ciclo se obtém uma mistura de arabinogalactanas e galactomananas.

O resíduo de café insolúvel (79 %, w/w) continua rico em polissacarideos (46-55% w/w), sendo enriquecido em glucose ao longo dos ciclos.

A maioria dos aminoácidos continuam no resíduo, mesmo após os tratamentos com microondas. Também a composição permanece inalterada, com excepção para as percentagens dos ácidos glutâmico e aspártico, cuja quantidade aumenta significativamente após os tratamentos.

A maioria do óleo remanesce no resíduo após os tratamentos. Uma elevada capacidade antioxidante (AOC) foi encontrada no óleo extraído do resíduo de café inicial, supernadantes, e extractos de resíduo finais. O óleo extraído após o primeiro ciclo de extracção apresenta uma diminuição da sua AOC quando comparada com a actividade do resíduo inicial, sendo detectada uma redução considerável após o segundo ciclo, ainda assim com valores superiores quando comparados com os valores de AOC apresentados por amostras comerciais de óleo de azeitona.

Todos os supernadantes apresentam uma AOC superior a qualquer um dos resíduos, incluindo o resíduo inicial e os resíduos obtidos após os vários tratamentos com microondas. Quando comparados com o resíduo inicial de café antes de qualquer tratamento com microondas, ao qual corresponde uma actividade de 15 g_{soluto}/ $L_{solução}$, o supernadante obtido após o primeiro ciclo de extracção apresentou um AOC de 5 g_{soluto} /$L_{solução}$, reduzindo a sua actividade para metade após o segundo ciclo.

Foram identificados ~170 compostos no espaço de cabeça do óleo obtido a partir do resíduo de café, incluindo furanos e pirazinas, compostos identificados como sendo responsáveis pelo aroma semelhante a café.

Ainda no espaço de cabeça do óleo foram identificados novos compostos os quais são: 1-metil-ciclopentanol; fenilacetaldeído; propanoato de 2-furano metanol; acetato de 2-furano metanol, 2-acetil-3-metilpirazina; 1-etil-1H-pyrrole-2-carboxaldeído; 2-pineno; (1S)-2,6,6-trimetil biciclo(3,1,1)heptano-2-eno; p-xileno;1-fenil-etanona; 5-metil-2(3H)-furanona.

abstract

The present work, studied the effect of microwave extraction on the chemical composition of the coffee residue. The analysis of the coffee residue included: sugars and amino-acids content (by GC-FID), fat content (by Soxhlet extraction), coffee residue oil volatile composition (GCxGC), and the antioxidant capacity (by spectrophotometry) of the oil, supernatants, and residues after microwave extractions.

The microwave extractions were performed in two 8-minutes cycles reaching 200°C. Both supernatants and residues were examined for their mass yield, polysaccharides and amino acids content and composition, fat content and antioxidant capacity.

The microwave extraction yields about 20-25% of mass, of which 22-38% are water soluble sugars. The polysaccharides composition changes from the first to the second cycle, being the arabinogalactans preferentially extracted during the first microwave cycle, and a mixture of arabinogalactans and galactomannans extracted to supernatant during the second cycle.

The insoluble coffee residue (79 %, w/w) remains rich in polysaccharides (20-39%, w/w), being enriched in glucose over the cycles.

Most of the amino acids remain in the residues, even after the microwave treatments. The composition remains almost unchanged, with the exception for the percentage of glutamic and aspartic acid, which rise significantly after the treatments.

Most of the oil is also maintained in the residue after the treatments. The oil extracted from the coffee initial residue, supernatant, and final residue extracts, all have high antioxidant capacity (AOC). The oil obtained after the first cycle of microwave extraction slightly lowered its AOC when compared to the initial activity, while a considerably reduction was detected after the second cycle, which is still higher that the AOC showed by commercial olive oil samples.

The supernatants all have higher AOC when compared to the residues, both the initial as the ones after the microwave treatments. When compared to the initial residue, which correspondent EC_{50} is 15 $g_{solute}/L_{solution}$ before microwave extraction, the supernatant after the first microwave extraction cycle has an AOC of 5 $g_{solute}/L_{solution}$, reducing its activity to half after the second microwave cycle.

In the headspace of the oil obtained from the coffee residue ~170 compounds were identified, including furans and pyrazines, which are compounds identified as being responsible for the coffee-like aroma. Among the new compounds, which were found in head-space of coffee oil are: 1-methyl-cyclopentanol; phenyl acetaldehyde; 2-furanmethanol, propanoate; 2-furanmethanol, acetate; 2-acetyl-3-methylpyrazine; 1-ethyl-1H-pyrrole-2carboxaldehyde; 2-pinene; (1S)-2,6,6-trimethylbicyclo(3,1,1)hept-2-ene; p-xylene; 1-phenyl-ethanone; 5-methyl-2(3H)-furanone.

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Abbreviation / Nomenclature

[DPPH]	[g/L]	DPPH concentration
1C MW		first cycle of microwave extraction
2C MW		second cycle of microwave extraction
AA		amino acids
AOC		antioxidant capacity
Ara		arabinose
Arg		arginine
Asp		aspartic acid
AV		*Aloe vera*
DPPH		free radical 2,2-diphenyl-1-picrylhydrazyl
EC$_{50}$	$\left[\dfrac{g_{solute}}{L_{solution}}\right]$	concentration of a compound required for 50% reduction of DPPH initial free radical concentration
Gal		galactose
GC		gas chromatography
Glc		glucose
Glu		glutamic acid
Gly		glycine
Ile		osileucine
IS		iInternal standard
Leu		leucine
LLE		liquid-liquid extraction
Lys		lysine
M		molecular mass
MAE		microwave-assisted extraction
Man		mannose
MW		microwave
Phe		phenylalanine
Pro		proline
R		residue
Ser		serine

Sn		supernatant
SPE		solid phase extraction
T	[°C]	temperature
t	[s]	time
Tyr		tyrosine
Val		valine
X_{DPPH}		conversion of DPPH

1. Overview / Introduction

Ever since its introduction in Europe in the second half of the sixteen century, coffee beans have been steadily growing in importance, due to their diffusion first as an expensive beverage in coffee houses and then as a common household item [1]. Nowadays, coffee is one of the most widely consumed beverages. According to the International Coffee Organization, 35.6 million bags of coffee were exported worldwide during the 5 months of October 2009 – February 2010, 7.1 million bags being exported just in February 2010 [2]. Among all of the known species, the most important are *Coffae arabica* (Arabica) and *Coffae canephora* (Robusta), which account, respectively, for about 75 and 24% of the total world coffee production; other (more than 90 species) account for only 1% of the total world coffee production [3; 4; 5].

Taking into consideration the huge amount of coffee residue produced all over the world, the reutilisation is of significant matter. It was suggested to use coffee residue as fertiliser, fuel [6] or an antioxidant material source [7], but these strategies have not been routinely implemented [8]. As the coffee residue is rich in polysaccharides with immunostimulatory activity, several strategies for its valorisation have now been considered [8]. Those polysaccharides can be used as a nutritional supplement in many products, such as in diary industry (yogurt, milk and functional beverages); breakfast cereals, biscuits, bread, and snacks; soft drinks, and mineral and prong water, as well as coffee beverages with higher and optimised content in functional polysaccharides [8].

The aim of this work was to define the coffee residue composition and the effects of the microwave extraction on it, as well as on the antioxidant properties. The content of sugars, proteins and fat was analysed before and after the microwave extraction both in supernatants and residues. The antioxidant properties were examined in the residues (fat and defatted), the supernatants and the oils, before and after the microwave extraction. The volatile composition of the coffee oil (before any microwave treatment) was also studied.

1.1. General coffee composition

The chemical composition of coffee brew is strongly influenced by the composition of the green bean, the roasting procedure, and the extraction conditions. Green coffee

beans are rich in carbohydrates, proteins, and phenolic compounds. Table 1 shows the general constituents of the two major coffee species: *Arabica* and *Robusta*.

Table 1. Average composition of green coffee (% dry matter) [9].

Constituent	Arabica	Robusta
carbohydrates	58.9	60.8
lipids	16.0	10.0
total amino acids	10.3	10.3
– of which free	0.5	0.8
chlorogenic acids	6.5	10.0
minerals	4.2	4.4
aliphatic acids	1.7	1.6
caffeine and purines	1.2	2.2
trigonelline	1.0	0.7
glycosides	0.2	traces

Coffee composition is changing; from the green bean, where the differences are mainly caused by its origin, through the roasting process, which can have different degrees and diverse conditions. During the roasting process the Maillard reactions take place between amino acids/proteins and reducing sugars, which results in the formation of flavour and coloured compounds [10]. These changes in the main groups of coffee compounds in two Arabica coffees can be seen in table 2.

Table 2. Chemical composition of HMWM of green and roasted Arabica coffees. [a] as % of dry and defatted coffee weight. [b] as % of HMWM weight. [c] anhydrosugar. [d] %N x 6.25. [e] as 3-CQA equivalents by the prussian blue method. HMWM – high molecular weight material [11]

coffee		HMWM[a]	sugar[b, c]	protein[b, d]	phenolics[b, e]
Costa Rica	green	7.56	15.0	43.7	6.8
	roasted	7.62	31.4	9.7	32.7
Brazil	green	0.05	18.6	34.3	10.5
	roasted	8.51	29.3	8.9	42.7

1.2. Microwave extraction

Among many extraction methods, hot compressed water is one of the most environmentally friendly physicochemical methods to hydrolyze biomass [12]. Microwave heating is a heating source that may be used to produce hot compressed water [13]. Microwave heating has been widely applied in organic chemistry [14; 15]. A continuous microwave system [16] has the potential to be applied to a larger semi-industrial scale process [13].

Microwave-assisted extraction (MAE) can extract nutraceutical products from plant sources in a faster manner than conventional solid-liquid extraction. The most important advantages of this micro-assisted reactions are the enhanced selectivity, much improved reaction rates, milder reaction conditions, and the formation of cleaner products with higher yields and minor wastes [17; 18; 19].

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Domestic and industrial microwaves generally operate at 2.45 GHz, and occasionally at 0.915 GHz in USA and at 0.896 GHz in Europe. Microwaves are transmitted as waves, which can penetrate biomaterials and interact with polar molecules such as water in biomaterials to create heat. Therefore, microwaves can heat a whole material with simultaneously depth penetration [20].

There are two types of commercially available MAE systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure. The pressure in the vessel essentially depends on the volume and the boiling point of the solvents. As MAE depends on the dielectric susceptibility of solvent and matrix, better recoveries can be obtained by moistening samples with a substance that possesses a relatively high dielectric constant such as water [20].

MAE offers a rapid delivery of energy to a total volume of solvent and solid plant matrix. Because water inside the plant matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from matrix, improving the recovery of nutraceuticals. The effect of microwave energy is thus strongly dependent on the dielectric susceptibility of both the solvent and the solid plant matrix [20].

The chosen solvent should possess a high dielectric constant and strongly absorb microwave energy. Solvents such as ethanol, methanol and water are sufficiently polar to be heated by microwave energy [21]. During extraction, the solvent volume must be adequate to ensure that the solid matrix is completely immersed [22]. Temperature is an important factor contributing to the recovery yield. Generally, the higher temperature of microwave-extraction is applied, the higher recovery yield is obtained. However, for the extraction of thermolabile compounds, high temperatures may cause the degradation of extracts [20].

The particle sizes of extracted materials are usually in the range of 100 μm – 2 mm [23; 20]. Well-chosen power can improve the extraction because the limiting step of the extraction is frequently the dispersion of substances out of the plant matrix. Consequently, larger surface area of a fine power provides contact between the plant matrix and the solvent [20].

1.3. Carbohydrates content

The polysaccharides present in coffee are responsible for many important properties of coffee infusions, such as the retention of coffee volatile substances [24; 11; 25; 8]. They also contribute to the organoleptic characteristics of the coffee brew, the viscosity and stability of espresso coffee foam, and thus the creamy sensation in the mouth, called 'body' [24; 26; 9; 25]. Recently, it has been shown that polysaccharides have beneficial biological activities. They undergo rapid fermentation in the human colon and in this way they contribute to the physiological effects generally associated with fibre fermentation [27; 28; 29; 8].

Coffee mannans are structurally similar to the bioactive acetylated mannans from *Aloe vera* (AV) [8]. The acetylated mannans extracted from AV have several beneficial biological activities, such as the decrease of cholesterol levels in mammals, macrophage activation and stimulation of T-lymphocyte cells, and action against infectious and tumour diseases. The structure similarity of coffee and AV mannans seem to be the cause of the possible immunostimulatory activity of coffee polysaccharides [8]. These mannans have also prebiotic activity and dietary fibre properties. Due to their indigestibility and high molecular weight, mannans are not expected to be absorbed by the gut, but can stimulate the mucosal immune system, as has been reported for the nondigestible oligosaccharides. In the human colon, coffee mannans undergo rapid fermentation producing mannooligosaccharides and short chain fatty acids (SCFAs). Nevertheless, dietary fibre fermentation is known to improve mineral absorption, produce several products that allow reducing gut infections, increase the levels of intestinal SCFAs, suppression of colon cancer initiation, and alleviation of constipation [30; 31; 32; 33].

In green coffee bean the majority are carbohydrates (60%), lipids (13%), amino acids (10%) and chlorogenic acid (8%) [9]. Carbohydrates are present in green coffee both

as soluble and as insoluble polysaccharides (polymers composed of mannose, galactose, glucose, and with some arabinose) (40-50% db), oligosaccharides (5-10% db), mainly sucrose, and traces of reducing sugars [9].

The water-soluble polysaccharides extracted from ground roasted coffee are a major component of the soluble material present in coffee brews [34]. The monosaccharides composition (on dry matter, db) for both *Arabica* and *Robusta* species is presented in table 3, where the major monosaccharides are mannose and galactose.

Table 3. Difference in the water-soluble polysaccharides composition from roasted beans according to the coffee specie: Arabica and Robusta. carbohydrates (% of monosaccharide on dry matter) [9].

Sugar	Arabica	Robusta
Total	43.9	47.3
Mannose (Man)	22.0	22.0
Galactose (Gal)	11.0	13.1
Glucose (Glc)	7.2	8.2
Arabinose (Ara)	3.7	4.0

Arabinogalactans, polysaccharides consisting of arabinose and galactose composing units, are the major group of polysaccharides extracted with hot water from green coffee beans [11; 25]. During the roasting process the increase in temperature increases the amount of the coffee polysaccharides extracted [35; 11]. The main polysaccharides found in the roasted coffees are galactomannans (69%), polysaccharides consisting of a mannose backbone with galactose, and type II arabinogalactans (28%) [11; 36].

The coffee residue resultant from coffee beverages preparation is composed by the materials that were not extracted with hot water. The structural features of galactomannans and mannans in the residue (eg. decreasing branching) are also distinct [37]. The sugar analysis of coffee residue (table 4) shows that mannose (57%) is the main sugar in the residue, followed by galactose (26%), glucose (11%) and arabinose (6%) [8].

Table 4. Sugar composition of Brazilian Arabica coffee residue [8].

Sample	sugar composition (% mol)				total sugars (%)
	Ara	Man	Gal	Glc	
Coffee residue	6	57	26	11	35

$\beta-1 \rightarrow 4$ linked polysaccharides have low solubility in water, due to intramolecular hydrogen linkages. The increase of the degree of polymerisation increases their

insolubility, whereas the increase of the number of branching residues or acetyl groups in those polymer decreases the insolubility [8; 38].

The composition of galactomannans presenting immunostimulatory activity, those extracted to the coffee infusion and recovered from the coffee residue, are shown and compared in table 5 [38].

Table 5. Carbohydrate composition of Brazil Arabica coffee infusion and residue with presenting immunostimulatory activity. [a] values from Nunes et al. [36]; [b] total molar percentage obtained by methylation analysis; [c] values are the molar percentage obtained by sugar analysis [38].

sugar	coffee infusion [a] (mol%)		coffee residue (mol %)	
Ara	0.6[b]	2[c]	7.6	6
Man	91.8	89	69.1	69
Gal	6.1	7	22.0	23
Glc	1.8	2	1.2	2

1.4. Amino acids content

Amino acids together with carbohydrates are important, because they are colour and aroma precursors in the Maillard reaction during roasting process. Green coffee beans contain 8.7-12.2 % (w/w) proteins and can be divided into the water-soluble (50 %) and the water-insoluble proteins (50 %). The water-soluble coffee proteins end up in the brew, whereas the other half end up in the residue [9].

Recent studies reported that the total free amino acid concentration does not change significantly with the chemical reactions occurring during drying, fermentation, and storage. However, during the roasting process, the minor free amino acid fraction is degraded, and only vestigial amounts are present in the roasted coffee bean and brew [5].

The high temperatures (~200°C) achieved during roasting process are expected to have an effect on the proteins present in coffees. The protein content changes from 12 % in the green beans to 10 % after roasting, which means that during the roasting process 21 % of all proteins is lost (taking the weight loss of the beans into account). The content of arginine (-93 %, w/w), lysine (-87 %), serine (-58 %), threonine (-36 %), histidine (-35 %), and asparagins/aspartic acid (-24 %) decrease during roasting, which is connected to their hydrophility [10].

In experiments with fresh beans and imitation of dry processing procedure, the quality of the peptides remained practically unchanged, while the composition changed

significantly [39]. Protein content in green (12%) and roasted (10.1%) coffee is shown in table 6.

Table 6. Protein content in different Arabica coffee fractions, on dry basis [10].

coffee sample	protein (%, w/w)
green bean	12.0
roasted beans	10.1
brew	10.1

Changes in the amino acids composition in different fractions of coffee: green bean, roasted bean and brew are shown in table 7. Although half of the proteins in green beans are water soluble, only 10 % of the proteins present in the roasted beans are extracted into the brew. This occurs because proteins become less water soluble due to chemical reactions occurring during roasting process. Part of the proteins are also degraded, and their amino acids converted into aroma compounds [10].

Table 7. Amino acid composition (mol %) of Arabica coffee fractions. [a] nd = not determined. [b] total amount of amino acids in % (w/w) [10].

AA	green bean	roasted bean	brew
Ala	7	9	8
Cys	nd [a]	nd	nd
Asx	10	10	10
Glx	18	22	29
Phe	4	5	4
Gly	11	12	13
His	2	2	2
Ile	4	5	4
Lys	6	1	0
Leu	9	11	9
Met	1	1	1
Pro	6	7	8
Arg	4	0	0
Ser	5	3	3
Thr	4	3	2
Val	6	8	5
Trp	nd	nd	nd
Tyr	2	2	2
total % (w/w) [b]	**12.0**	**10.1**	**6.4**

1.5. Oil content

Lipids consist of a wide group of compounds, most of which are not soluble in water. Lipids commonly are divided into oils and fats. Oils refer to lipids that are liquids at

room temperature, while fats refer to these that are solid. The oil is commonly extracted in a Soxhlet apparatus, using *n*-hexane as solvent.

The total lipid content of Arabica is around 15%, but levels up to 17 % have been measured. In Robusta the average is 10 or 11.5 %, but can be as low as 7 %. As for the total oil content in the Arabica varied between 10.8 % and 13.2 % [9; 40]. As it is shown in table 8, the main components of the coffee lipids are the triacylglycerides (~75 %), followed by diterpene fatty acid esters amounting up to 20 % [40]. Other components of the lipid fraction of coffee beans include free fatty acids, sterols, tocopherols and free diterpene esters, among others represented in table 8 [9].

Table 8. Lipid content in green beans, Arabica coffee [41].

Constituent	% total lipids
triacylglycerides	70-80
free fatty acids	0.5-2.7
diterpene esters	15-19
free diterpenes	0.1-1.2
triterpenes, sterols, and sterol esters	1.4-3.2
5-hydroxytryptamides and derivatives	0.3-1.0
tocopherols	0.3-0.7
phosphatides	0.3

Tocopherols, which are present in the coffee oil as minor components, are known to have antioxidant properties [4]. Coffee oil contains also pentacyclic diterpenes as typical lipid constituents which have not been detected in any other food [42]. Quantitatively important diterpenes in coffee oil are cafestol, kahweol and 16-O-methylcafestol (figure 1) [40; 43]. Cafestol and kahweol can be naturally found only in coffee, where they are present in the unsaponifiable lipid fraction [42; 43].

The diterpenes in coffee oil are responsible for various (both positive and negative) physiological effects in humans. The relation between coffee consumption and coronary heart disease is well know. It was postulated that an intake of 10 mg of cafestol and kahweol *per day* raises the serum cholesterol level by 0.05 g/L. Intake of cafestol and kahweol causes an increase in a low-density lipoprotein (LDL) cholesterol, triglycerides, and alanine aminotrasferase (ALT) activity in abnormal subjects [40]. In contrast, it was shown that the diterpenes may increase the activity of glutathione-S-transferase (GST), resulting in an inceased degradation of toxic substances, and protection against aflatoxin B1 induced genotoxicity [40; 43].

R = H : Free Diterpene
R = Fatty acids : Diterpene Esters
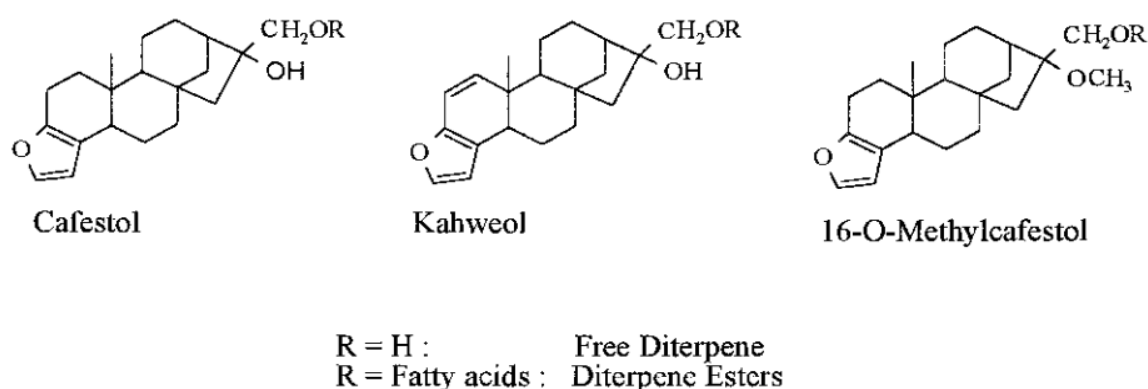
Figure 1. Molecular structural formula of the diterpenes of coffee.

Roasting has little influence on the percentage composition of the diterpene ester fraction [40]. Roasted coffee oil has been widely used as a flavour source in food and cosmetics. Moreover, a reduction in the diterpene levels of roasted coffee oil significantly increases its stability and sensorial profile, decreasing its hypercholesterolemic effect [43]. About 23 % of the diterpene esters present in the powder can be found in the beverage. In espresso coffee there are up to 2.5 % of the initial concentration [40].

1.6. Antioxidant capacity

Antioxidants are substances in foods that significantly decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans [44], but also includes dietary antioxidants, and components that prevent fats from becoming rancid as well. Effective antioxidants are radical scavengers that break down radical chain reactions between oxygen and the substrates [45]. Antioxidants, including polyphenolic compounds, are believed to be a prevention of oxidative stress related diseases [46; 47; 45], like for example: inflammation, cardiovascular disease, neuronal diseases, cancer, aging-related disorders [48; 49; 50; 45].

There are two main types of antioxidant capacity assays: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfers (ET). ET-based assays include the total phenols assay by Folin-Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and DPPH. The

ET-based assays involve one redox reaction with the oxidant as an indicator of the reaction end point. They are based on the following electron transfer reaction [45]:

probe (oxidant) + e (from antioxidant) → reduced probe + oxidized antioxidant
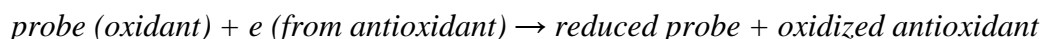
Those assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change is correlated with the sample's antioxidant concentrations. The change of absorbance (ΔABS) is plotted against the antioxidant concentration to give a linear curve [45].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (figure 2) assay is an easy and accurate method to measure the antioxidant capacity of fruit and vegetable juices or extracts [51; 45]. The free radical DPPH has been widely used to test the free radical-scavenging ability of various dietary antioxidant polyphenols [48].

In this work, the free radical-scavenging activities of the samples were evaluated by determining their abilities to chemically reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is one of stable organic nitrogen radicals and has a maximum UV-VIS absorption at 515 nm. Upon reduction, the solution colour fades. The reaction progress is monitored by a spectrophotometer. The antioxidant activity expressed as the amount of antioxidants able to reduce the initial DPPH$^{.}$ concentration to 50 % (EC_{50}), given in terms of moles of antioxidant per mole of DPPH$^{.}$ [48; 45].

1: Diphenylpicryldrazyl (free radical)   2: Diphenylhydrazine (nonradical)
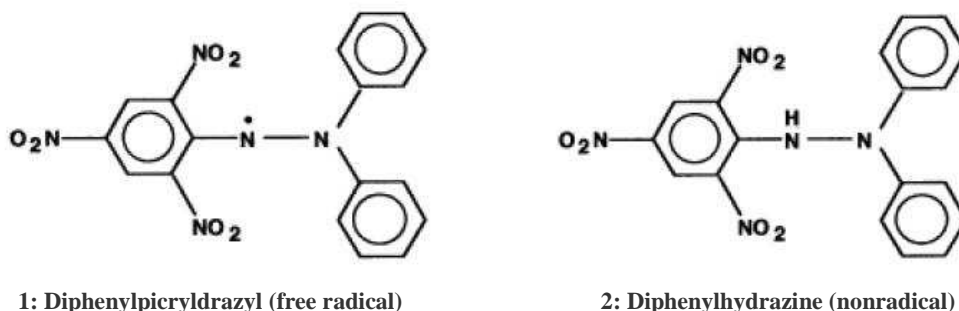
Figure 2. Chemical structure of DPPH [52].

1.7. Volatile compounds

Volatile compounds are responsible for aroma. It might be said that coffee aroma is the most important attribute to specialty coffee. The number of aroma compounds found in coffee increases every year. Today the number is well over 800. The perception of coffee aroma is dependent upon both the concentration of the compound and its odour threshold.

It is probable that a relatively small group of compounds that share both a high concentration and a low odour threshold make up the fragrance we know as coffee aroma.

There are several chemical processes that affect the development of volatile compounds in coffee, such as [9]:

1) Maillard or/and non-enzymatic browning reaction between nitrogen containing substances, amino acids, proteins, as well as trigonelline, serotonine, and carbohydrates, hydroxy-acids and phenols on the other;
2) Strecker degradation;
3) Degradation of individual amino acids, particularly sulfur amino acids, hydroxy amino acids, and proline;
4) Degradation of trigonelline;
5) Degradation of sugar;
6) Degradation of phenolic acids, particularly the quinic acid moiety;
7) Minor lipid degradation;
8) Interaction between intermediate decomposition products.

There are many compounds identified, eg.: 150 aliphatic compounds (inter alia: 56 carbonyl and 9 sulfur containing compounds), 20 alicyclic compounds together with 10 ketones, 60 aromatic benzenoid compounds including 6 phenols; 300 heterocyclic compounds including: 74 furans, 10 hydrofurans, 37 pyrroles, 9 pyridines, 2 quinolines, 70 pyrazines, 10 quinoxalines, 3 indoles, 23 thiophens, 3 thiophenones, 28 thiazoles, 28 oxazoles [53].

The furans are found to be the most predominant group of compounds amongst those contributing to coffee aroma. They typically have caramel-like odors since they result from the pyrolysis of sugars. Furans produce key aroma notes when secondary reactions take place with sulfur containing compounds [9].

The pyrazines are the second most abundant class of aromatic compounds and contribute to the roasted, walnut, cereal, cracker, or toast-like flavors in coffee. Along with thiazoles, the pyrazines have the lowest odor threshold and therefore significantly contribute to the coffee aroma. Next, the pyrroles are responsible for some of the sweet, caramel-like, and mushroom-like aromas in coffee. Conversely, the thiophenes are known to have a meaty aroma and are thought to be produced from Maillard reactions between sulfur containing amino acids and sugars. Thiazoles have an even smaller presence in the

overall aroma and are said to be formed via sugar degradation [9]. Some of aroma compounds found in coffee are shown in table 9.

Table 9. Volatile Arabica coffee compounds identified in the split-flow GC×GC experiment [54].

Peak	Compound	Peak	Compound
1	3-Hexanone	26	1-Hydroxy-2-butanone
2	2,3-Pentanedione	27	3-Ethylpyridine
3	Phenol	28	2-Ethyl-6-methylpyrazine
4	Hexanal	29	2-Ethyl-5-methylpyrazine
5	3-Penten-2-one(E)	30	d,l-Butandiol diacetate
6	Acetyl butyryl	31	Trimethylpyrazine
7	n-Methylpyrrole	32	n-Propylpyrazine
8	3,4-Hexanedione	33	2,6-Diethylpyrazine
9	2-Vinyl-5-methylfuran	34	Tetrahydro-2-furanmethanol
10	Pyridine	35	Vinylpyrazine
11	2,4,5-Trimethyloxazole	36	2,3-Dimethyl-2-cyclopenten-1-one
12	Pyrazine	37	2,5-Dimethyl-3-ethylpyrazine
13	Furan-2-methoxymethyl	38	2,3-Diethylpyrazine
14	3-Methyl-3-buten-1-ol	39	1-Octen-3-ol
15	2-Methylpyrazine	40	2,5-Diethylpyrazine
16	4-Methylthiazole	41	2,6-Dimethyl-3-ethylpyrazine
17	Acetoin	42	Furfural
18	2,3-Octanedione	43	Acetol acetyl
19	3-Methylpyridine	44	2-Furfurylmethylsulfide
20	Acetol	45	2-Butyl-3-methylpyrazine
21	2,5-Dimethylpyrazine	46	2,3-Diethyl-6-methylpyrazine
22	2,6-Dimethylpyrazine	47	2,3,4-Trimethyl-2-cyclopenten-1-one
23	2-Ethylpyrazine	48	Furaneol
24	2,3-Dimethylpyrazine	49	Furfuryl formate
25	2-Methyl-5-cyclopenten-1-one	50	2-Acetylfuran

1.7.1. Comprehensive two-dimensional gas chromatography

As the coffee samples are very complex comprehensive two-dimensional gas chromatography (GCxGC) was performed.

GCxGC employs two orthogonal mechanisms to separate the constituents of the sample within a single analysis. This technique is based on the application of the two GC columns coated with different stationary phases, one apolar and one polar, connected in series through a modulator. This interphase cuts small portions of the first dimension eluted by cryofocusing, and re-injects them onto the second column. Each first dimension peak is modulated several times, which allows the preservation of the first dimension separation. The second column is very short and narrow, and consequently each modulated portion is "flash" separated before the next modulation start [55; 56; 57].

2. Experimental

2.1. General composition

2.1.1. Sample preparation

The coffee residue was collected from local cafeteria. The coffee which was used
was Delta Cafés Platina (LOTE/ BA-1156, with expiring date 08/2011).

2.1.2. H_2O content - humidity

The water content percentage was determined in triplicate by the method of oven
drying at 105 °C till the constant mass (8 h).

2.1.3. Ashes

The ashes content percentage was determined by the method of oven burning at
550°C till the constant mass (8h).

2.2. Microwave extraction

To 20 g of thawed, wet samples 60 mL of water were added and mixed using a
magnetic stirrer. The microwave extraction was carried out at 200°C. Two cycles of
microwave extraction were performed. A first microwave extraction was performed on
coffee residue, which was called the first cycle of microwave extraction. As after the
chemical analysis it occurred that a high percentage of the polysaccharides remained in
residue a second microwave extraction was performed, which was called the second cycle
of microwave extraction. Temperature conditions are showed on the figure 3 (A – target
conditions, B – real conditions). Figure 3 also refers the input power used during the
heating process. The first cycle was performed with 6 reactors, whereas the second cycle
was performed only with 4 reactor, which had influence on the effective temperature plot.
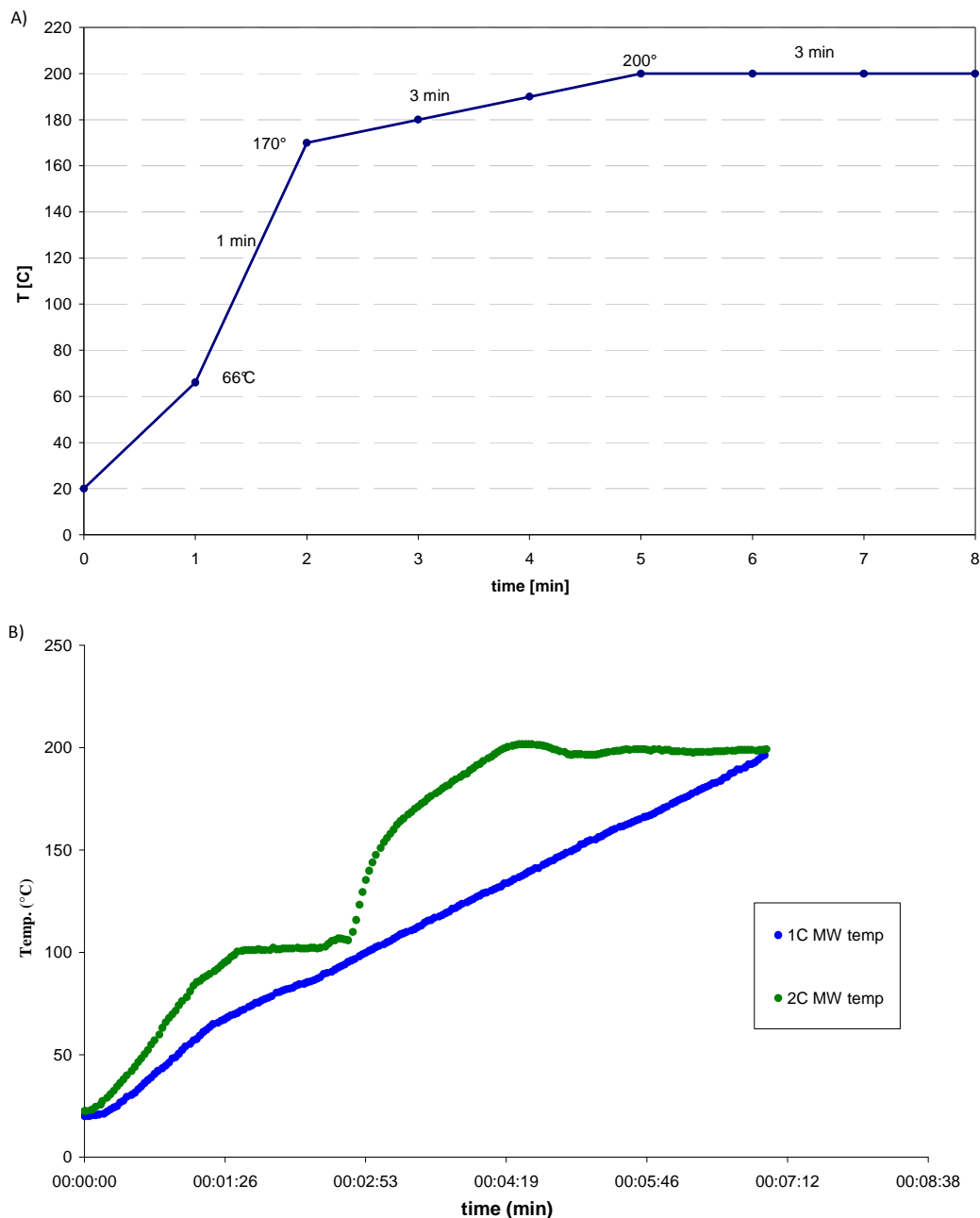
Figure 3. A) Target conditions during microwave extractions. B) The real microwave extraction condition

After the extraction, the resulting samples were centrifuged (15 000 rpm, 4 °C, 20 min). Supernatants were decanted from residues and filtered. Both supernatants and residues were freeze-dried. In order to define content of sugars and amino acids, as well as the antioxidant capacity, further experiments were carried out. Antioxidant capacity was measured by the DPPH method on both supernatant and residues, as well as in the oil and in the defatted residue. The schemes shown below represent the experimental design.
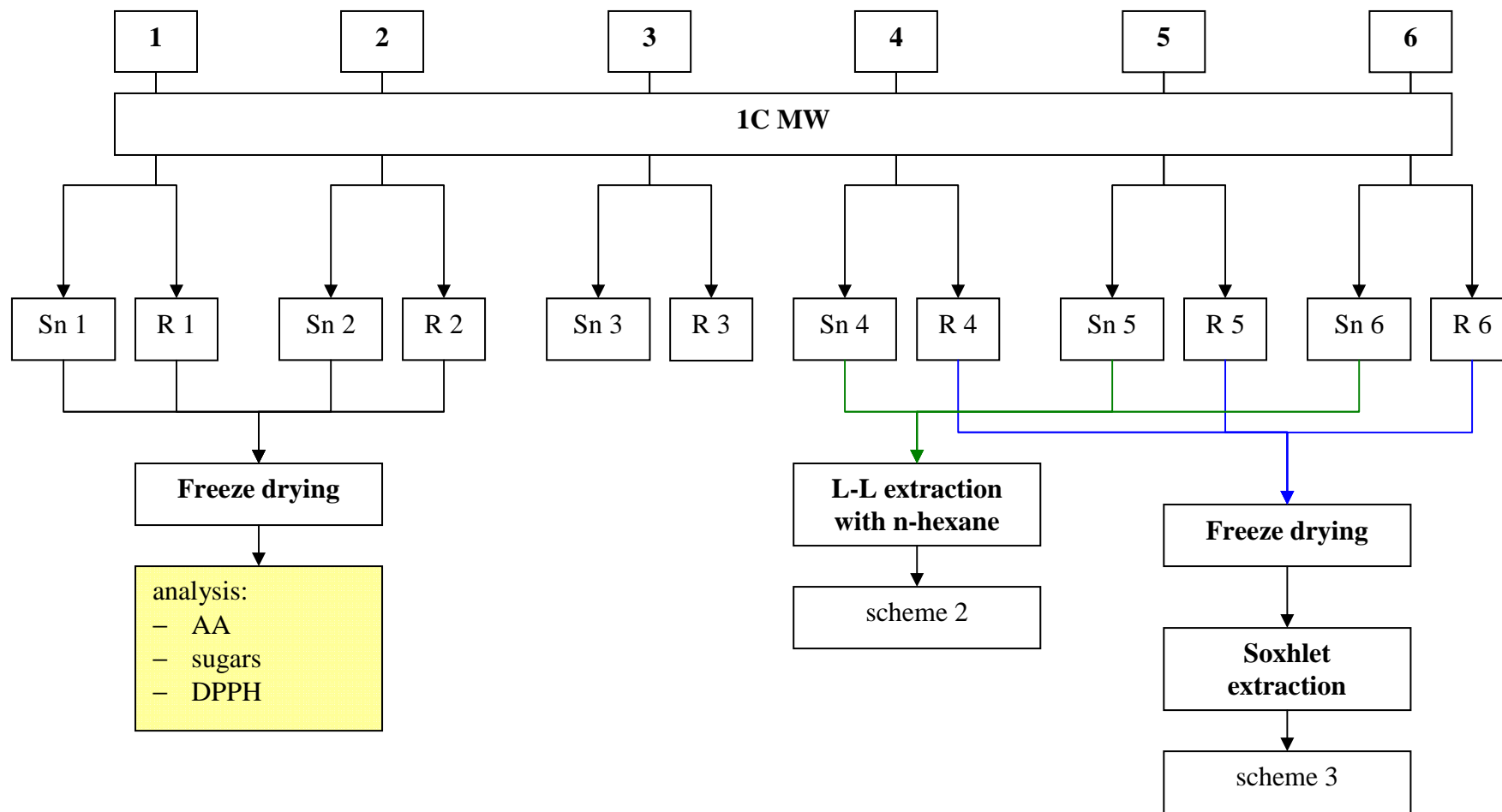
Scheme 1. Microwave extraction 1ˢᵗ cycle. R – residue; Sn – supernatant; 1C MW – first cycle of microwave extraction; L-L – liquid-liquid extraction; AA – amino acids; 1-6 – number of reactor.
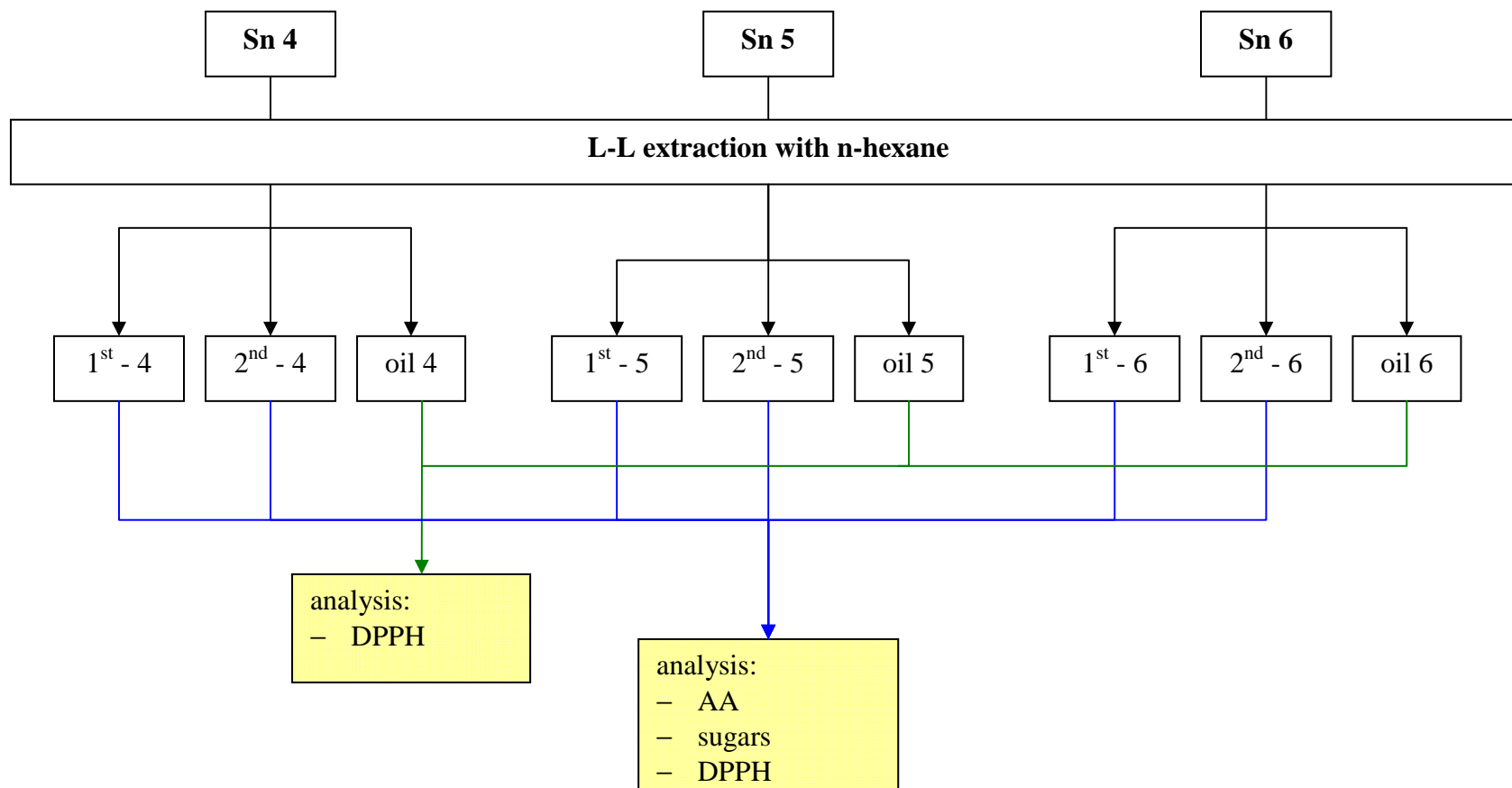
Scheme 2. Liquid-liquid extraction of supernatants after 1st cycle microwave extraction. Sn – supernatant; L-L – liquid-liquid extraction; 1st – aqueous phase after liquid-liquid extraction; 2nd – water-oil interphase after liquid-liquid extraction; AA – amino acids.
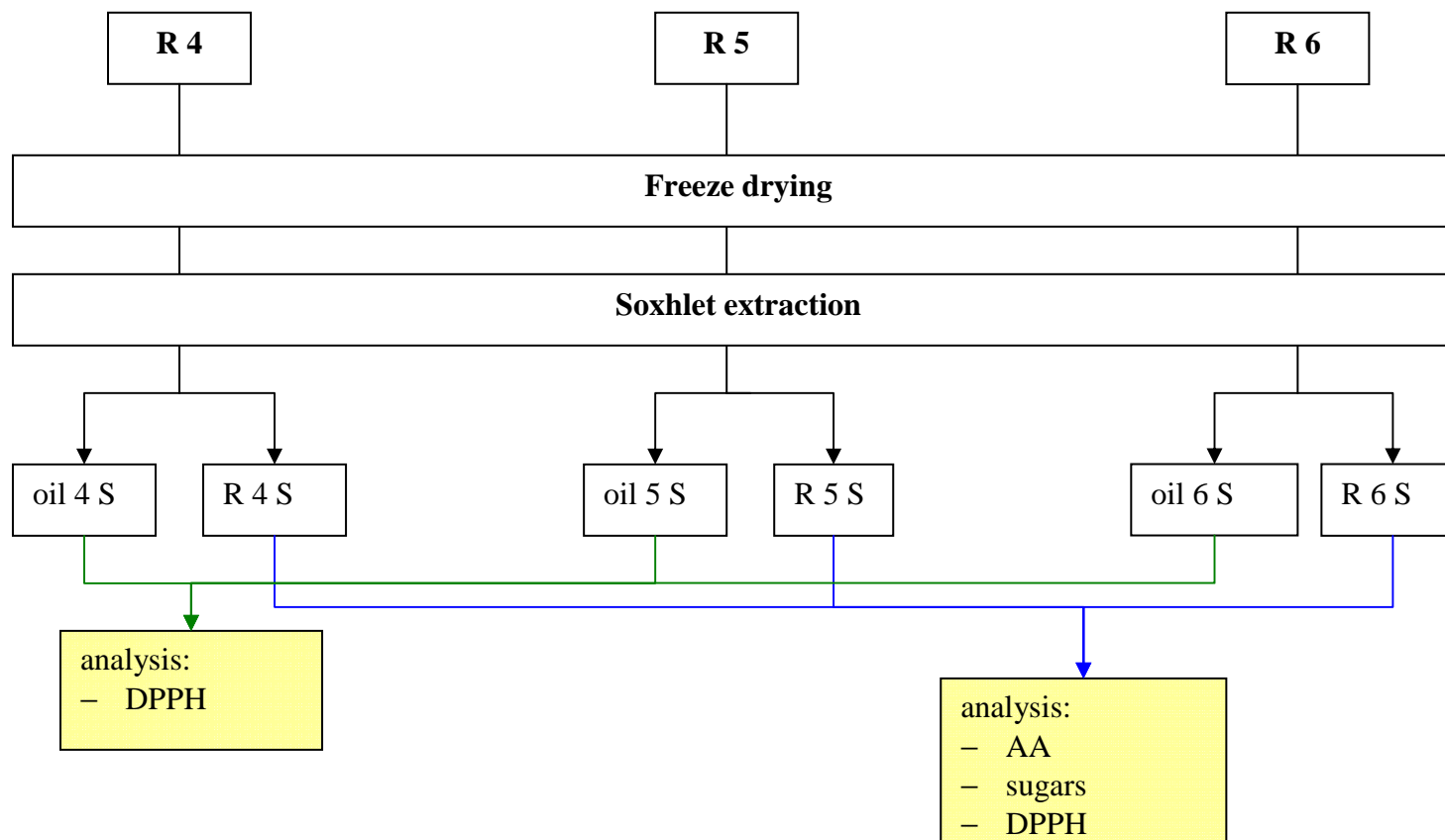
Scheme 3. Freeze drying and Soxhlet extraction of residues after the 1st cycle of microwave extraction. R – residue; R 4 S – R 6 S – defatted residues after Soxhlet extraction; AA – amino acids.
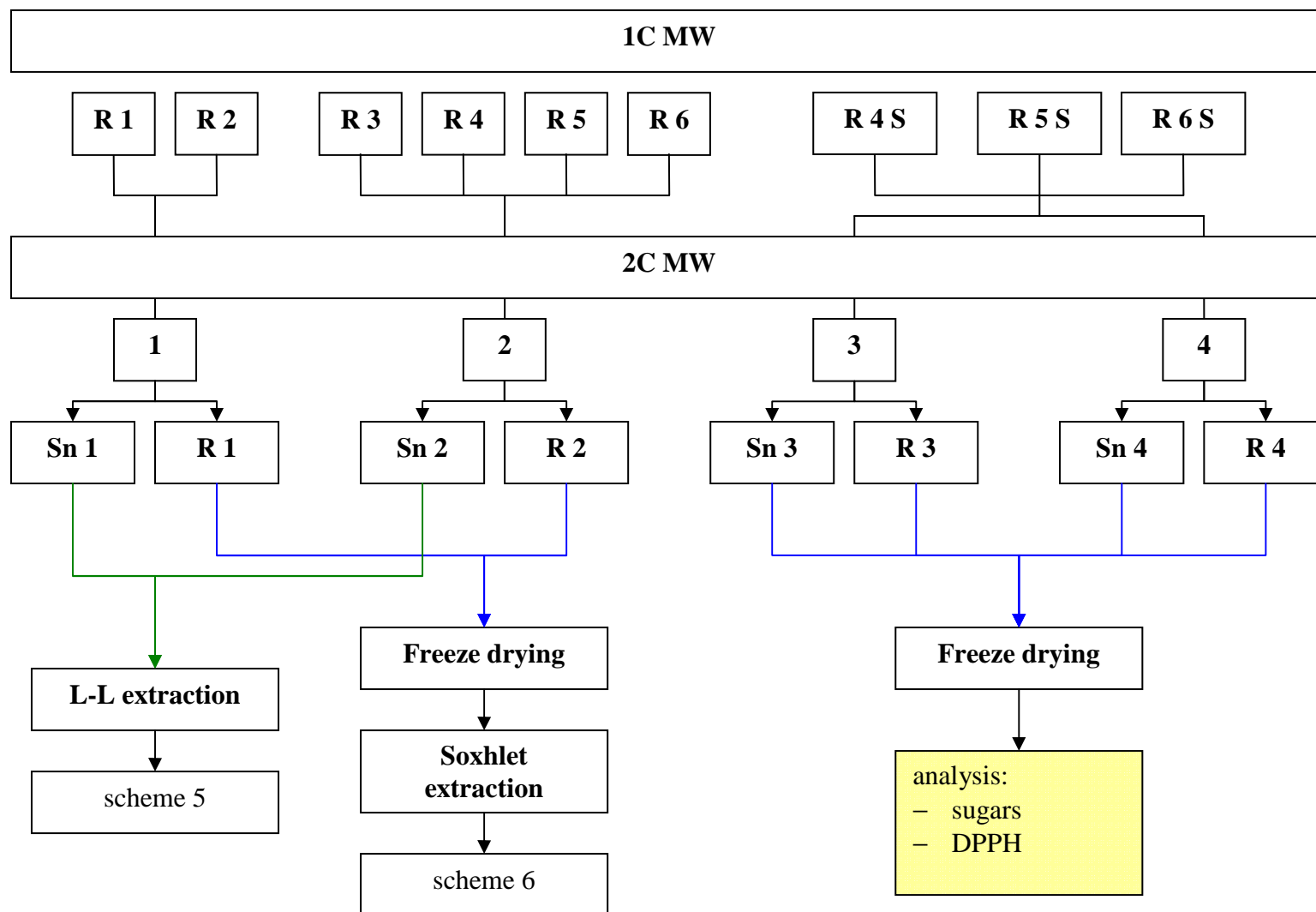
Scheme 4. Microwave extraction, 2nd cycle. R – residue; Sn – supernatant; 1C MW – first cycle of microwave extraction; 2C MW – second cycle of microwave extraction; L-L – liquid-liquid extraction.
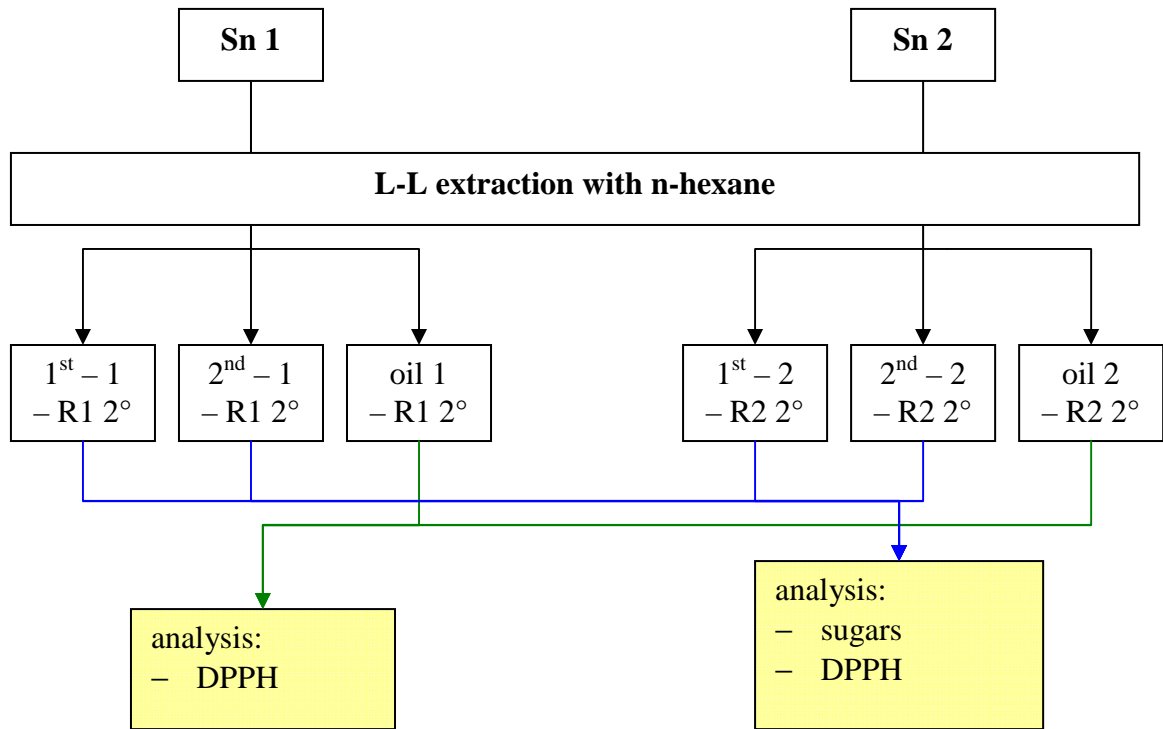
Scheme 5. Liquid-liquid extraction of supernatants after the 2nd cycle of microwave extraction. Sn – supernatant; L-L – liquid-liquid extraction; 1st – aqueous phase after liquid-liquid extraction; 2nd – water-oil interphase after liquid-liquid extraction.
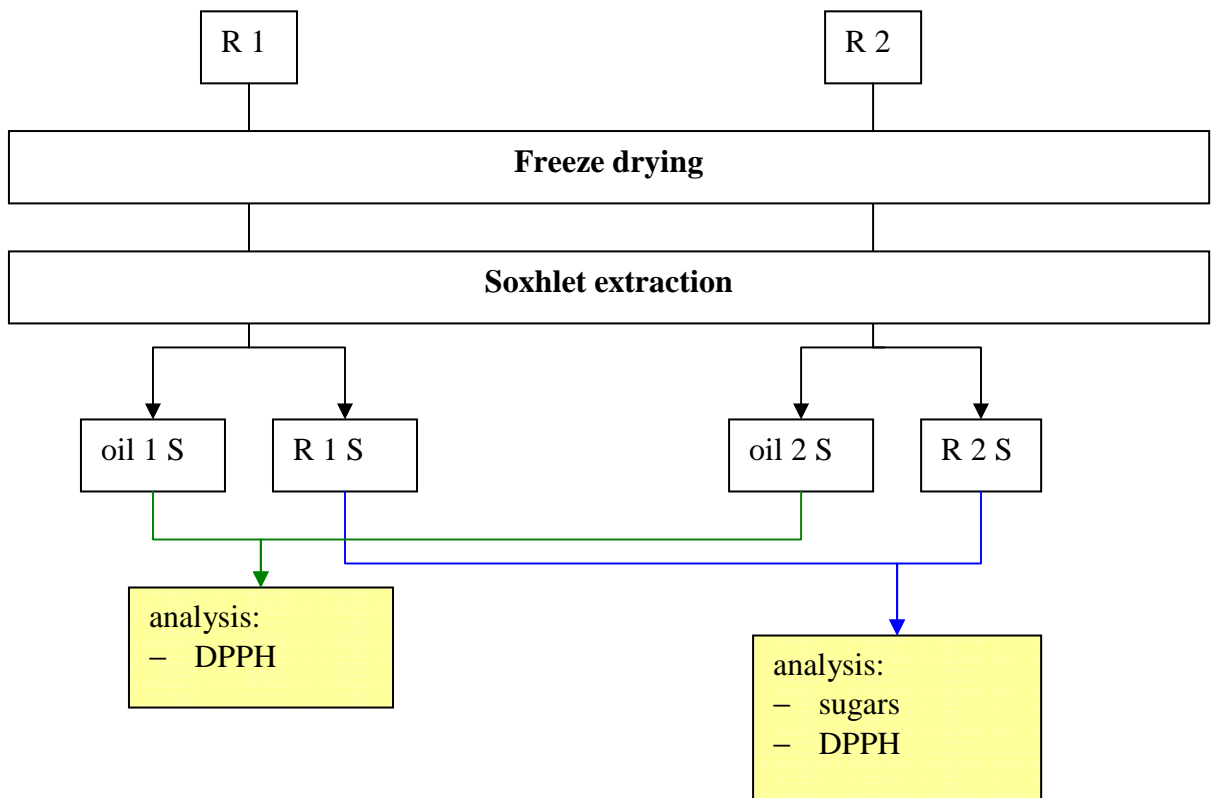
Scheme 6. Liofilisation and Soxhlet extraction of residues after the 2nd cycle of microwave extraction. R – residue; R 1 S; R 2 S – defatted residues after Soxhlet extraction.

2.3. Carbohydrates content

To carry out hydrolysis, about 2 mg of coffee samples were weighted in a 10 mL tubes, and 200 μL of 72 % H_2SO_4 were added [58]. After incubation for 3 h at room temperature with stirring every 30 min, the process was stopped by adding 1.1 mL of distillate water and incubated for another 1 h at 120°C. The tubes were cooled down in an ice bath.

2.3.1. GC-FID analysis

The reduction and acetylation process was conducted. After adding 200 μL of internal standard (2-deoxy-glucose (0.9675 g/L)), 1 mL of sample was transferred to other tube and neutralized with 400 μL NH_3 25 %. 200 μL of 15 % (m/v) $NaBH_4$ in 3 M NH_3 (150 mg $NaBH_4$ for 1 mL 3M NH_3) was added to samples and incubated for 1 h at 30°C. After cooling down the tubes in an ice bath and adding 2x50 μL of acetic acid, 300 μL of sample were transferred to sovirel tubes. Keeping samples in cold, 450 μL of 1-methylimidazole and 3 mL of acetic anhydride were added, mixed on vortex, and incubated for 30 min at 30°C. Later again in an ice bath, 3 mL of distillate water and 2.5 mL of dichloromethane were added. The alditol acetates were extracted to the organic phase by agitating the samples, and later separated by centrifugion (5 min, 4000 rpm, 4°C). Afterwards, the aqueous phase was removed by using the vacuum. Addition of distillate water and dichloromethane, mixing, centrifuging and removing the aqueous phase was repeated. Later the organic phase was washed with 3 mL of distillate water, mixed, and centrifuged, after which the aqueous phase was completely removed. After transferring the organic phase to tubes specific for the *SpeedVac*, the dichloromethane was evaporated. Afterwards, for two times 1 mL of anhydrous acetone was added and evaporated. Reduced and acetylated samples were stored in the close tubes in an anhydrous ambient [59].

The alditol acetates were dissolved in 50 μL of anhydrous acetone. 2 μL were injected in the Gas Chromatograph (GC) with capillary column DB-225 (30 m lenght, 0.25 mm internal diameter, 0.15 μm film thickness). GC analyses were performed with a Clarus 400 Gas Chromatograph equipped with a flame ionization detector and with an HPChemStation acquisition system. The carrier gas (nitrogen) flow rate was 1.2 mL/min. 2 μL of each sample was injected in split mode (split flow 48 mL/min).

Injector temperature was 220°C, detector temperature was 230°C. The temperature program used started at 200°C with the ramp of 40°C/min until 220°C, then held isothermally for 7 min, second ramp of 20°C/min until 230°C and finally held isothermally for 1 min performing a total 9 min program (figure 4).
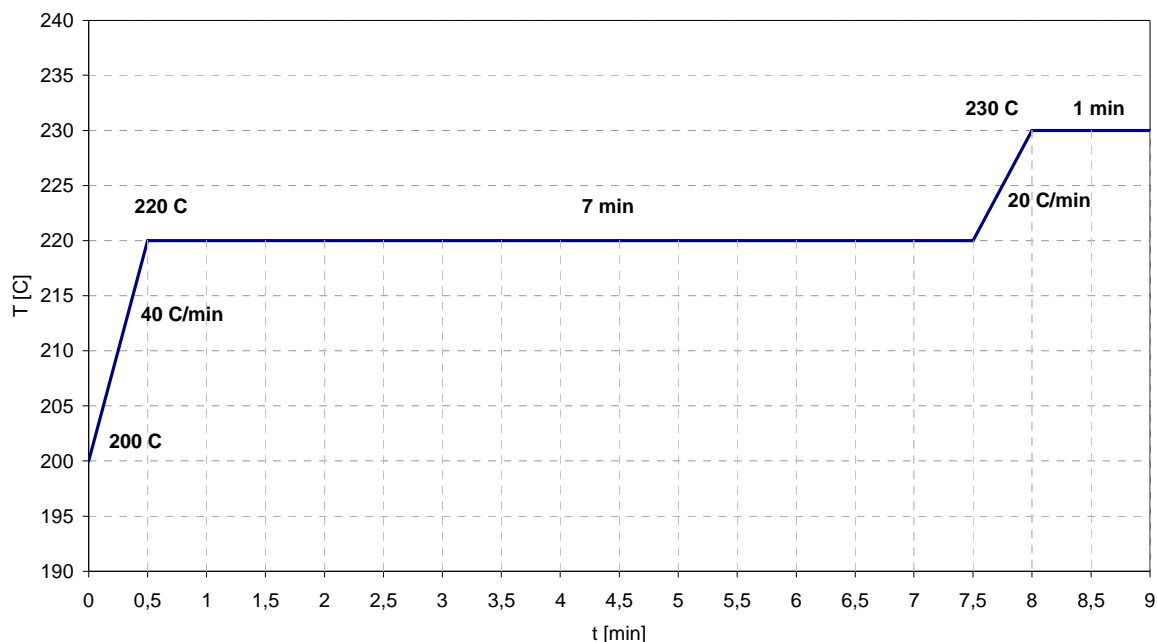
Figure 4. Oven temperature program of GC-FID for carbohydrates.

Identification of peaks was carried out by comparing their retention times with those of pure standards. Quantification was carried out by the internal standard method. All analyses were carried out in duplicates.

2.4. Amino acids content

To carry out hydrolysis 2 mL of 6 M HCl was added to 20 mg dry samples and keep in 110°C for 24 hours. After cooling down, 500 μL of internal standard (norleucine solution of 0.5 mol/mL in 0.1 M HCl) was added and evaporated to dryness in the *SpeedVac*. After dissolving in 1 mL of 0.1 M HCl, the samples were filtrated with 45 μL filters [60].

Derivatization was conducted. After evaporating until dryness in the *SpeedVac*, 200 μL of solution of isobutanol-HCl were added and heated in 120°C for 10 minutes, vortex and heated for another 30 minutes in the same temperature. The samples were

cooled down to the room temperature and the excess of the reagent was evaporated in the *SpeedVac*. 200 µL of butylated hydroxytoluene (BHT) were added and solvent was removed in the *SpeedVac*. After addition of 100 µL of heptafluorobutyl anhydride, mixture was heated for 10 minutes at 150°C. After cooling down to room temperature and evaporating the excess of the reagent in the *SpeedVac*, 50 µl of ethyl acetate were dissolved and immediately analyzed by liquid gas chromatography (GC-FID) [61; 62].

The temperature program is shown in figure 5.
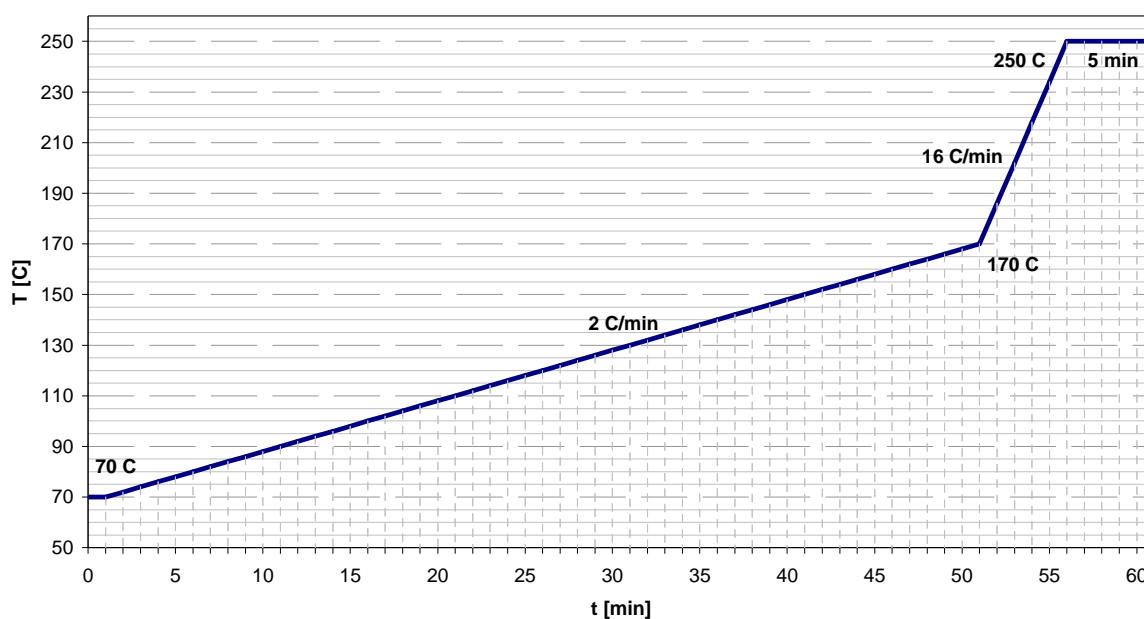
Figure 5. Oven temperature program of GC-FID for amino acids.

2.5. Oil content

2.5.1. Soxhlet method

In order to extract the oil (and other lipophylic compounds) from a solid mixture, a solid-liquid extraction was carried out in a specialized piece of glassware in-between a flask and a condenser called the Soxhlet extractor (figure 6). The refluxing solvent repeatedly washed the solid extracting the desired compound into the flask [63].

10 g of sample (dried coffee residue) were weighted in filter paper thimble and placed in a 500 mL Soxhlet chamber. The extraction was carried out using distillate petroleum ether as solvent at 55°C for 4 h (as 1 cycle took 2 min, there were approximately 120 cycles carried out). After extraction, the samples were cooled in the air for 30 min, the solvent was evaporated by reduced pressure evaporation to constant weight.
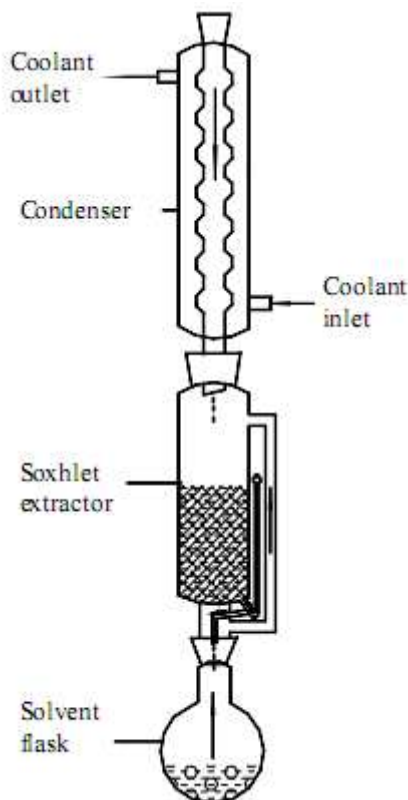
Figure 6. Experimental Soxhlet extraction apparatus [63].

2.5.2. Total free radical scavenger capacity of the oil

The antioxidant capacity of the oil samples was evaluated by the total free radical scavenger capacity (RSC) following the methodology described by Espín *et al.* [64]. Accordingly, the RSC is the variation of the concentration of DPPH (free radical), previously dissolved in ethyl acetate, after 60 min of reaction with the samples:

$$RSC = [DPPH]_0 - [DPPH]_f$$

where the initial [DPPH]$_0$ and final [DPPH]$_f$ concentrations are spectrophotometrically measured at 515 nm. The antioxidant capacity (AOC) of the oil from coffee residue extracted is then expressed in terms of tocopherol equivalents, i.e. the concentration of a tocopherol solution which gives rise to the same RSC as shown in figure 7.

Calibration curve as tocopherol equivalents [65]:

$$AOC = 43.4 \cdot RSC$$
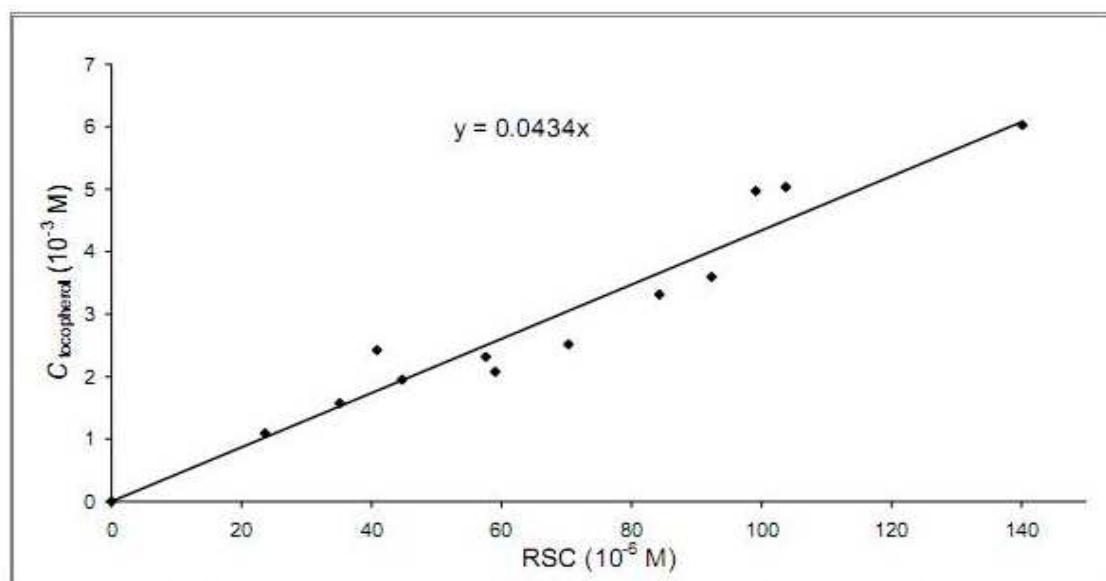
Figure 7. Calibration curve for the antioxidant capacity: tocopherol concentration against radical scavenger capacity [65].

Oil samples (50 mg) were taken and added directly to 3.95 mL of a DPPH solution in ethyl acetate with concentration $1.5*10^{-4}$ M. The oil was utilized without any pre-treatment. The reaction mixture was shaken vigorously and allowed to react at room temperature (22°C) in the dark. After 60 min, the concentration of the remaining DPPH was determined colorimetrically using a glass cuvette at 515 nm, by blanking against an appropriate control (mixture without the radical). A double beam ultraviolet-visible (UV-Vis) spectrophotometer (Lambda 35, Perkin-Elmer, USA) was used to read samples absorbance.

The AOC of the oil is expressed as the concentration of an equivalent tocopherol solution. Each result presents the mean and the standard deviation for a minimum of three experiments [66].

2.6. Coffee residue antioxidant capacity

Dry coffee samples were diluted in water (30 g/L H_2O) and saponified [67]. The DPPH solution (3.9 mL, 25 mg/L) in ethanol was mixed with sample solution (0.1 mL) [45]. The reaction mixture was shaken vigorously and allowed to react at room temperature (22°C) in the dark. After 30 min, the concentration of the remaining DPPH was determined colorimetrically at 515 nm, by blanking against an appropriate control (mixture without the

radical). A double beam ultraviolet-visible (UV-Vis) spectrofotometer (Lambda 35, Perkin-Elmer, USA) was used to read samples absorbance [45].

The calibration curve was produced by measuring standard solutions of DPPH radical in ethanol in the concentration range 0–100 μg/mL. The calibration curve for DPPH is presented in Figure 8 [45].
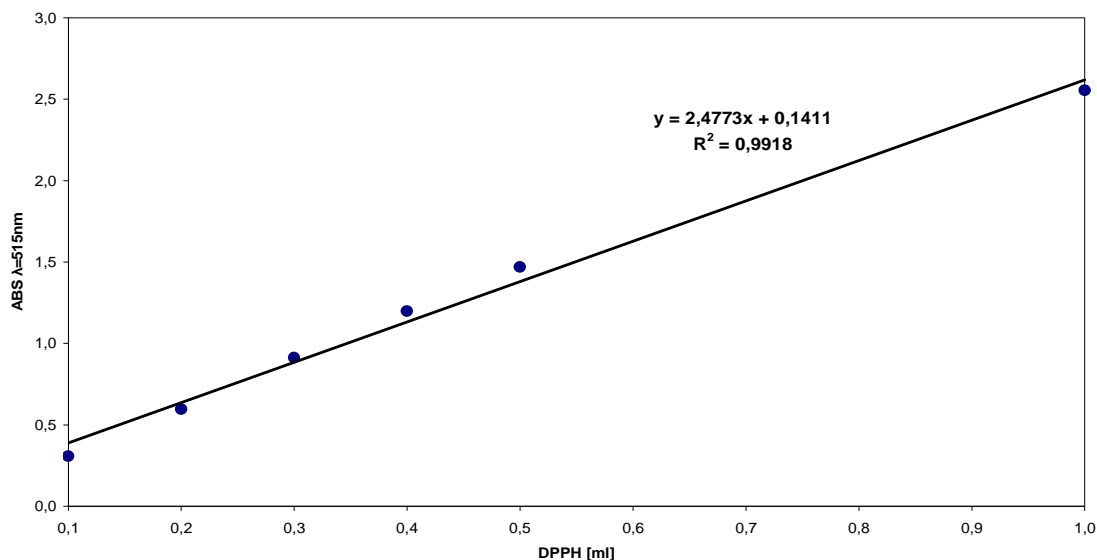
Figure 8. Calibration curve to determine DPPH radical concentration.

The percentage of the DPPH remaining is calculated as [48]:

$$\%DPPH_{rem} = 100 \cdot \frac{[DPPH]_{rem}}{[DPPH]_{T=0}}$$

where %DPPH$_{rem}$ is proportional to the antioxidants concentration, and the concentration that causes a decrease in the initial DPPH concentration by 50% is defined as EC$_{50}$ [48; 45]. The radical scavenging activities were expressed as EC$_{50}$ values, which were calculated from a logarithmic curve measured for all samples.

2.6. Oil Volatile compounds - GCxGC

An SPME triple phase 50/30 μm fiber (divinylbenzene / carboxen / polydimethylsiloxane), purchased from Supelco Inc. was used for the extraction of volatiles from the coffee residue. The fiber was conditioned according to manufacturer recommendations prior to use.

Oil samples were placed in a sealed 2 mL vial, and heated for 15 min at 40°C (pre-incubation). The SPME needle was inserted into the vial, and the fiber exposed to the headspace above the coffee sample for 45 min at 40°C. After sampling, the SPME coating fibre was manually introduced into the GC × GC–ToFMS injection port at 250°C and kept for 3 min for desorption. The injection port was lined with a 0.75 mm I.D. splitless glass liner. Splitless injections were used (30 s). LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC × GC–ToFMS system consisted of an Agilent GC 6890N gas chromatograph, with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven. The detector was a high-speed ToF mass spectrometer. An HP-5 30 m × 0.32 mm I.D., 0.25 μm film thickness (J&W Scientific Inc., Folsom, CA, USA) was used as first-dimension column and a DB-FFAP 0.79 m × 0.25 mm I.D., 0.25 μm film thickness (J&W Scientific Inc., Folsom, CA, USA) was used as a second-dimension column. The carrier gas was helium at a constant flow rate of 2.50 mL/min. The primary oven temperature was programmed from 40°C (during 2 min), then to 45°C with a rate of 0.50°C/min (0 min), and finally to 220°C with rate of 40.00°C/min (during 2 min). The secondary oven temperature was programmed from 70 (2 min), through 75°C (0 min) at 0.5°C/min, to 240°C (1 min) at 40°C/min. The MS transfer line temperature was 250°C and the MS source temperature was 250°C. The modulation time was 8 s; the modulator temperature was kept at 20°C offset (above primary oven). The ToF-MS was operated at a spectrum storage rate of 125 spectra/s. The mass spectrometer was operated in the EI mode at 70 eV using a range of m/z 33–350 and the voltage was −1695 V. Total ion chromatograms (TIC) were processed using the automated data processing software ChromaTOF (LECO) at S/N threshold 10. Contour plots were used to evaluate the separation general quality and for manual peak identification. A signal-to-noise threshold of 50 was used. Two commercial databases (Wiley 275 and US National Institute of Science and Technology (NIST) V. 2.0 – Mainlib and Replib) were used [68].

A manual inspection of the mass spectra was done, combined with the use of additional data, such as the retention index (RI) value. For the determination of the RI, a C_8–C_{20} n-alkanes series was used. Furthermore, 73% of peaks has similarity > 800, which was also checked to decide whether a peak was correctly identified or not [57].

Verification of the volatile coffee residue profile was achieved through duplicate sample analysis [57].

3. Results and discussion

3.1. General composition

Table 10 shows the general composition of different coffee fractions. The results showed that coffee residue humidity equals approximately 60 %, while ashes (mineral residue after burning) are only 0.5 %. Drying curves of three different samples and average can be seen in figure 9. The main compounds after drying the coffee residue are: sugars (80 %), lipids (13 %) and proteins (5 %). After Soxhlet extraction, in defatted coffee residue the composition does not change significantly.

Table 10. Coffee general composition [9; 40]. [a] dry basis.

Material	Literature (green been)	Residue before MW	
		Residue	**Defatted**
H_2O		60.76 %	
dry material		39.24 %	
ashes		0.50 %	
fat [a]	10.8-13.2 %	12.94 %	
sugars [a]	43.0-48.3 %	79.50 %	89.34 %
proteins [a]	8.7-12.2 %	4.83 %	5.28 %
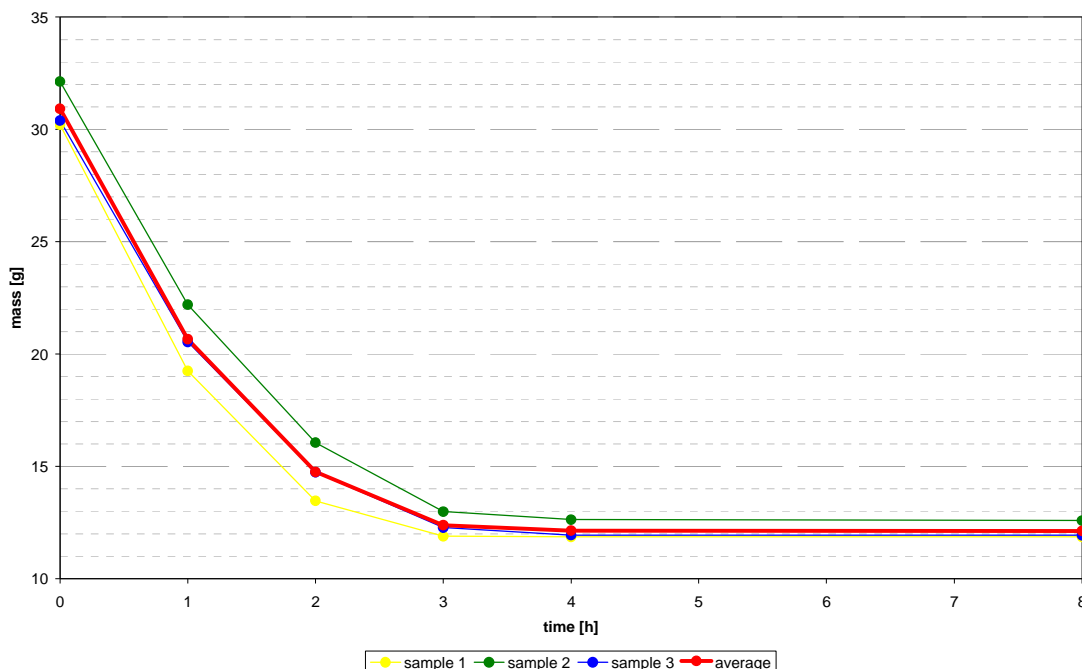
Figure 9. Drying curves of coffee residue samples.

The oil content in residue (13 %) is similar to the literature data for green bean (10.8 % - 13.2 %) [9; 40].

Sugar content in coffee residue (80%) is higher than in literature (45 %) [9], however those literature data refer to green bean. Furthermore, the results here show that previous hydrolysis methodologies were not sufficient and therefore the results for the sugar content have been underestimated. The incubation conditions were change from 3h at 100°C [58] to 1h at 120°C, and from 1 M to 2 M for the acid concentration (data not published).

Amino acids content in coffee residue (5 %) correspondent to approximately half the value given by literature, however the literature data is given for green bean (9-12 %), which is in accordance with the assumption that 50% of amino acids are soluble in water and extracted to the supernatant during the preparation of coffee infusion [9].

3.2. Microwave extraction

During microwave extraction, up to 20 % of mass is extracted to supernatant (see scheme 7). High temperature treatment leads to degradation of complex substances as well as decreasing branching in polysaccharides and peptides. After the extraction, still up to 80 % of mass remains in the residue.
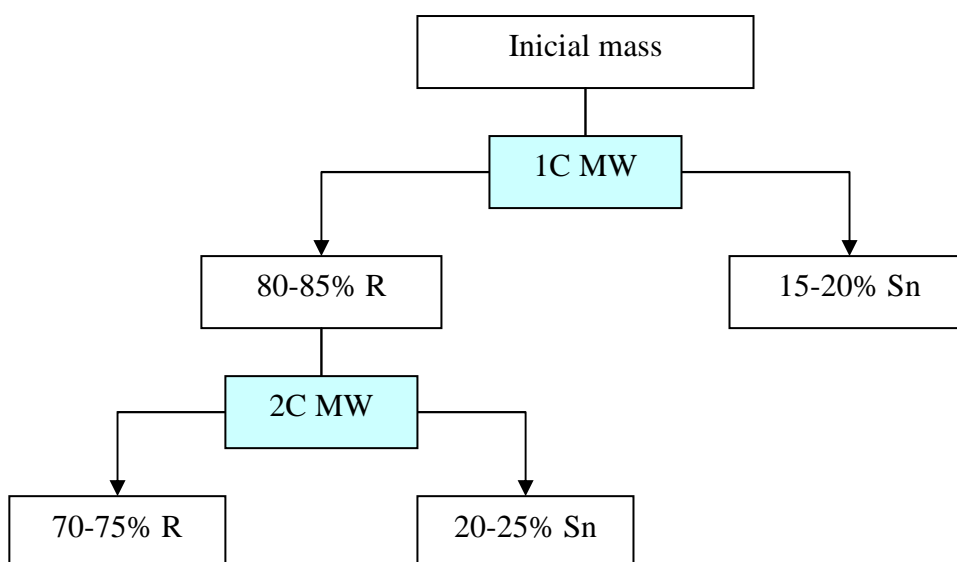
Scheme 7. Microwave extraction according to mass content. 1C MW – fist cycle of microwave extraction, 2C MW – second cycle of microwave extraction

3.3. Carbohydrate content

The carbohydrates individual composition (in the supernatant and residue), before and after two cycles of microwave extraction, are given in scheme 8.

```
                        ┌──────────────────┐
                        │    (Sugars)      │
                        │      79 %        │
                        └──────────────────┘
        ┌──────────────┬──────────┴────────┬──────────────┐
   ┌─────────┐   ┌─────────┐         ┌─────────┐    ┌─────────┐
   │   Ara   │   │   Man   │         │   Gal   │    │   Glc   │
   │ 6.5±0.7%│   │48.0±0.8%│         │26.0±0.2%│    │19.5±0.1%│
   └─────────┘   └─────────┘         └─────────┘    └─────────┘
        └──────────────┴───────┬───────────┴──────────────┘
                     ┌────────────────────┐
                     │       1C MW        │
                     └────────────────────┘
              ┌─────────────┴──────────────┐
        ┌──────────┐                 ┌──────────┐
        │    Sn    │                 │    R     │
        │  38.3%   │                 │  39.2%   │
        └──────────┘                 └──────────┘
```

Sn				R			
Ara	Man	Gal	Glc	Ara	Man	Gal	Glc
12.4	31.9	48.8	6.9	5.2	49.9	24.1	20.8
±1.1%	±0.3%	±1.1%	±0.2%	±0.6%	±2.2%	±2.6%	±0.8%

```
                     ┌────────────────────┐
                     │       2C MW        │
                     └────────────────────┘
              ┌─────────────┴──────────────┐
        ┌──────────┐                 ┌──────────┐
        │    Sn    │                 │    R     │
        │  22.5%   │                 │  20.0%   │
        └──────────┘                 └──────────┘
```

Sn				R			
Ara	Man	Gal	Glc	Ara	Man	Gal	Glc
5.4	41.4	38.8	14.3	2.0	53.0	12.5	32.5
±1.1%	±8.2%	±5.0%	±11%	±0.3%	±1.8%	±1.2%	±2.9%
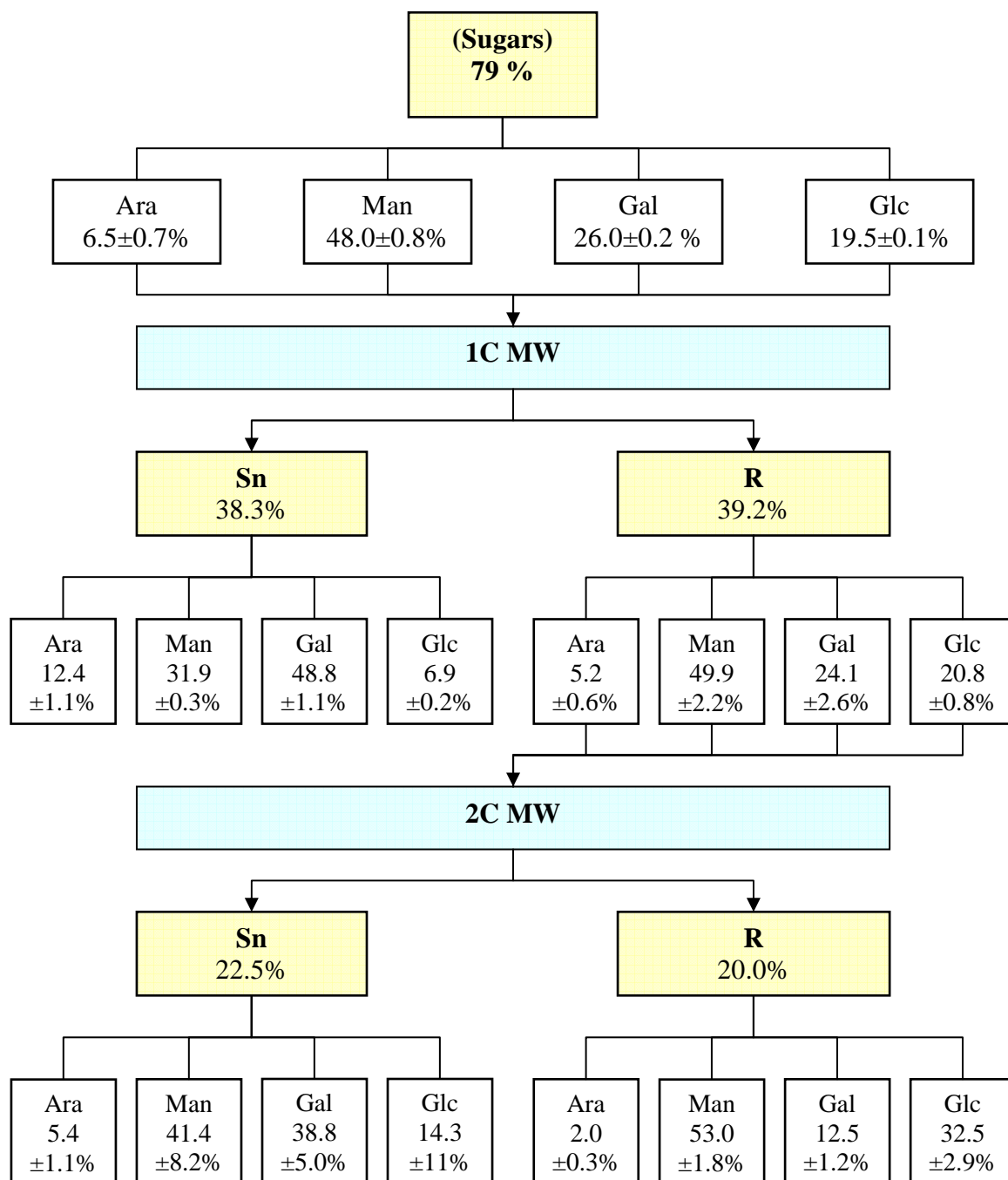
Scheme 8. Sugar composition in coffee residue while microwave extraction. 1C MW – first cycle of micowave extraction; 2C MW – second cycle of micowave extraction; Sn – supernatant; R – residue

The molar percentage of arabinose (Ara: 12.4 %) and galactose (Gal: 48.8 %) in the supernatant suggests that arabinogalactans are the main polysaccharides extracted during the first microwave cycle. Additionally the decrease in molar percentage of those monosaccharides in the remaining residue (Ara from 6.5 % to 5.2 % and Gal from 26.0 % to 24.1 %) is observed. The molar percentage of mannose (Man) in the supernatant was higher after the second cycle of microwaves (41.4 %) than after first cycle (31.9 %), together with still a significant content of galactose (38.8 %) and arabinose (5.4 %), which imply that a mixture of arabinogalactans and galactomannans are extracted. The sugar composition in the residue is getting richer in glucose (Glc) from 19.5 % before microwave extraction to 32.5% after the second cycle, which remains in the residue as cellulose.

During the first and second cycles of microwave extraction both arabinogalactans and galactomannans are extracted. This is suggested by the high content of both arabinose and manose correspondently, while galactose is contributing to both arabinogalactans and galactomannans and therefore cannot be used to distinguish them. However, in the second cycle is notorious a certain decrease in the content of arabinose, while manose is increasing, which means that the content on galactomannans must be improved during this second cycle. Literature, suggests that arabinogalactans are more sensible to temperature treatments, and therefore agrees with the higher content on these polysaccharides during the first cycle of treatment. However, after the major extraction of arabinogalactans during the first cycle, the extraction of the galactomannans seems to be facilitated during the second extraction cycle, observed by the higher content in manose.

The increase on the extraction of galactomannans can be caused by depolymerisation, such hypothesis must be however confirmed. This may happen during microwave extraction because of the high temperature treatment, analogous to what is happening during the roasting process. It was however shown that during the roasting process, where high temperature is also used, the galactomannans extracted with hot water as high molecular weight material, are less branched than those present in the green coffee extracts. As a result, the galactomannans are also becoming less soluble because of the lowering branching. The final solubility results as an equilibrium between the two factors: depolymerisation and disbranching. As the galactomannans are more soluble in hot water after the microwave treatment, it must result that depolymerisation has a higher impact.

Another possible explanation for the higher yields obtained after the microwave extraction may be caused by a modification in the coffee structure during microwave extraction, reflected in the increase of the accessibility of water to the polysaccharides, and as a result increasing the galactomannans extraction [11; 36].

Glucose is considered the most stable saccharide [13], and remains as the major monossacaride residue in the solid after the microwave heating (even at 230°C), as part of the cellulose matrix.

3.4. Amino acids content

The amino acids content was determined on the residue before and after the first cycle of microwave extraction, as well as in the defatted residue.

As shown in scheme 9, amino acids content in coffee residue was 5.6%, which increases to 5.6 % when considering an oil dry basis (12.9% of oil, w/w), in agreement with the results for the defatted coffee residue (5.3%). After the first cycle of microwave extraction most of the amino acids remain in the residue, with a total content of 5.9 % (w/w), value that has been simultaneously corrected according to the microwave extraction yield (80% of the initial mass remains in the residue) and assuming an oil dry basis.

```
                          ┌─────────────────────┐
                          │     total AA        │
                          │    5.6±0.6% [a]      │
                          └─────────────────────┘
  ┌─────────────────────────┐         │
  │ defatted (after Soxhlet) │◄────────┤
  │      5.3±1.6%            │         │
  └─────────────────────────┘    ┌────┴────┐
                   ┌──────────────┐    ┌──────────────────┐
                   │ Sn 1C MW [b] │    │   R 1C MW        │
                   │   0.3% [c]   │    │  5.9±1.1% [a, c]  │
                   └──────────────┘    └──────────────────┘
                      ┌───────────────────────────┐  │
                      │ defatted (after Soxhlet)   │◄─┘
                      │        5.1% [c]            │
                      └───────────────────────────┘
```
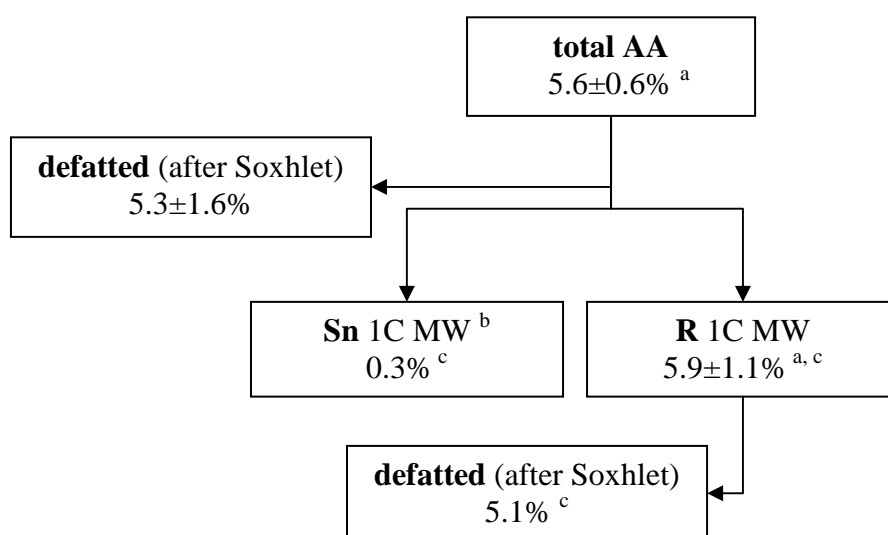
Scheme 9. Amino acids content [a] oil dry basis; [b] performed only on one sample; [c] assuming the extraction yield (20% for the Sn, 80% for R); AA – amino acids, R – residue, Sn – supernatant; 1C MW – first cycle microwave.

The amino acids composition is given in Table 11 while the correspondent experimental design is represented in scheme 10.

The results show a similar amino acids composition in the coffee residue before and after the first cycle of microwave extraction.

The major amino acids are: glutamic acid, leucine, aspartic acid, alanine, valine, glycine, proline and isoleucine, similarly to the composition given in the literature for both roasted beans and brew (see table 11) [10]. The only significant difference after the microwave extraction is due to the molar percentage of glutamic acid which rises significantly after the microwave extraction when compared with the remaining fractions.

The total amount of amino acids in each extract is also given in table 11, although these amounts are only representative for each fraction. The amino acids composition was only given for one sample, for a more complete analysis further trials would be necessary.

Table 11. Amino acid composition (Mol %) of different coffee fractions. [a] total amount of amino acids in % (w/w). AA – amino acids; R – residue; Sn – supernatant; 1C MW – first cycle of microwave extraction; 2C MW – second cycle of microwave extraction; tr. – traces.

AA	Literature data [10]		R	After 1C MW	
	roasted bean	brew		Sn	R
Ala	9	8	11	10	6
Asx / Asp	10	10	11	13	15
Glx / Glu	22	29	28	37	40
Phe	5	4	3	tr.	3
Gly	12	13	8	8	5
Ile	5	4	5	5	4
Pro	7	8	6	6	4
Ser	3	3	1	1	2
Thr	3	2	2	1	2
Val	8	5	7	7	6
Total % (w/w) [a]	10.1	6.4	4.9	1.3	6.5

total AA 4.89±0.59% [a]					
Glu 27.6±2.1% [b]	Asp 11.2±1.4%	Val 7.1±1.9%	Pro 6.2±0.7%	Phe 2.9±0.3%	Ser 1.3±0.7%
Leu 18.6±1.4%	Ala 10.6±0.4%	Gly 7.8±0.7%	Ile 5.0±2.7%	Thr 1.6±0.1%	Tyr traces

Sn 1C MW [c] 1.25% [a]					
Glu 37.1%	Asp 12.8%	Val 7.3%	Pro 5.7%	Phe traces	Ser 1.1%
Leu 12.4%	Ala 10.3%	Gly 7.6%	Ile 4.5%	Thr 1.3%	Tyr traces

R 1C MW 6.46±1.13% [a]					
Glu 39.5±4.2%	Asp 14.6±0.7%	Val 5.6±1.2%	Pro 4.2±2.4%	Phe 3.4±0.4%	Ser 1.5±0.1%
Leu 12.8±1.4%	Ala 5.9±1.1%	Gly 5.2±0.8%	Ile 4.3±0.8%	Thr 1.5±0.1%	Lys 1.6±0.2%
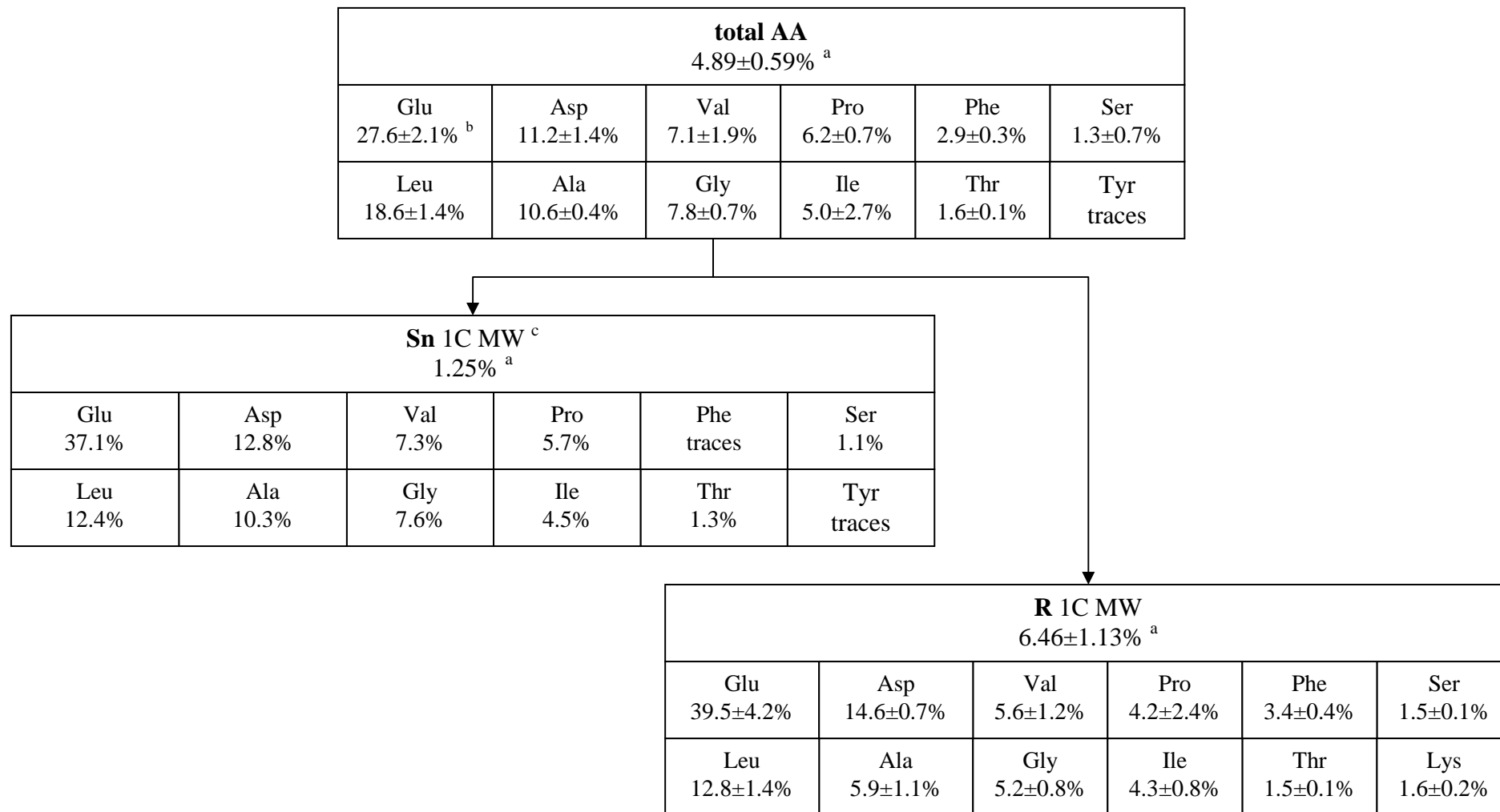
Scheme 10. Amino acids composition after first cycle of microwaves. [a] percentage of total amino acids in initial sample; [b] amino acids composition in mol % [c] performed on one sample; 1C MW – fisr cycle of micowaves; AA – amino acids, Sn – supernatant; R – residue.

3.5. Oil content

The oil content obtained from the coffee residue throughout a solid-liquid extraction in a Soxhlet apparatus, and after low pressure evaporation of the solvent equals 13 % (table 12), which confirm literature data (table 13) [9].

Table 12. Fat analysis by low pressure evaporation of solvent. [a] initial mass content (w/w).

time [h]	sample			average
	1	2	3	
0[a]	10.0323	10.0520	10.0340	10.0394
1	1.5374	1.3803	1.3998	1.4393
2	1.2901	1.2897	1.2813	1.2870
3	1.2954	1.3058	1.2974	1.2995
%	12.9123 %	12.9904 %	12.9300 %	12.9443 %

Table 13. Oil content in coffee samples. 1C MW – fist cycle of microwave extraction; 2C MW – second cycle of microwave extraction; R – residue; Sn – supernatant [9; 40].

Literature	1C MW		2C MW	
green bean	R	Sn	R	Sn
10.8 – 13.2 %	13.85 %	1.32 %	12.43 %	1.02 %

The oil content in the residue remains practically unchanged after the microwave extractions (1st and 2nd cycles) as the amount that is transferred to the supernatant is very low compared to the remaining.

3.5.1. Antioxidant capacity (AOC) of oil

To compare the antioxidant capacity of the coffee oil with other sources of oil samples referred in the literature [65], the results were presented in terms of tocopherol equivalents, i.e. the concentration of tocopherol solution which gives rise to the same total free radical scavenger capacity.

All coffee samples show high antioxidant capacity as presented in figure 10. The highest AOC was obtained for the oil extracted from the coffee residue before any microwave treatment. Such oil sample, showed an AOC three times higher when compared to the literature results given for grape seed oil, and up to six times higher than the olive oil

(a commercial sample tested during this essay), which are known for their antioxidant properties.

After the first cycle of microwave extraction, the AOC lowers from $30*10^{-3}$ M to $24*10^{-3}$ M in tocopherol equivalents, while after the second cycle the AOC further decreases to $8*10^{-3}$ M (figure 10). It is assumed that the AOC of the samples is decreasing due to the degradation of the antioxidant compounds present during the heating treatments.
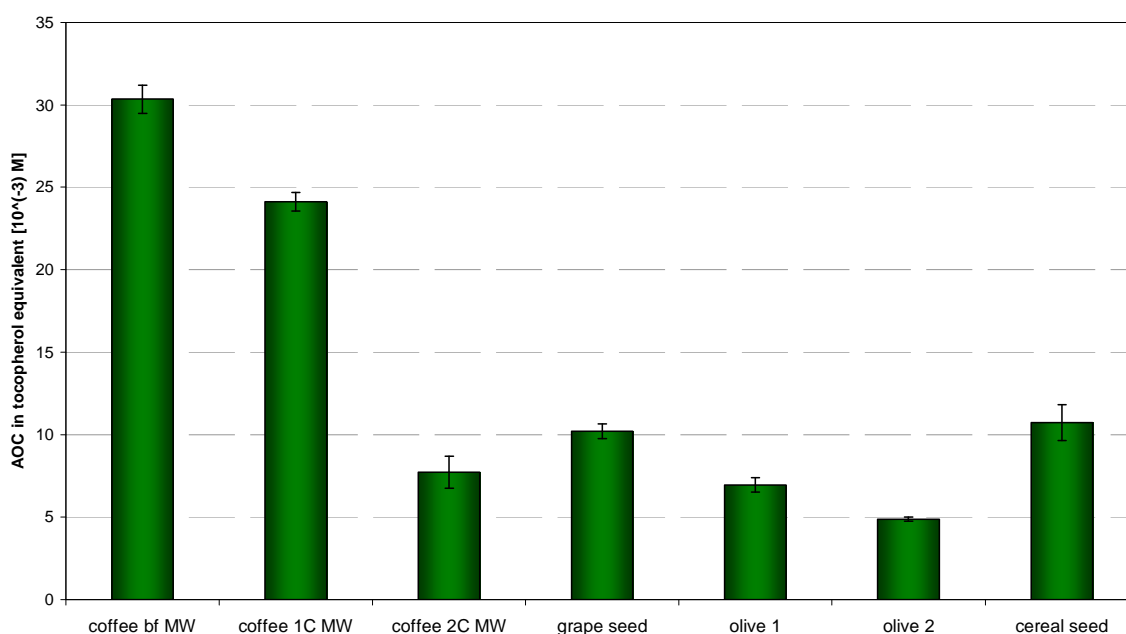
Figure 10. Antioxidant capacity in tocopherol equivalents of different coffee fractions with comparison with olive oil, grape seeds oil and commercial cereal seed oil Frigi. AOC – antioxidant capacity; bf MW – oil extracted from the coffee residue before microwave extraction; 1C MW – oil extracted from the coffee residue after the first cycle of microwave extraction; 2C MW – oil extracted from the coffee residue after the second cycle of microwave extraction.

3.5.2. Antioxidant capacity of hydrophilic supernatants and residues

For the solid samples, considering both the supernatants and the remaining residues, the AOC was expressed as EC_{50}, which is the most common methodology. The EC_{50}, corresponds to concentration of sample necessary to decrease by 50% the initial DPPH concentration, and was calculated from a logarithmic curve of DPPH conversion vs. sample concentration (figure 11). The higher the EC_{50} is, the higher the concentration it is

necessary to reduce the initial DPPH to 50%, and consequently the lower amount of antioxidants are in the sample, as a result the AOC is lower.
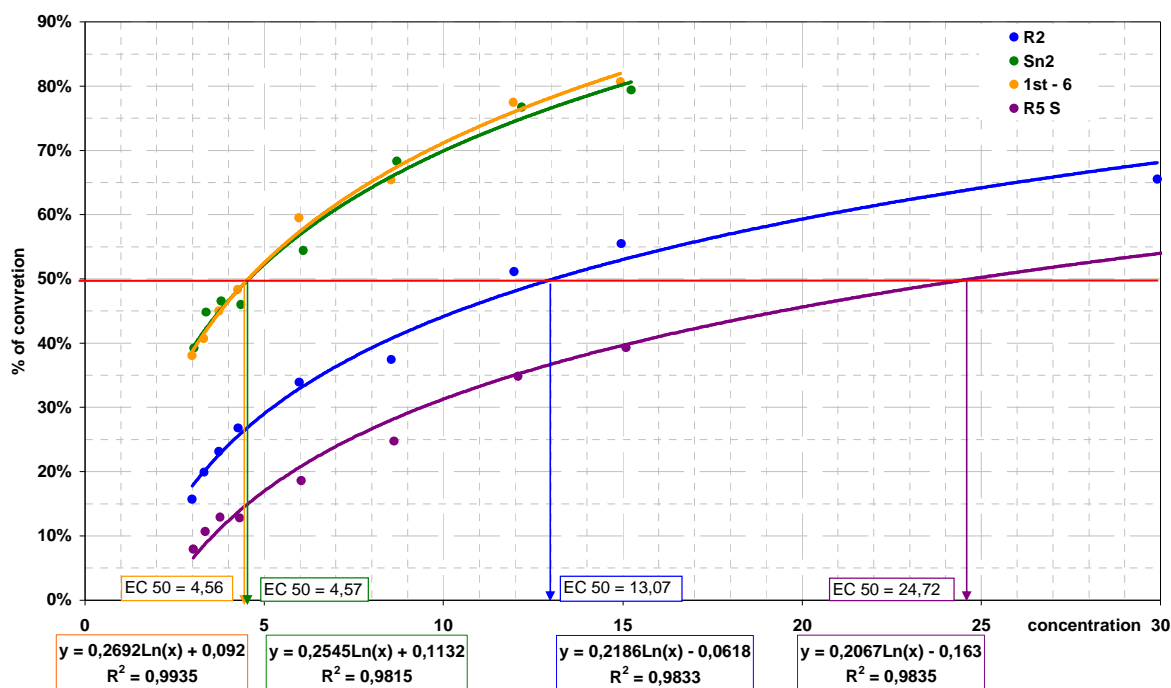
Figure 11. Example of calculating EC$_{50}$ - conversion of DPPH after microwave extraction.

The comparison of the AOC of different coffee fractions (namely the initial residue and the residues obtained after both the 1st and 2nd microwave extractions, R, the extracted supernatants after the 1st and 2nd microwave extractions, Sn, the residue obtained after the oil being extracted, and an aqueous extract obtained after submitting the aqueous supernatants to a liquid-liquid extraction with *n*-hexane) is shown in figure 12. In comparison with the residues, the supernatants have the highest AOC. Such results may be explained by the solubilisation of several compounds with antioxidant activity in water, which are easily extracted to the supernatants with the microwave treatments. Such effect, being less manifested during the 2nd cycle, as many compounds have already been extracted during the 1st cycle. As a result, the AOC of the 2nd supernatant (after the 2nd microwave cycle) reduces its activity to half when compared to the supernatant obtained during the 1st microwave cycle. Such compounds are hydrophilic, therefore solubilised in the water phase after the liquid-liquid extraction, which explains the higher AOC given in this fractions.

In terms of the different residues, the differences are not so clear. It seems that after the microwave extraction the residues obtained tend to decrease their activity compared to

the initial residue without treatment, which is in agreement with the higher activity given by the supernatants, however such tendency is not statistically significant due to the high deviations obtained in the essay.

Compared to the initial residue, also the residue obtained after the soxhlet extraction tends to decrease its activity, which is also in accordance with the assumption that lipophylic compounds with antioxidant activity are being extracted to the oil, which has shown an interesting activity (figure 10).
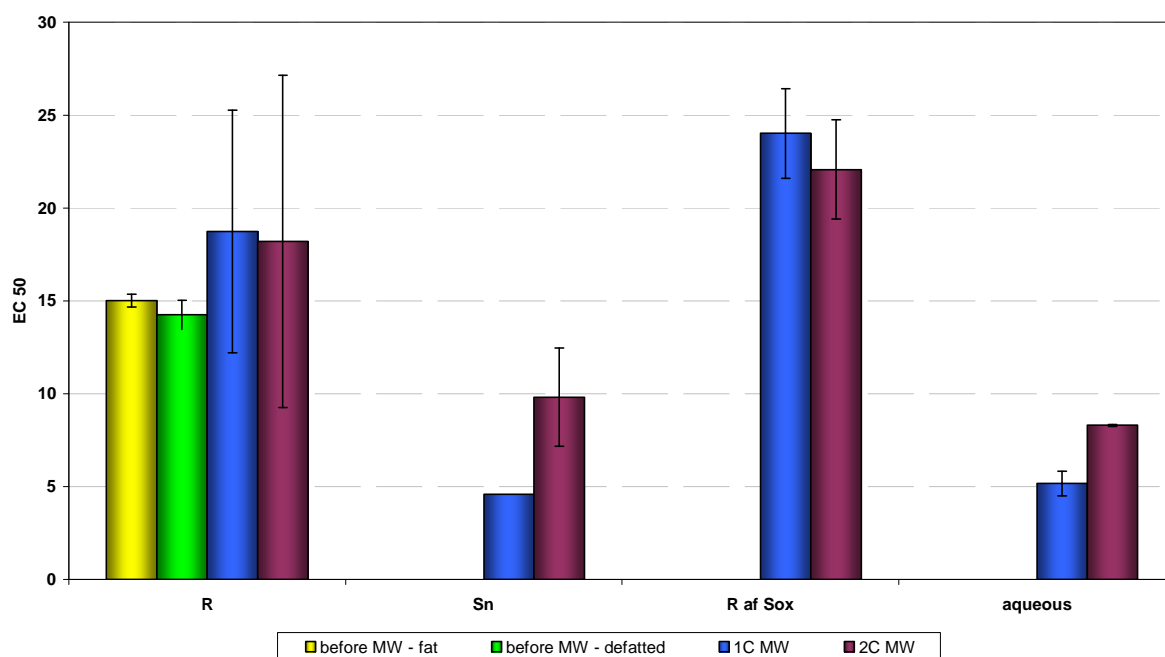
Figure 12. Comparison of the scavenging activity of phenolic compounds in coffee samples. R – residue; Sn – supernatant; R af Sox – defatted residue after Soxhlet extraction; aqueous – aqueous phase after liquid-liquid extraction; MW – microwave; 1C MW – samples after first cycle of microwave; 2C MW – samples after second cycle of microwaves.

3.6. Volatile compounds

During the oil extractions, a pleasant smell related to coffee, has been detected while manipulating the different samples. As a result, it appeared interesting to analyse the oil volatile composition and infer to its relationship with literature given data for the volatile composition of the coffee brew, by performing a two dimensional gas chromatography (GCxGC) analysis.

In the headspace of residue coffee oil samples, 170 compounds (table 14) were identified. The complex 3D view of the GCxGC plot (figure 13), was transformed to 2D

chromatogram (figure 14), where the intense of the pick is shown by changes of colour from blue to green and red as the peak intensity is increasing.
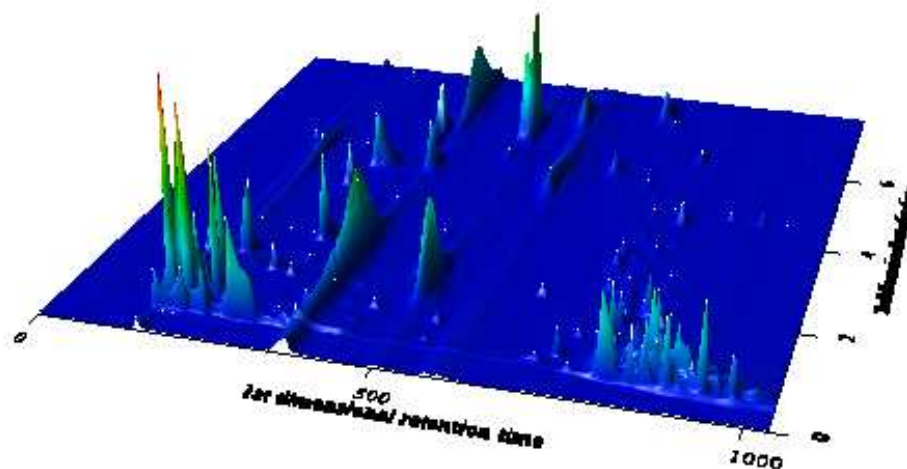
Figure 13. 3D view of GCxGC plot of coffee oil .
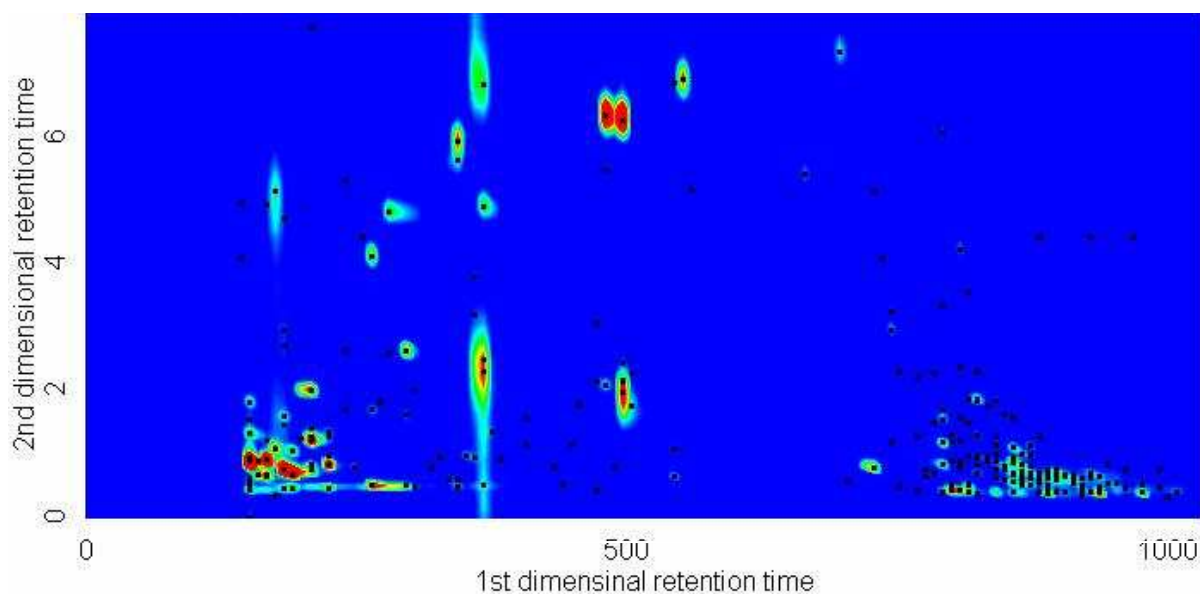
Figure 14. Chromatogram of headspace of coffee oil.

Figure 15 shows the main families in headspace of oil sample, after peak analysis. The correspondent data are presented in table 14.
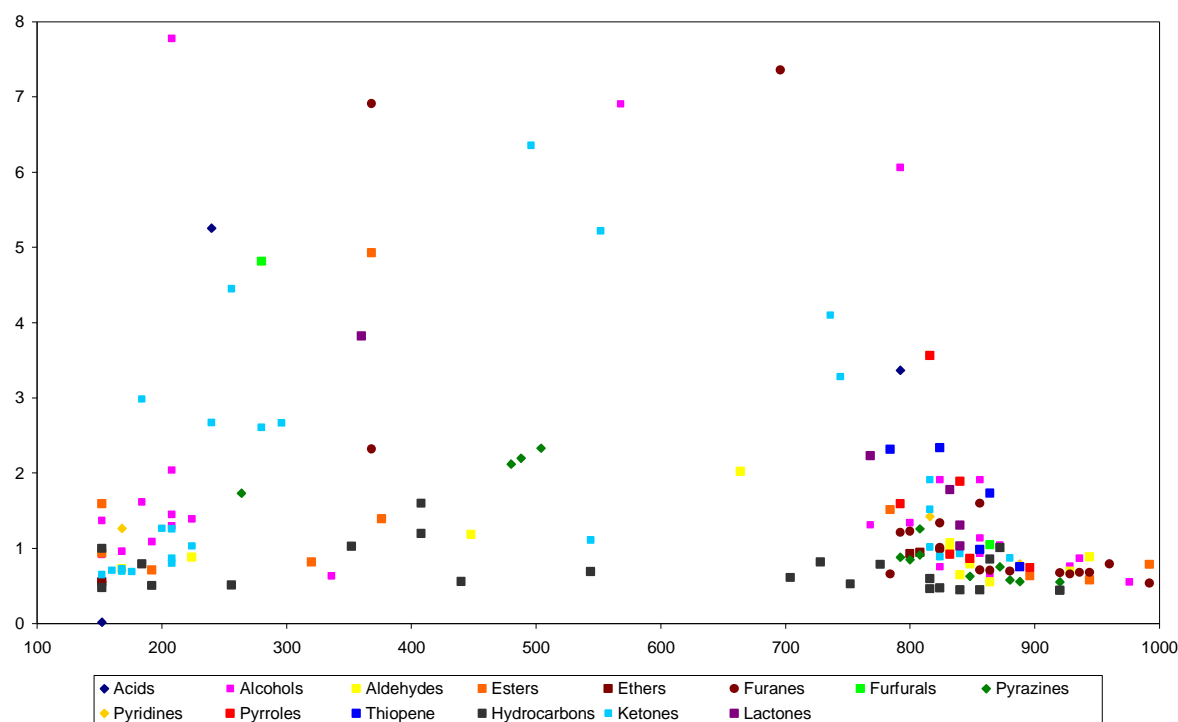
Figure 15. Main families in GCxGC of coffee oil.

Among volatile compounds we can identify polar and apolar compounds, respectively appearing in high and low second dimensional retention times. Low polarity shows: hydrocarbons (figure 16), pyrazines (figure 17), thiopens (figure 18), pyrroles (figure 19), pyridines (figure 20), aldehydes (figure 21), esters (figure 24), alcohols (figure 25). High polarity shows: diols (figure 25), ketons (figure 23), diesters (figure 24) and acids (figure 26). The first dimensional retention time shows volatility of compounds, the most volatile the compound is, the earlier it comes out.

Hydrocarbons have the lowest 2nd dimensional retention time, between 0.440 and 1.600 seconds (figure 16), as they are the least polar, because of the lack of the functional groups. Alifatic hydrocarbons appear between 0.440 s and 0.608 s, where the number of carbons increases with the increase of 2nd dimensional retention time. The double bond between carbons makes hydrocarbons more polar, and their 2nd dimensional retention time is between 0.856 s and 1.008 s. The cyclic hydrocarbons have 2nd dimensional time between 0.480 s and 0.816 s. The most polar among hydrocarbons are those with aromatic ring, which appear between 0.592 s and 1.600 s in 2nd dimensional retention time. In all cases the number of carbons in each subfamily increases with raise of 1st dimensional retention time.
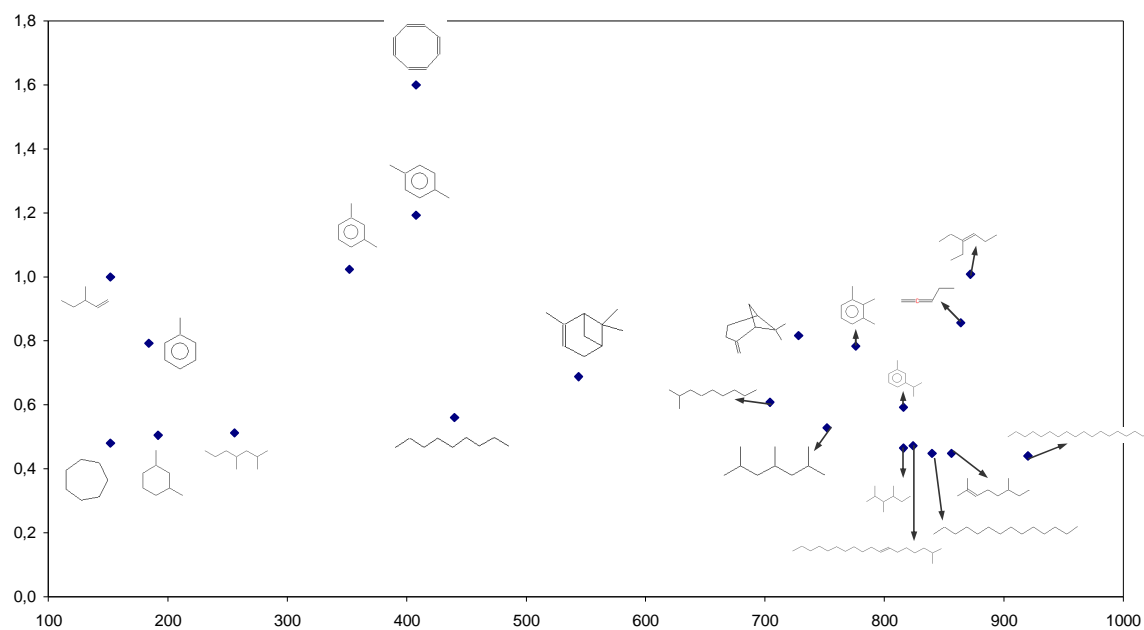
Figure 16. Hydrocarbons in the oil extracted from coffee residue identified in GCxGC.

Pyrazines appears in the 2nd dimensional retention time between 0.552 s and 2.328 s (figure 17). Pyrazines are heterocyclic aromatic compounds with two nitrogen atoms in the six-membered aromatic ring.
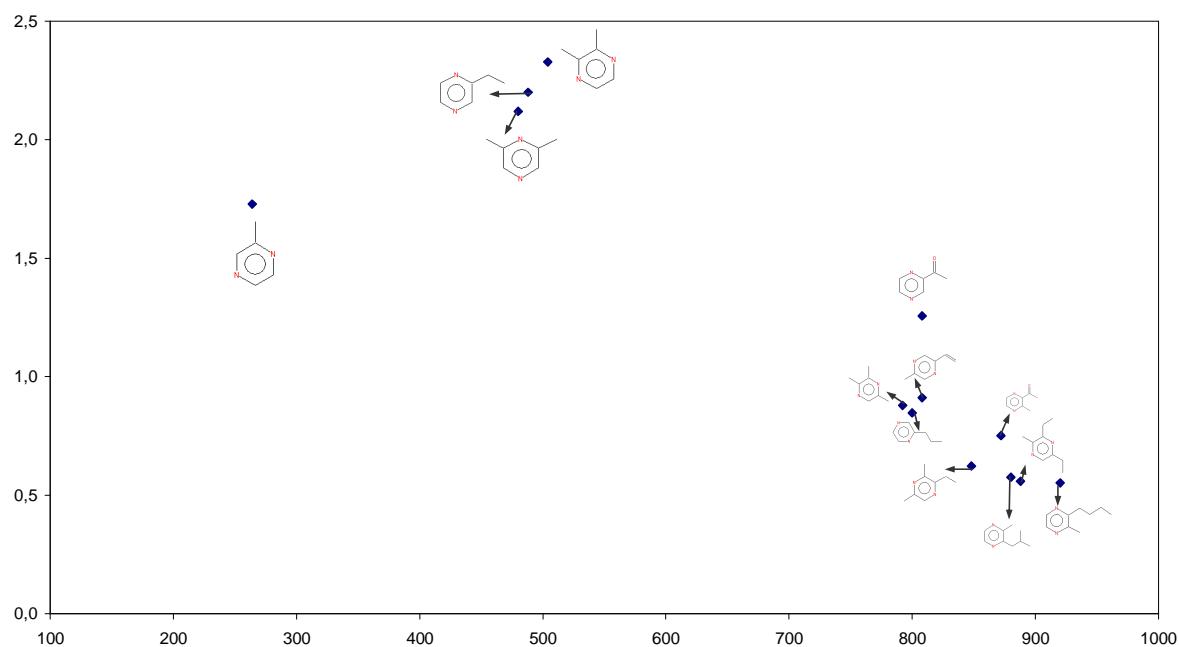
Figure 17. Pyrazines in the oil extracted from coffee residue identified in GCxGC.

Thiophens appear in 2nd dimensional retention time between 0.752 s and 2.336 s (figure 18). They are heterocyclic compounds with one sulphur atom in a flat five-membered ring. The 1st dimensional retention time is high, between 784 s and 888 s, which confirms the low volatility of thiopens.
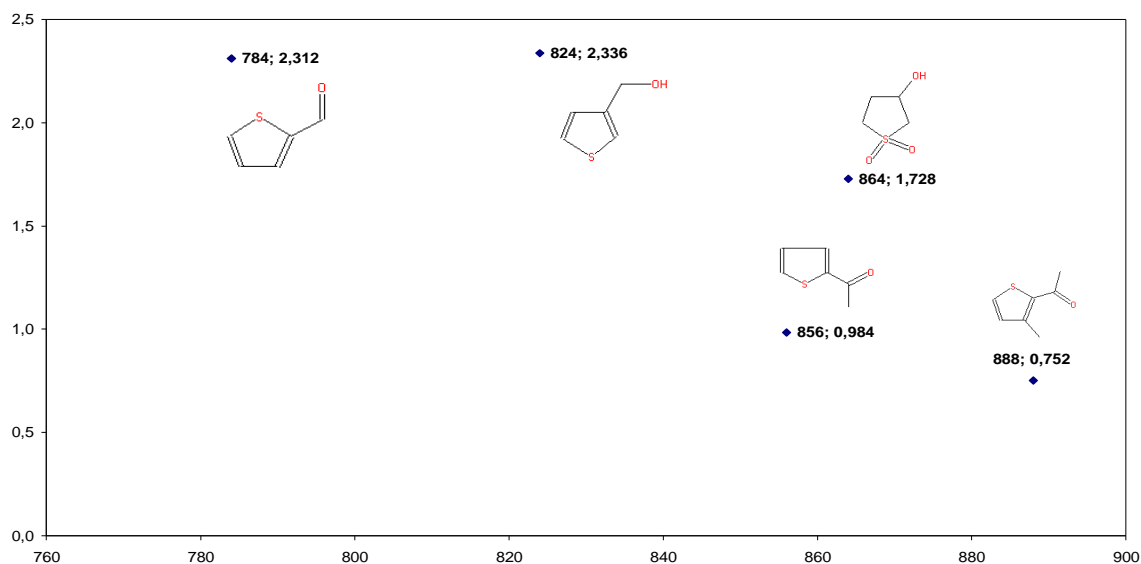
Figure 18. Thiophens in the oil extracted from coffee residue identified in GCxGC.

Pyrroles are another heterocyclic group with five-membered ring, which contain one nitrogen atom, they appear in 2nd dimensional retention time between 0.744 s and 3.560 s (figure 19). The 1st dimensional retention time is high, between 782 s and 896 s, which confirms the low volatility of pyrroles.
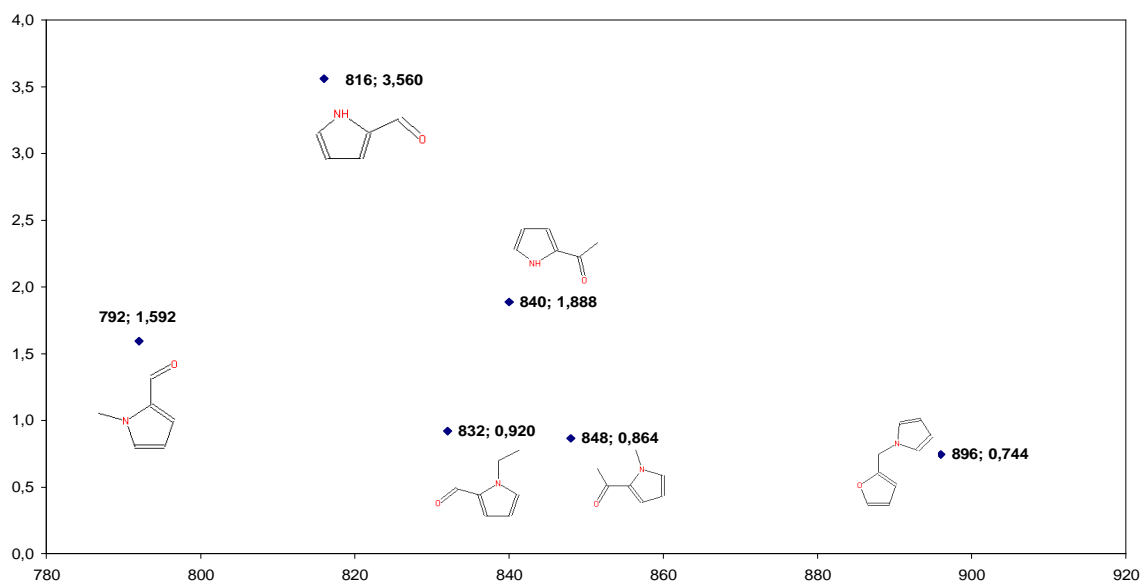
Figure 19. Pyrroles in the oil extracted from coffee residue identified in GCxGC.

Pyridines appear in 2nd dimensional retention time between 0.792 s and 1.424 s (figure 20) and they are six-membered heterocyclic compounds with one nitrogen atom.
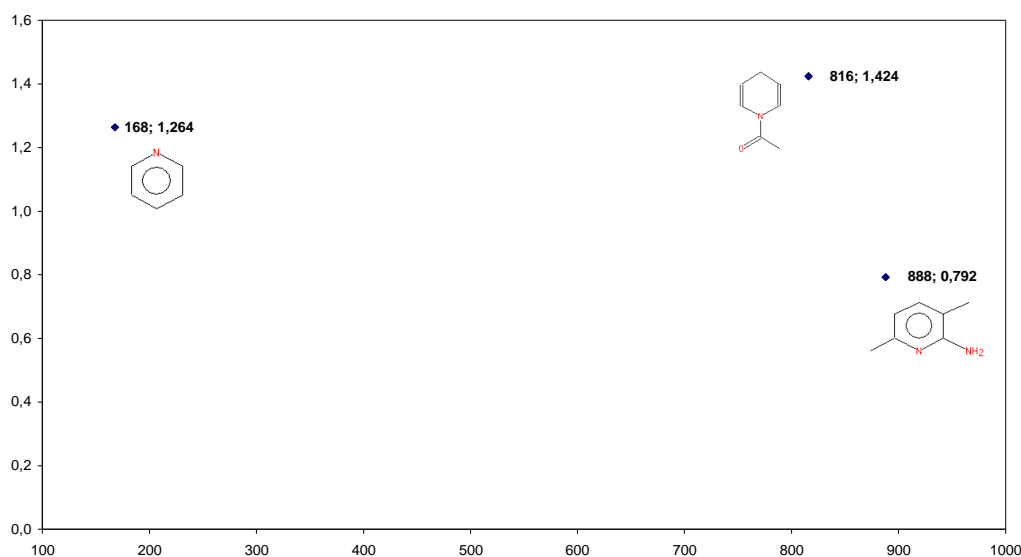
Figure 20. Pyridines in the oil extracted from coffee residue identified in GCxGC.

Aldehydes appear in 2nd dimensional retention time between 0.552 s and 2.024 s (figure 21), as they are not very polar.
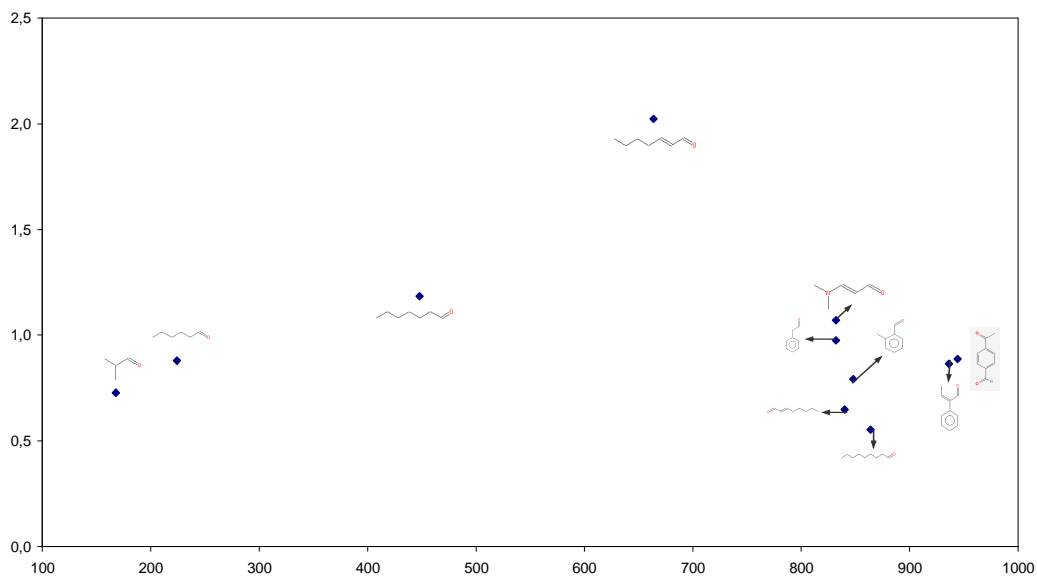
Figure 21. Aldehydes in the oil extracted from coffee residue identified in GCxGC.

Furanes appear in 2nd dimensional retention time between 0.536 s and 7.360 s (figure 22). The lowest 2nd dimensional retention time have simple furans, which appear between 0.536 s and 0.656 s, whereas the highest 2nd dimensional retention have furanes with additional hydroxyl or carbonyl groups.
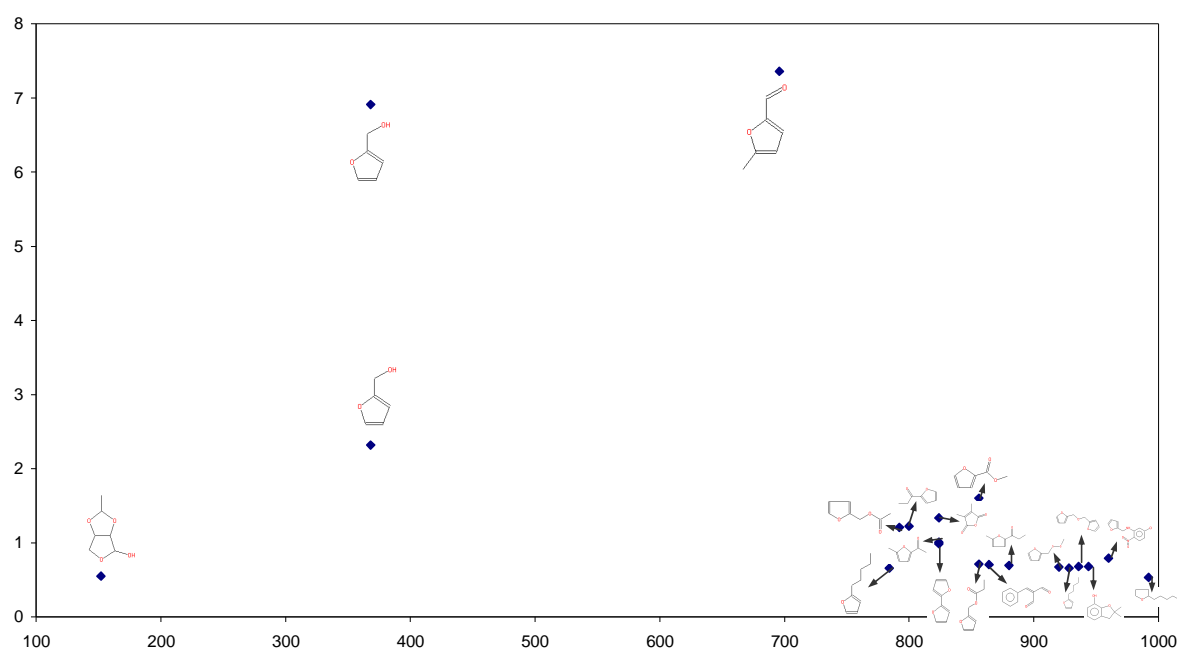
Figure 22. Furanes in the oil extracted from coffee residue identified in GCxGC.

Ketones show a big variety in both dimensional retention times. The 2nd dimensional retention time is between 0.648 s and 6.352 s (figure 23). The simple ketones have low 2nd dimensional retention time between 0.648 s and 0.800 s, cyclic ketones and those with double bond between carbon atoms have medium values between 0.888 s and 2.608 s, whereas ketones with hydroxyl, carbonyl or ether functional group have high values between 0.688 s and 6.352 s.
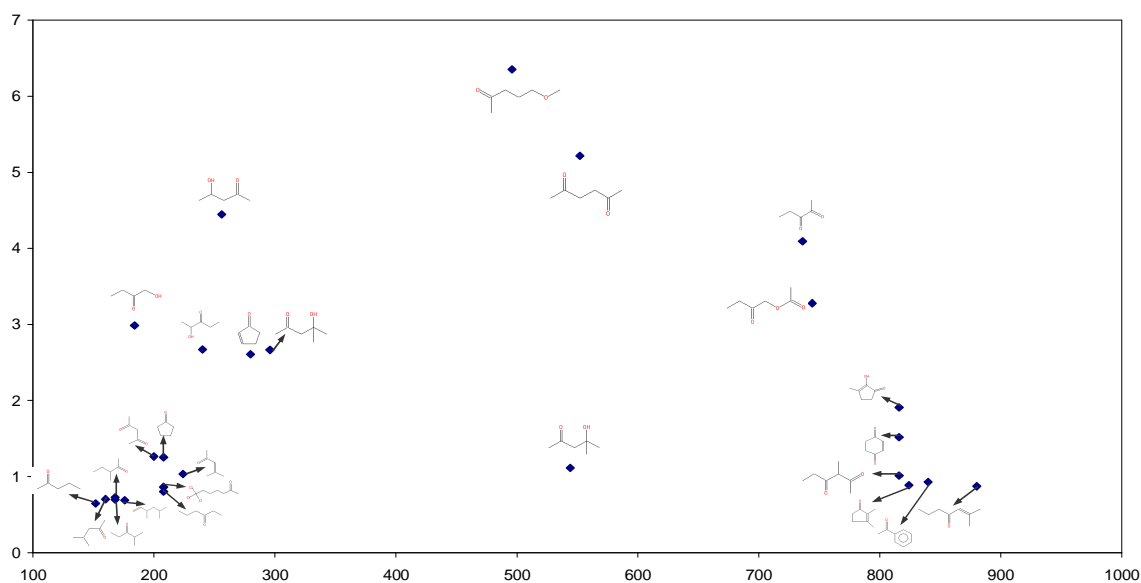
Figure 23. Ketones in the oil extracted from coffee residue identified in GCxGC.

Esters appear in low 2nd dimensional retention time, between 0.576 s and 1.592 s
(figure 24), except for diesters, which have much higher values (up to 4.928 s). Esters with
additional carbonyl functional group have 2nd dimensional retention time around 1.500 s.
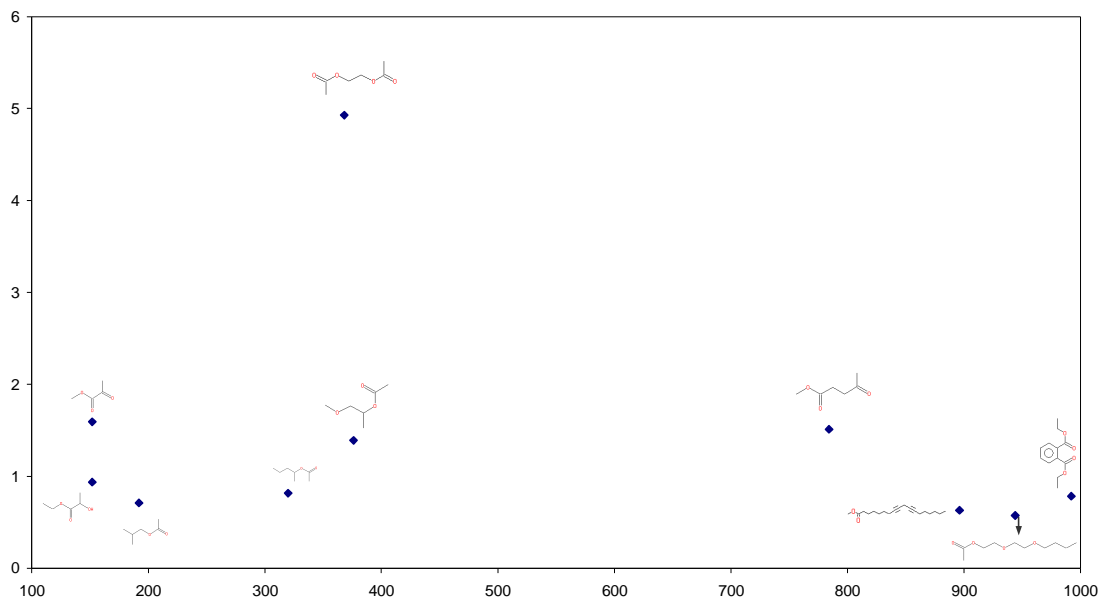
Figure 24. Esters in the oil extracted from coffee residue identified in GCxGC.

Alcohols appear in quite low 2nd dimensional retention times (figure 25), between
0.552 s and 2.040 s, apart from diols, which have two hydroxyl functional groups, which
highest their polarity, they appear between 6.064 s and 7.776 s. Phenols come out in high
1st dimensional retention time, according to the presence of the aromatic ring.
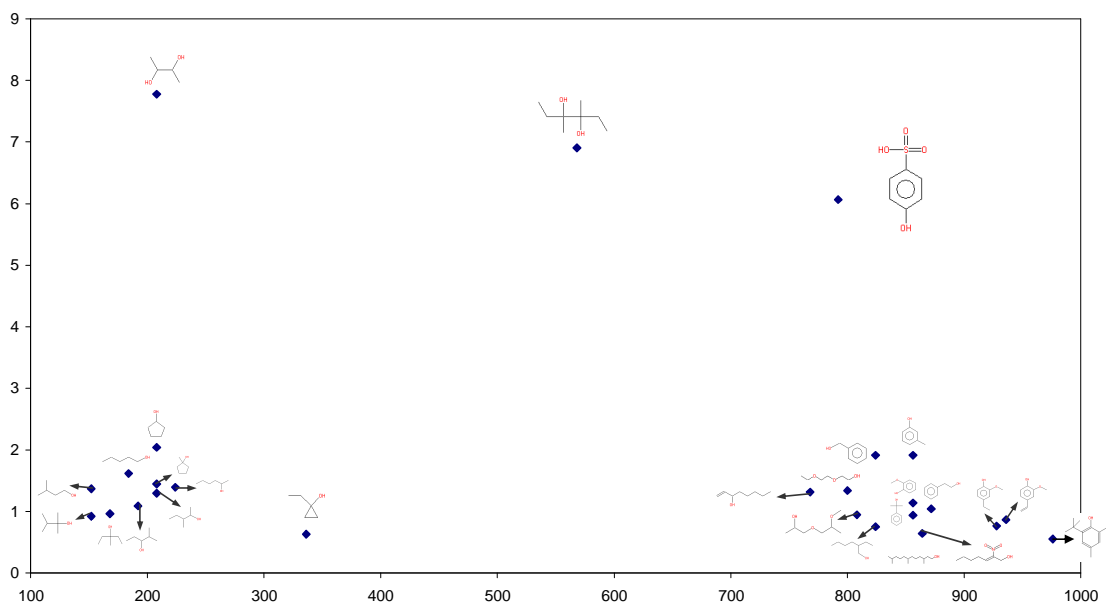
Figure 25. Alcohols in the oil extracted from coffee residue identified in GCxGC.

According to the high polarity of carboxyl group (–COOH), acids appear in high second dimensional retention time (figure 26). Propanoic acid appeared in very low 2nd dimensional retention time (0.016), because of wrap-around effect. Wrap around is an undesirable consequence of compounds not completing elution from the second column during the modulation cycle in which they were injected [69].
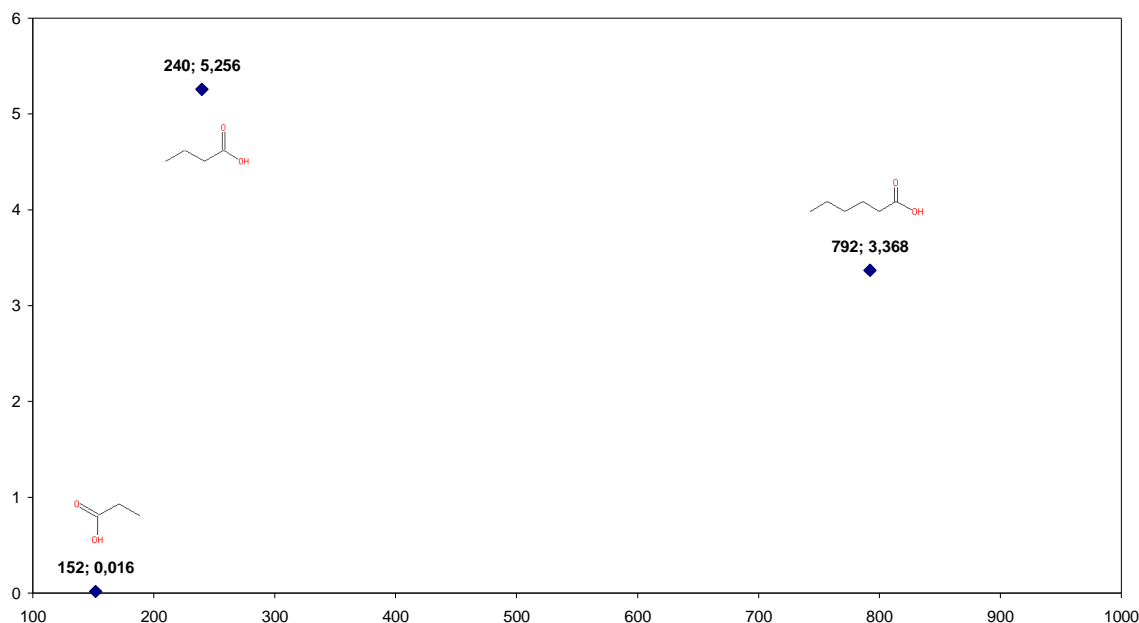
Figure 26. Acids in coffee oil, identified in GCxGC.

Among the heterocyclic compounds which are responsible for aroma 19 furans, 13 pyrazines and 5 thiopens were identified. Some of the compounds are responsible for typical coffee-like aroma: furanes (2-furanmethanethiol), pyrazines (3,5-diethyl-2-methyl-pyrazine; trimethyl-pyrazine; 2-ethyl-5-methyl-pyrazine) [54]. The following compounds have been identified in the oil as responsible for the roasted odor: ethyl-2methoxy-phenol; pyrazines (2-ethyl-3,5-dimethyl-pyrazine; 2-(n-propyl)-pyrazine; trimethyl-pyrazine; 2-methyl-pyrazine; 2,6-dimethyl-pyrazine; ethyl-pyrazine; 2,3-dimethyl-pyrazine), thiopen (1-(2-thienyl)-ethanone). Acids (butanoic, hexanoic and propanoic) and aldehydes (hexanal) are mainly responsible for the buttery smell, which is also important in coffee. Aldehydes have related to the fatty odor (heptanal; 2-heptenal; 2-octenal), whereas warm, sweet, caramellic odor has been related to aldehydes (2-methyl benzaldehyde); ester (propanoic acid – 2-oxo methyl ester), furanes (2-furanmethanol; 1-(2-furanyl)-1-propanone), pyranes (3-hydroxy-2-methyl- 4H-pyran-4-one), pyrazines (2-butyl-3-methylpyrazine; acetylpyrazine); pyrroles (1-methyl-1H-pyrrole-2-carboxaldehyde; 1-(1H-

pyrrol-2-yl)-ethanone; 1H-pyrrole-2-carboxyaldehyde); ketones (2-hydroxy-3-methyl-2-cyclopenten-1-one; 2,5-hexanedione). Hazelnut-like odor (1-(5-methyl-2-furanyl)-1-propanone) and earthy, mouldy odor (2-pentyl-furan; 1-(1-methyl-1H-pyrrol-2-yl)-ethanone; 2,3-dimethyl-2-cyclopenten-1-one; 2,4-pentanedione) are also important in total coffee aroma. Finally, also the fruity (ethylidene-benzeneacetaldehyde; acetic acid – 2-methylpropyl ester; 4-methyl-2-pentanone), smoky and woddy odors (2-methoxy-phenol) have been also taking part in total coffee aroma [93].

Among the new compounds, which were found in head-space of coffee oil extracted from the residue, but have not been identified in the coffee brew are: 1-methyl-cyclopentanol; phenyl acetaldehyde; 2-furanmethanol, propanoate; 2-furanmethanol, acetate; 2-acetyl-3-methylpyrazine; 1-ethyl-1H-pyrrole-2carboxaldehyde; 2-pinene; (1S)-2,6,6-trimethylbicyclo(3,1,1)hept-2-ene; p-xylene; 1-phenyl-ethanone; 5-methyl-2(3H)-furanone. Further analysis however, would have to be performed to identified the concentrations and the individual impact of these new compounds in the total aroma of the oil.

Table 14. Volatile compounds identified by GCxGC/ToF-MS in headspace of the oil extracted from the coffee residue. [1]Dtr[a] – fist dimensional retention time; [2]Dtr[a] – second dimensional retention time in seconds (s); RI_{lit} – retention index in the literature (authors listed below); RI_{calc} – retention index obtained through the modulated chromatogram; CAS – chemical abstracts service registry number; SD – standard deviation.

[1]Dtr[a]	[2]Dtr[a]	Compound	CAS	Simila-rity	Family			RI_{lit}	RI_{calc}	Peak aread (*10^{-5}) 1	2	Average	SD	R.S.D. (%)
152	0,016	Propanoic acid	79-09-4	914	Acids			741	769[70]	19,77	15,62	17,69	2,93	16,58
792	3,368	Hexanoic acid	142-62-1	954	Acids			1085	1015	4,34	1,36	2,85	2,11	73,87
240	5,256	Butanoic acid	107-92-6	963	Acids			856	814[70]	7,41	11,20	9,31	2,68	28,83
					Acids Sum					31,52	28,18			
336	0,632	1-ethylcyclopropanol	57872-31-8	834	Alcohols	Alifatic	-		858	2,64	3,94	3,29	0,92	27,91
824	0,752	1-Hexanol, 2-ethyl-	104-76-7	945	Alcohols	Alifatic	-	1029	1056[71]	7,99	5,22	6,61	1,96	29,67
152	0,920	2-Butanol, 2,3-dimethyl-	594-60-5	918	Alcohols	Alifatic	-		769	204,03	222,19	213,11	12,84	6,03
168	0,960	3-Pentanol, 3-methyl-	77-74-7	966	Alcohols	Alifatic	-		777	110,99	113,54	112,26	1,80	1,61
192	1,088	3-Pentanol, 2-methyl-	565-67-3	944	Alcohols	Alifatic	-		788	24,86	23,38	24,12	1,05	4,35
208	1,296	2-Pentanol, 3-methyl-	565-60-6	928	Alcohols	Alifatic	-		796	21,20	21,41	21,31	0,15	0,72
152	1,368	1-Butanol, 3-methyl-	123-51-3	902	Alcohols	Alifatic	-	706	769[70]	8,22	29,95	19,08	15,37	80,52
224	1,392	2-Hexanol	626-93-7	992	Alcohols	Alifatic	-	803	804[72]	10,75	8,43	9,59	1,64	17,10
184	1,616	1-Pentanol	71-41-0	960	Alcohols	Alifatic	-	735	785[70]	15,44	9,67	12,55	4,08	32,54
208	1,448	Cyclopentanol, 1-methyl-	1462-03-9	910	Alcohols	Alifatic	Cyclic	796	796[70]	3,04	3,33	3,19	0,21	6,48
208	2,040	Cyclopentanol	96-41-3	915	Alcohols	Alifatic	Cyclic	788	797	83,82	79,34	81,58	3,16	3,88
568	6,904	DL-3,4-Dimethyl-3,4-hexanediol	32388-94-6	723	Alcohols	Alifatic	Diol	1000	941	116,15	127,63	121,89	8,12	6,66
208	7,776	2,3-Butanediol	513-85-9	958	Alcohols	Alifatic	Diol	769	799[73]	5,23	3,51	4,37	1,21	27,76
808	0,944	2-Propanol, 1-(2-methoxypropoxy)-	13429-07-7	883	Alcohols	Alifatic	dioxide		1034	-	0,96	-	-	-
800	1,336	Ethanol, 2-(2-ethoxyethoxy)-	111-90-0	960	Alcohols	Alifatic	dioxide	1012	1024	1,57	1,62	1,59	0,04	2,32
864	0,640	2-Nitrohept-2-en-1-ol	104313-51-1	770	Alcohols	Alifatic	nitro		1121	-	14,07	-	-	-
768	1,312	1-octen-3-ol	3391-86-4	946	Alcohols	Alifatic	with =	991	995[74]	1,29	0,56	0,93	0,52	55,70
976	0,552	butyl hydroxy toluene	128-37-0	753	Alcohols	Aromatic	-	1512	1553[73]	3,03	3,47	3,25	0,31	9,60
856	0,936	Benzenemethanol, dimethyl-	617-94-7	705	Alcohols	Aromatic	-	1080	1102[70]	10,60	8,21	9,41	1,69	17,95

872	1,040	Benzeneethanol	60-12-8	864	Alcohols	Aromatic	-	1116	1142[72]	2,57	1,42	1,99	0,82	40,93
824	1,912	Benzenemethanol	100-51-6	947	Alcohols	Aromatic	-	1043	1058[75]	19,86	13,54	16,70	4,47	26,79
856	1,912	Phenol, 3-methyl-	108-39-4	950	Alcohols	Aromatic	-	1084	1104[76]	1,29	-	-	-	-
792	6,064	Benzenesulfonic acid, 4-hydroxy-	98-67-9	978	Alcohols	Aromatic	Diol		1019	14,16	9,51	11,84	3,29	27,79
928	0,760	Phenol, 4-ethyl-2-methoxy-	2785-89-9	930	Alcohols	Aromatic	oxide	1285	1303[77]	6,15	7,60	6,87	1,02	14,83
936	0,864	2-Methoxy-4-vinylphenol	7786-61-0	878	Alcohols	Aromatic	oxide	1282	1336	2,22	-	-	-	-
856	1,136	Phenol, 2-methoxy-	90-05-1	962	Alcohols	Aromatic	oxide	1091	1102[76]	15,34	10,37	12,86	3,51	27,33
					Alcohols Sum					**692,43**	**722,86**			
864	0,552	Nonanal	124-19-6	936	Aldehydes	Alifatic	-	1108	1121[78]	11,26	13,95	12,60	1,90	15,08
224	0,880	Hexanal	66-25-1	940	Aldehydes	Alifatic	-	803	804[74]	105,07	66,02	85,54	27,61	32,28
448	1,184	Heptanal	111-71-7	943	Aldehydes	Alifatic	-	882	907[70]	5,53	3,59	4,56	1,37	30,15
832	1,072	2-Propenal, 3-(dimethylamino)-	927-63-9	786	Aldehydes	Alifatic	amino		1068	2,91	-	-	-	-
840	0,648	2-Octenal, (E)-	2548-87-0	921	Aldehydes	Alifatic	with =	1034	1078[70]	5,66	2,21	3,94	2,44	61,93
664	2,024	2-Heptenal, (E)-	18829-55-5	945	Aldehydes	Alifatic	with =	942	967[70]	9,03	-	-	-	-
848	0,792	Benzaldehyde, 2-methyl-	529-20-4	742	Aldehydes	Aromatic	-	1066	1090[73]	3,19	1,98	2,59	0,86	33,02
832	0,976	Phenyl acetaldehyde	122-78-1	815	Aldehydes	Aromatic	-	1012	1067[70]	1,57	1,76	1,67	0,13	8,04
928	0,696	Benzeneacetaldehyde, ethylidene-	4411-89-6	674	Aldehydes	Aromatic	with =	1237	1302[70]	2,01	-	-	-	-
					Aldehydes Sum					**146,24**	**89,51**			
144	5,304	2-Pentanamine, 4-methyl-	108-09-8	915	Amines	Alifatic	-	732	767	5,44	-	-	-	-
					Amines Sum					**5,44**	**0,00**			
192	0,712	Acetic acid, 2-methylpropyl ester	110-19-0	959	Esters	Alifatic	-	741	788[70]	74,97	65,71	70,34	6,55	9,31
320	0,816	2-Pentanol, acetate	626-38-0	952	Esters	Alifatic	-	820	850	2,69	2,22	2,45	0,33	13,47
368	4,928	1,2-Ethanediol, diacetate	111-55-7	956	Esters	Alifatic	Diester		875	109,22	80,47	94,84	20,33	21,43
944	0,576	Ethanol, 2-(2-butoxyethoxy)-, acetate	124-17-4	820	Esters	Alifatic	dioxide	1361,6	1369[70]	3,48	6,48	4,98	2,12	42,61
152	0,936	Propanoic acid, 2-hydroxy-, ethyl ester	97-64-3	893	Esters	Alifatic	Hydroxyl	787	769[70]	33,23	29,51	31,37	2,63	8,37
376	1,392	1-Methoxy-2-propyl ester of acetic acid	108-65-6	980	Esters	Alifatic	oxide		877	2,48	4,64	3,56	1,53	42,96
784	1,512	Pentanoic acid, 4-oxo-, methyl ester	624-45-3	927	Esters	Alifatic	oxide		1002	2,40	1,52	1,96	0,62	31,66
152	1,592	Propanoic acid, 2-oxo-, methyl ester	600-22-6	864	Esters	Alifatic	oxide	722	770	2,06	0,89	1,48	0,83	56,02

896	0,632	8,11-Octadecadiynoic acid, methyl ester	18202-23-8	652	Esters	Alifatic	with =		1202	15,03	8,75	11,89	4,44	37,32
992	0,784	Diethyl Phthalate	84-66-2	947	Esters	Aromatic	Diester	1585	1636[79]	0,62	1,04	0,83	0,30	35,56
					Esters Sum					246,16	201,23			
152	0,552	Ethane, 1,1-diethoxy-	105-57-7	734	Ethers	Alifatic	diether	730	769[80]	-	3,53	-	-	-
808	0,944	Propane, 1,2-dimethoxy-	7778-85-0	883	Ethers	Alifatic	diether		1034	1,45	-	-	-	-
800	0,928	Dipropylene glycol monomethyl ether	34590-94-8	880	Ethers	Alifatic	Hydroxyl	983	1023	1,50	0,96	1,23	0,38	30,76
					Ethers Sum					2,95	4,49			
776	2,576	1H-Imidazole, 1-methyl-	616-47-7	932	Heterocyclic	Alcaloid	Imidazol		998	212,00	-	-	-	-
888	0,704	4,5-Dihydrooxazole-5-one, 4-chloromethylene-2-phenyl-	14848-36-3	549	Heterocyclic	Azoles	Oxazoles		1181	3,06	-	-	-	-
304	2,024	Ethanone, 1-(3,3-dimethyloxiranyl)-	4478-63-1	909	Heterocyclic	Epoxide	oxide	780	843	4,58	5,33	4,96	0,52	10,59
784	0,656	Furan, 2-pentyl-	3777-69-3	917	Heterocyclic	Furanes	-	1001	1000[72]	3,12	-	-	-	-
864	0,704	Benzylidenemalonaldehyde	82700-43-4	688	Heterocyclic	Furanes	Aromatic		1121	5,78	2,91	4,35	2,02	46,59
936	0,680	Furan, 2,2'-[oxybis(methylene)]bis-	4437-22-3	891	Heterocyclic	Furanes	difuran	1292	1336[70]	6,58	6,71	6,65	0,09	1,39
824	0,984	2,2'-Bifuran	5905-00-0	707	Heterocyclic	Furanes	difuran	1065	1056	1,93	1,40	1,67	0,37	22,27
856	0,712	2-Furanmethanol, propanoate	623-19-8	646	Heterocyclic	Furanes	ester	1084	1101[73]	7,60	-	-	-	-
792	1,208	2-Furanmethanol, acetate	623-17-6	929	Heterocyclic	Furanes	ester	991	1012[81]	22,00	15,81	18,91	4,37	23,14
152	0,552	endo-2,3-O-Ethylidene-á-d-erythrofuranose	77519-84-7	580	Heterocyclic	Furanes	Furanose		769	3,01	-	-	-	-
960	0,792	(5-Chloro-2-nitro-phenyl)-furan-2-ylmethyl-amine	347355-88-8	865	Heterocyclic	Furanes	Halogens		1455	1,86	1,57	1,71	0,20	11,78
944	0,680	7-Benzofuranol, 2,3-dihydro-2,2-dimethyl-	1563-38-8	703	Heterocyclic	Furanes	Hydroxyl	1391	1369[70]	7,01	-	-	-	-
368	2,320	2-Furanmethanol	98-00-0	969	Heterocyclic	Furanes	Hydroxyl	819	874[70]	484,32	523,06	503,69	27,40	5,44
880	0,696	1-Propanone, 1-(5-methyl-2-furanyl)-	10599-69-6	674	Heterocyclic	Furanes	oxide		1161	1,99	-	-	-	-
824	1,008	2-Acetyl-5-methylfuran	1193-79-9	846	Heterocyclic	Furanes	oxide		1057	3,19	2,29	2,74	0,64	23,26
800	1,224	1-Propanone, 1-(2-furanyl)-(CAS)	3194-15-8	905	Heterocyclic	Furanes	oxide		1023	2,52	1,70	2,11	0,58	27,52
824	1,336	2,5-Furandione, 3,4-dimethyl-	766-39-2	918	Heterocyclic	Furanes	oxide		1057	9,28	5,79	7,53	2,47	32,77
696	7,360	5 methyl furfural	620-02-0	968	Heterocyclic	Furanes	oxide	924	977[70]	83,85	49,09	66,47	24,58	36,99

920	0,672	Furan, 2-[(methyldithio)methyl]-	57500-00-2	677	Heterocyclic	Furanes	Sulfour	1243	1277	3,57	-	-	-	-
864	1,048	2-Furanmethanethiol	98-02-2	595	Heterocyclic	Furfurals	Thiol		1122	5,76	6,40	6,08	0,45	7,38
280	4,816	Furfural	98-01-1	959	Heterocyclic	Furfurals		794	833[70]	189,77	116,86	153,31	51,56	33,63
864	0,640	3-Methoxyhex-1-ene	108811-41-2	577	Heterocyclic	Nitrile			1121	4,05	-	-	-	-
272	1,840	Methoxycyclopentaneacetonitrile	99765-38-5	912	Heterocyclic	Nitrile			827	1,90	-	-	-	-
824	1,880	2H-Pyran-2-one, 5,6-dihydro-	3393-45-1	947	Heterocyclic	Pyranes	oxide		1058	1,38	0,73	1,06	0,46	43,60
872	1,168	4H-Pyran-4-one, 3-hydroxy-2-methyl-	118-71-8	926	Heterocyclic	Pyranes	hydroxyl	1111	1142[82]	10,41	5,86	8,14	3,22	39,54
920	0,552	2-Butyl-3-methylpyrazine	15987-00-5	706	Heterocyclic	Pyrazines	-		1276	2,13	1,83	1,98	0,21	10,65
888	0,560	Pyrazine, 3,5-diethyl-2-methyl-	18138-05-1	851	Heterocyclic	Pyrazines	-	1206	1181	14,78	6,74	10,76	5,68	52,83
880	0,576	2-Isobutyl-3-methylpyrazine	13925-06-9	613	Heterocyclic	Pyrazines	-	1114	1161[70]	2,29	-	-	-	-
848	0,624	Pyrazine, 2-ethyl-3,5-dimethyl-	13925-07-0	708	Heterocyclic	Pyrazines	-	1083	1090[77]	3,15	22,43	12,79	13,64	106,59
800	0,848	Pyrazine, 2-(n-propyl)-	18138-03-9	887	Heterocyclic	Pyrazines	-	980	1023	0,92	-	-	-	-
792	0,880	Pyrazine, trimethyl-	14667-55-1	855	Heterocyclic	Pyrazines	-	999	1012[73]	12,77	-	-	-	-
264	1,728	2-methyl pyrazine	109-08-0	965	Heterocyclic	Pyrazines	-	826	823[73]	21,13	14,04	17,59	5,01	28,50
480	2,120	Pyrazine, 2,6-dimethyl-	108-50-9	919	Heterocyclic	Pyrazines	-	880	913[70]	27,98	19,51	23,74	6,00	25,25
488	2,200	Pyrazine, ethyl-	13925-00-3	950	Heterocyclic	Pyrazines	-	881	918	5,32	-	-	-	-
504	2,328	Pyrazine, 2,3-dimethyl-	5910-89-4	887	Heterocyclic	Pyrazines	-	894	922	5,19	3,39	4,29	1,28	29,77
872	0,752	2-Acetyl-3-methylpyrazine	23787-80-6	641	Heterocyclic	Pyrazines	oxide	1080	1141[73]	15,50	7,47	11,48	5,68	49,48
808	1,256	Acetylpyrazine	22047-25-2	783	Heterocyclic	Pyrazines	oxide	1017	1035	3,09	3,74	3,42	0,47	13,64
808	0,912	Pyrazine, 2-ethenyl-5-methyl-	13925-08-1	778	Heterocyclic	Pyrazines	with =	984	1034	1,75	0,98	1,37	0,54	39,56
888	0,792	2-Pyridinamine, 3,6-dimethyl-	823-61-0	655	Heterocyclic	Pyridines	amino	1212	1182	2,67	2,19	2,43	0,34	14,10
816	1,424	4(H)-Pyridine, N-acetyl-	67402-83-9	666	Heterocyclic	Pyridines			1046	1,21	-	-	-	-
496	2,176	2,5-Dimethylpyrimidine	22868-76-4	799	Heterocyclic	Pyrimidines			920	6,33	-	-	-	-
896	0,744	1H-Pyrrole, 1-(2-furanylmethyl)-	1438-94-4	912	Heterocyclic	Pyrroles	furan	1199	1202	18,06	13,45	15,76	3,26	20,69
848	0,864	Ethanone, 1-(1-methyl-1H-pyrrol-2-yl)-	932-16-1	779	Heterocyclic	Pyrroles	oxide	1050	1090[70]	7,51	4,28	5,90	2,28	38,75
832	0,920	1H-Pyrrole-2-carboxaldehyde, 1-ethyl-	2167-14-8	783	Heterocyclic	Pyrroles	oxide	1067	1067[70]	2,40	1,61	2,00	0,56	27,71
792	1,592	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	1192-58-1	922	Heterocyclic	Pyrroles	oxide	955	1015	16,99	9,31	13,15	5,43	41,30
840	1,888	Ethanone, 1-(1H-pyrrol-2-yl)-	1072-83-9	950	Heterocyclic	Pyrroles	oxide	1972	1080[75]	8,77	4,44	6,61	3,06	46,38
816	3,560	1H-Pyrrole-2-carboxaldehyde	1003-29-8	945	Heterocyclic	Pyrroles	oxide	988	1049	3,36	2,60	2,98	0,54	18,02
816	0,544	l-Limonene	5989-54-8	947	Heterocyclic	Terpenes		1030	1045[77]	12,60	11,10	11,85	1,07	8,99

864	1,728	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	13031-76-0	805	Heterocyclic	Thiopene	dioxide		1124	1,26	-	-	-	-
824	2,336	3-Thiophenemethanol	71637-34-8	940	Heterocyclic	Thiopene	hydroxyl	1037	1058	3,88	2,53	3,21	0,96	29,96
888	0,752	2-Acetyl-3-methylthiophene	13679-72-6	662	Heterocyclic	Thiopene	oxide	1119	1181	2,11	-	-	-	-
856	0,984	Ethanone, 1-(2-thienyl)-	88-15-3	931	Heterocyclic	Thiopene	oxide	1086	1102[73]	2,93	1,31	2,12	1,14	53,99
784	2,312	2-Thiophenecarboxaldehyde	98-03-3	926	Heterocyclic	Thiopene	oxide	983	1003[70]	2,22	1,48	1,85	0,52	27,94
					Heterocyclic Sum					**1288,84**	**879,95**			
856	0,448	2-Octane, 2,6-dimethyl	4057-42-5	753	Hydrocarbons	Alifatic	-		1101	15,25	47,82	31,54	23,03	73,02
816	0,464	Hexane, 2,3,4-trimethyl-	921-47-1	798	Hydrocarbons	Alifatic	-		1045	12,47	-	-	-	-
256	0,512	Heptane, 2,4-dimethyl-	2213-23-2	937	Hydrocarbons	Alifatic	-	788	819	7,56	-	-	-	-
752	0,528	Heptane, 2,4,6-trimethyl	2613-61-8	889	Hydrocarbons	Alifatic	-	916	911	3,03	3,22	3,13	0,13	4,14
440	0,560	Nonane	111-84-2	705	Hydrocarbons	Alifatic	-	899	904[73]	5,04	2,27	3,65	1,96	53,75
704	0,608	Nonane, 2-methyl-	871-83-0	906	Hydrocarbons	Alifatic	-	962	977[83]	6,15	6,19	6,17	0,02	0,40
152	0,480	Cycloheptane	291-64-5	863	Hydrocarbons	Alifatic	Cyclic		769	-	9,42	-	-	-
192	0,504	Cyclohexane, 1,3-dimethyl-, trans-	2207-03-6	814	Hydrocarbons	Alifatic	Cyclic	842	788	35,80	32,21	34,01	2,54	7,46
544	0,688	(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	7785-26-4	933	Hydrocarbons	Alifatic	Cyclic	938	933[84]	1,94	7,28	4,61	3,78	82,01
728	0,816	2-pinene	127-91-3	930	Hydrocarbons	Alifatic	Cyclic	981	984[85]	14,23	51,64	32,93	26,45	80,31
864	0,856	1,2-Pentadiene	591-95-7	747	Hydrocarbons	Alifatic	with =		1122	12,79	8,38	10,58	3,12	29,47
152	1,000	1-Pentene, 3-methyl-	760-20-3	805	Hydrocarbons	Alifatic	with =		769	24,74	25,50	25,12	0,54	2,14
872	1,008	3-Ethyl-3-hexene	16789-51-8	758	Hydrocarbons	Alifatic	with =		1142	2,63	1,32	1,97	0,93	47,15
816	0,592	Benzene, 1-methyl-3-(1-methylethyl)-	535-77-3	919	Hydrocarbons	Aromatic	-	1013	1045[70]	-	1,54	-	-	-
776	0,784	Benzene, 1,2,3-trimethyl-	526-73-8	920	Hydrocarbons	Aromatic	-	998	998[86]	0,79	0,72	0,75	0,05	6,51
184	0,792	Benzene, methyl-	108-88-3	966	Hydrocarbons	Aromatic	-	770	785[72]	219,09	209,50	214,29	6,78	3,16
352	1,024	Benzene, 1,3-dimethyl-	108-38-3	974	Hydrocarbons	Aromatic	-	867	865[87]	10,66	9,43	10,05	0,87	8,63
408	1,192	p-Xylene	106-42-3	969	Hydrocarbons	Aromatic	-	888	892[78]	2,62	2,18	2,40	0,31	13,13
408	1,600	1,3,5,7-Cyclooctatetraene	629-20-9	957	Hydrocarbons	Aromatic	-	880	893	4,41	-	-	-	-
					Hydrocarbons Sum					**379,21**	**418,61**			
168	0,696	3-Pentanone, 2-methyl-	565-69-5	921	Ketones	Alifatic	-		777	29,39	24,70	27,05	3,32	12,26
160	0,704	2-Pentanone, 4-methyl-	108-10-1	938	Ketones	Alifatic	-	733	773[88]	32,98	25,45	29,21	5,33	18,23

168	0,728	2-Pentanone, 3-methyl-	565-61-7	565-61-7	Ketones	Alifatic	-		777	-	35,97	-	-	-
208	0,800	3-Hexanone	589-38-8	885	Ketones	Alifatic	-	756	796[70]	9,45	6,57	8,01	2,04	25,45
824	0,888	2,3-Dimethyl-2-cyclopenten-1-one	1121-05-7	791	Ketones	Alifatic	Cyclic		1056	2,96	1,83	2,39	0,80	33,49
208	1,256	Cyclopentanone	120-92-3	969	Ketones	Alifatic	Cyclic	767	796[89]	86,45	75,03	80,74	8,07	10,00
816	1,912	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	80-71-7	963	Ketones	Alifatic	Cyclic		1047	6,34	3,77	5,05	1,82	35,99
280	2,608	2-Cyclopenten-1-one	930-30-3	936	Ketones	Alifatic	Cyclic		832	2,96	2,10	2,53	0,61	24,04
816	1,016	3-Methyl-2,4-hexanedione	4220-52-4	856	Ketones	Alifatic	diketones		1045	1,27	0,76	1,01	0,36	35,63
200	1,264	2,4-Pentanedione	123-54-6	945	Ketones	Alifatic	diketones	783	792[90]	5,77	4,23	5,00	1,09	21,85
552	5,216	2,5-Hexanedione	110-13-4	978	Ketones	Alifatic	diketones	933	937[90]	4,97	3,76	4,37	0,86	19,64
208	0,864	2-Heptanone, 7,7,7-trichloro-	154264-40-1	895	Ketones	Alifatic	Halogens		796	12,91	9,91	11,41	2,12	18,57
176	0,688	pentanal, 2,4-dimethyl	27944-79-2	833	Ketones	Alifatic	hydroxyl		781	7,34	-	-	-	-
296	2,664	2-Pentanone, 4-hydroxy-4-methyl-	123-42-2	965	Ketones	Alifatic	hydroxyl	840	839[91]	97,58	69,26	83,42	20,03	24,01
240	2,672	2-Hydroxy-3-pentanone	5704-20-1	853	Ketones	Alifatic	hydroxyl	817	812	1,47	1,40	1,44	0,05	3,13
184	2,984	1-Hydroxy-2-butanone	5077-67-8	910	Ketones	Alifatic	hydroxyl	798	786	4,19	4,01	4,10	0,13	3,06
256	4,448	2-Pentanone, 4-hydroxy-	4161-60-8	840	Ketones	Alifatic	hydroxyl	849,4	821[70]	6,09	4,22	5,15	1,33	25,73
816	1,520	2-Cyclohexene-1,4-dione	4505-38-8	804	Ketones	Alifatic	oxide	1032	1046[70]	1,74	1,07	1,40	0,48	34,04
744	3,280	2-Butanone, 1-(acetyloxy)-	1575-57-1	910	Ketones	Alifatic	oxide		989	8,00	5,45	6,72	1,80	26,75
496	6,352	2-Pentanone, 5-methoxy-	17429-04-8	731	Ketones	Alifatic	oxide		921	277,67	-	-	-	-
880	0,872	2-Hepten-4-one, 2-methyl-	22319-24-0	674	Ketones	Alifatic	with =		1162	0,98	-	-	-	-
224	1,032	3-Penten-2-one, 4-methyl-	141-79-7	954	Ketones	Alifatic	with =	801	804[86]	4,74	2,98	3,86	1,24	32,19
840	0,928	Ethanone, 1-phenyl-	98-86-2	940	Ketones	Aromatic	-	1065	1079[73]	3,15	2,82	2,98	0,24	7,98
					Ketones Sum					**608,41**	**285,28**			
840	1,032	2(3H)-Furanone, 5-ethyldihydro-	695-06-7	706	Lactones				1055	1079[86]	1,12	0,73	0,92	0,27	29,57
840	1,304	2H-Pyran-2-one, tetrahydro-	542-28-9	940	Lactones				1079	5,52	2,88	4,20	1,87	44,49
832	1,776	2(5H)-Furanone, 5-methyl-	591-11-7	946	Lactones				1069	3,73	1,95	2,84	1,26	44,36
768	2,232	2(3H)-Furanone, dihydro-5,5-dimethyl-	3123-97-5	866	Lactones			991,7	966[70]	3,85	2,03	2,94	1,29	43,80
360	3,824	2(3H)-Furanone, 5-methyl-	591-12-8	943	Lactones			920	871[92]	3,78	6,15	4,96	1,68	33,76
					Lactones Sum					**17,99**	**13,73**			

360	0,976	2-Pentanol, nitrate	21981-48-6	783	Nitrate	Alifatic	-	912	869	1,13	1,67	1,40	0,38	26,83
					Nitrate Sum					1,13	1,67			
344	5,616	Hydroperoxide, 1-ethylpropyl	24254-57-7	835	Peroxide				864	35,70	35,70	35,70	0,00	0,00
344	5,928	Hydroperoxide, 1-methylbutyl	14018-58-7	943	Peroxide				864	104,17	130,43	117,30	18,57	15,83
					Peroxide Sum					139,87	166,14			
					Total Sum					3560,19	2811,64			

4. Results and discussion

The coffee residue composition is rich in polysaccharides (80 %, dry basis), which can be further extracted using microwaves. The other main compounds are lipids (13 %) and proteins (5 %).

During the first cycle of microwave extraction, arabinogalactans are the main polysaccharides extracted, whereas during the second cycle the amount of extracted galactomannans increases surmounting significantly the amount of arabinogalactans.

After the microwave extraction most of the amino acids remain in residue (5.9 %), and only 1 % (w/w) of the supernatant content being amino acids, without significant change in the composition.

The oil content is about 13% of the total weight, remaining mainly in the residue after the microwave extractions. In general, the antioxidant properties of the oil surmount other oils such as the grape seed and olive oil, even after the microwave treatments which clearly affects the AOC of the resulting oil. Antioxidant activity is also shown by the coffee residues and supernatants, the last one being the highest. It is expected that some of the compounds possessing antioxidant properties are being extracted during the microwave treatments, which would simultaneously cause the increasing of the supernatants and decreasing of the coffee residues AOC.

The oil obtained from the coffee residue had an enjoyable and characteristic coffee smell, for which mainly furanes, pyrazines, pyranes and ketons have been hold responsible.

These results can be the basis for future applications in the extraction of water soluble polysaccharides using microwaves. In addition, the pleasant coffee aroma associated to a high antioxidant capacity of coffee oil, even after the microwave treatment, has shown a high potential for future applications, e.g. in food products and cosmetics.

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