The lipid fraction of the coffee bean

Karl Speer and Isabelle Kölling-Speer

*Institute of Food Chemistry, Technische Universität Dresden, Germany. *Corresponding author: karl.speer@chemie.tu-dresden.de*

The lipid fraction of coffee is composed mainly of triacylglycerols, sterols and tocopherols, the typical components found in all common edible vegetable oils. Additionally, the so-called coffee oil contains diterpenes of the kaurene family in proportions of up to 20 % of the total lipids. Diterpenes are of interest because of their analytical and physiological effects. The composition of the main lipid components of the two most important coffee species, *Coffea arabica* and *Coffea canphora* var. Robusta is presented. In addition, the influences of typical processes like roasting and steaming on selected lipid components as well as the effects of the storage of green coffee beans under different conditions will be described. Furthermore, new findings regarding the 5-hydroxytryptamides, the main parts of the coffee wax located on the outer layer of the bean and the recently identified components coffeadiol and arabiol I will also be discussed.
Key words: *Coffea*, coffee oil, coffee wax, diterpenes, 5-hydroxytryptamides.

A fração lipídica da semente de café: A fração lipídica do café é composta principalmente de triacilgliceróis, esteróis e tocoferóis, componentes típicos encontrados em todo óleo vegetal comestível comum. Adicionalmente, o chamado oleo de café contém diterpenos da família dos kaurenos, em proporção de até 20 % dos lipídeos totais. Diterpenos são de interesse por causa de seus efeitos fisiológicos. As composições dos principais componentes lipídicos das duas espécies mais importantes de café, *Coffea arabica* e *Coffea canphora* var. Robusta são apresentadas. Também, serão descritas as influências de processos tais como torração e "steaming" sobre determinados components lipídicos, assim como os efeitos do armazenamento do café verde sob diferentes condições. Além disso, serão discutidas as novas descobertas sobre as 5-hidroxitriptamidas, os principais componentes da cera de café, localizada nas camadas externas da semente, e os compostos "coffeadiol" e "arabiol I", recentemente identificados.
Palavras-chave: *Coffea*, cera de café, diterpenos, óleo de café, 5-hidroxitriptamidas.

INTRODUCTION

The two most important coffee species, Coffea Arabica and Coffea canephora var. Robusta, contain between 7 and 17 % fat. The lipid content of green Arabica coffee beans averages some 15 %, whilst Robusta coffees contain much less, namely around 10 %. Most of the lipids, the coffee oil, are located in the endosperm of green coffee beans (Wilson et al., 1997); only a small amount, the coffee wax, is located on the outer layer of the bean.

Coffee oil is composed mainly of triacylglycerols with fatty acids in proportions similar to those found in common edible vegetable oils. The relatively large unsaponifiable fraction is rich in diterpenes of the kaurane family, mainly cafestol, kahweol and 16-O-methylcafestol, which have been receiving more and more attention in recent years due to their different physiological effects. Furthermore, 16-O-methylcafestol serves as a reliable indicator for Robusta coffee in coffee blends. Among the sterols, also a part of the unsaponifiable matter, various desmethyl-, methyl- and dimethylsterols have been identified. The composition of the lipid fraction of green coffee is given in table 1.

Coffee oil

Determination of total oil content: The yield of crude lipid is a function not only of the composition of the bean but also of the conditions of extraction, particularly particle size and surface area, choice of solvent and duration of extraction. One standard method is that given by the AOAC (1965). The

Table 1. Composition of lipids of green coffee (data from Maier, 1981)

Compounds	% dry matter
Triacylglycerols	75.2
Esters of diterpene alcohols and fatty acids	18.5
Diterpene alcohols	0.4
Esters of sterols and fatty acids	3.2
Sterols	2.2
Tocopherols	0.04 - 0.06
Phosphatides	0.1 - 0.5
Tryptamine derivatives	0.6 - 1.0

Soxhlet extraction is carried out over 16 h using petroleum ether (35°-50°C boiling range). In the method of the German Society for Lipid Science (DGF) published in 1952, the material is ground, then dried at 105°C for 30-35 min (if the moisture content exceeds 10 %), and extracted for 4 h with petroleum ether (40°–55 °C boiling range). Streuli (1970) treated the ground green coffee with acid prior to extraction; his method became an official Swiss method. In connection with the great differences in yield just mentioned, the term "coffee oil" needs to be defined more explicitly.

Isolation of coffee oil for detailed analysis: For obtaining a coffee oil to be used for studying its chemical composition in detail, direct solvent extraction without acid treatment is necessary. According to Picard et al. (1984), several authors used diethyl ether, petroleum ether with different boiling point ranges, n-hexane and a mixture of diethyl ether and n-hexane. The results are not homogeneous because they depend on the selected solvent. In some cases, polar or non-lipid substances such as caffeine were extracted.

Picard et al. observed that with increasing extraction time the oil content of a Robusta coffee slightly rose for extraction with hexane/diethyl ether for 6 and 8 h (11.4 and 11.6 %) and then slightly decreased for 10 and 12 h (11.0 and 10.9 %).

Furthermore, Folstar et al. (1975) demonstrated that the yield obtainable in solvent extraction depends on the particle size to which the coffee is finally ground.

Speer (1989) extracted ground coffee of a particle size smaller than 0.63 mm and used tertiary butyl methyl ether as extraction solvent instead of the very dangerous diethyl ether. His method was adopted as a part of the DIN method 10779 (1999) and described as follows: roasted coffee beans are coarsely ground in a regular coffee mill and passed through a 0.63 mm sieve. 5 g of the sieved material are then powdered together with sodium sulphate in a mortar and extracted with tertiary butyl methyl ether in a Soxhlet (4 h) siphoning 6-7 times per hour. The solvent is evaporated and the residue is then dried to constant weight (105°C). Longer extraction times (6, 8 or 10 hours) do not increase the lipid content. For green coffee beans, grinding in the mill is carried out together with dry ice.

Fatty acids

Total fatty acids and fatty acids in triacylglycerols: For the most part, the fatty acids are to be found in the combined state; most are esterified with glycerol in the triacylglycerols, some 20 % are esterified with diterpenes, and a small proportion is to be found in the sterol esters.

The total fatty acid composition of coffee oil has been the subject of many investigations (Wurziger, 1963; Calzolari and Cerma, 1963; Carisano and Gariboldi, 1964; Hartmann et al., 1968; Pokorny and Forman, 1970; Streuli, 1970; Roffi et al., 1971; Chassevent et al., 1974; Vitzthum, 1976; Lercker et al., 1996).

Folstar et al. (1975) and Speer et al. (1993) investigated the fatty acids in detail. They analysed the fatty acids in the triacylglycerols of coffee beans and in the diterpene esters (Kurzrock and Speer, 2001). The fatty acids in sterol esters were determined by Picard et al. (1984).

For separating the different lipid classes Folstar et al. (1975) used a Florisil column. Speer et al. (1993) isolated the triacylglycerols by means of gel permeation chromatography, transesterified them with potassium methylate and chromatographed the methylated fatty acids using a 60 m fused silica capillary column coated with RTX 2330 (table 2).

During roasting there were only small changes in the fatty acid composition (Vitzthum, 1976). Casal et al. (1997) and then Alves et al (2003) reported that in Arabica and Robusta coffee the roasting process increased the trans-fatty acid levels, specifically the contents of $C_{18:2ct}$ and $C_{18:2tc}$.

Nonetheless, their literature overview is incomplete; therefore, data from earlier publications could not consider in the discussion of the results.

Folstar (1985) studied the positional distribution of the fatty acids in the triglyceride molecule. A technique was used whereby sn-1,2 (2,3)-diglycerides, sn-2-monoglycerides and fatty acids were obtained from triacylglycerols through partial deacylation using pancreatic lipase. It was shown that the unsaturated acids, especially linoleic acid, are preferably esterified with the secondary hydroxyl position in glycerol.

Later, Nikolova-Damyanova et al. (1998) and Jham et al. (2003) analysed the composition of the major triacylglycerols in coffee lipids. The latter used RP-HPLC with a refractive index and RP-HPLC with a light scattering detector, respectively. No significant differences in the triacylglycerols compositions due to the type, origin and drying procedure were found.

Free fatty acids: The presence of free fatty acids (FFA) in coffee has been described by various authors (Kaufmann and Hamsagar, 1962; Calzolari and Cerma, 1963; Carisano and Gariboldi, 1964; Wajda and Walczyk, 1978). All their data are expressed by the acid value, a common but indirect determination procedure used in the analysis of fat. In the case of coffee this titration method is only very approximate for it includes not only the free fatty acids themselves but other acid compounds as well. Therefore, Speer et al. (1993) developed a method to determine the free fatty acids directly. Using the gel chromatographic system with BioBeads S-X3 mentioned above, the coffee lipids extracted with tertiary butyl methyl ether can be divided into three individual fractions: a fraction with the triacylglycerols, a fraction containing the diterpene fatty acid esters, and one with the free fatty acids. The latter were converted with BF_3/methanol and determined by capillary gas chromatography as methyl esters.

Nine different free fatty acids were detected, which are similarly distributed in the Robusta and Arabica coffees, respectively. In both coffee species the main fatty acids are $C_{18:2}$ and C_{16}. It was also possible to detect large proportions of C_{18}, $C_{18:1}$, C_{20} and C_{22}, but only minor traces of C_{14}, $C_{18:3}$ and C_{24}. Differences between Arabica and Robusta only become visible when their stearic acid and oleic acid content is compared on the chromatograms (figure 1).

Table 2. Fatty acids in triacylglycerols of green coffee beans (%).

	Folstar (1975) from dewaxed green beans	Speer (1993)	Speer (1993)
		Robusta (n=9)	Arabica (n= 4)
$C_{14:0}$	0.2	traces	traces
$C_{15:0}$		traces	traces
$C_{16:0}$	33.3	27.2-32.1	26.6-27.8
$C_{16:1}$		traces	traces
$C_{17:0}$		traces	traces
$C_{18:0}$	7.3	5.8-7.2	5.6-6.3
$C_{18:1}$	6.6	9.7-14.2	6.7-8.2
$C_{18:2}$	47.7	43.9-49.3	52.2-54.3
$C_{18:3}$	1.7	0.9-1.4	2.2-2.6
$C_{19:0}$		traces	traces
$C_{20:0}$	2.5	2.7-4.3	2.6-2.8
$C_{20:1}$		0.2-0.3	traces-0.3
$C_{21:0}$		traces	traces
$C_{22:0}$	0.5	0.3-0.8	0.5-0.6
$C_{23:0}$		traces	traces
$C_{24:0}$	traces	0.3-0.4	0.2-0.4

While the proportion of stearic acid is noticeably smaller than that of oleic acid in the Robustas, the percentages of these two acids in the Arabica coffees are almost equal. The ratio stearic acid/ oleic acid may give a first indication of Robusta in coffee blends.

The content of individual free fatty acids for freshly harvested green coffees seems to be very low. For a Brazilian coffee that was prepared fresh, both in a wet and dried manner, only contents of free fatty acids of about 1g.kg^{-1} were found, whereas a 10-year-old coffee from Brazil contained more than 30 g.kg^{-1}. From these data it was possible to conclude that fat-splitting enzymes exert a great influence. To prove this assumption Speer et al. (2004) analysed coffees of different ages with a modified lipase test kit. The test was based on the hydrolysis of a specific substrate, the 1,2-O-dilauryl-rac-glycero-3-glutaric acid-resorufin ester. The intensity of the red coloured resorufin (λ_{max} = 572 nm) is proportional to the activity of the lipase.

A lipase activity could be detected in all green coffees investigated, even in 10-year-old Brazilian coffees. This may be the reason for high contents of free fatty acids in older green coffees. To study this relation in detail, a raw coffee from Colombia was investigated. Aside from 25°C for

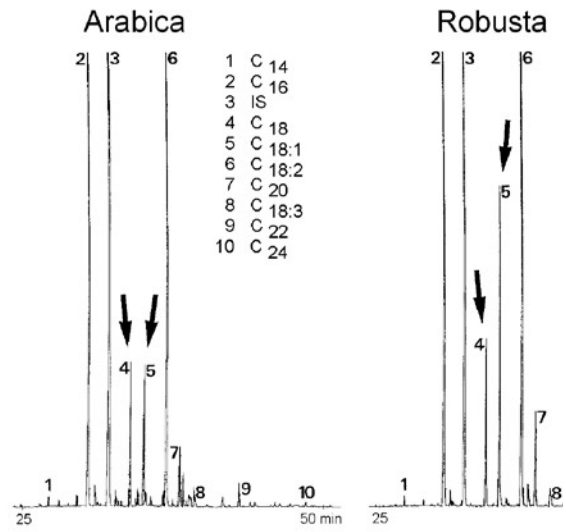

Figure 1.GC chromatograms of methylated free fatty acids. IS = Internal standard heptadecanoic acid ethyl ester. From Speer et al. (1993).

standard requirements, storage experiments were carried out at 12°C, a temperature which applies to most of the Hamburg storage warehouses. Exemplary experiments at 40°C were carried out as well, thereby simulating accelerated storage. Since changes may, to a large degree, be influenced by the water content, the effects of dry storage (water content 6.2 %) normal storage (water content 11.8 %) and wet storage (water content 13.5 %) were compared. Furthermore, the effects of storing raw coffee under a controlled atmosphere were investigated where the oxygen content was maintained at 2 % and 5 %. All in all, 1000 kg of raw coffee were packaged, for which we used special bags, filling each of them with 2 kg of raw coffee beans and the bags stored for 18 months.

The results of the raw coffees stored at 25°C are presented in detail in figure 2. For coffee with the original moisture content, a steady increase from 1.8 $g.kg^{-1}$ to approximately 3.8 $g.kg^{-1}$ could be detected within the 18 months. While the composition of the atmosphere apparently does not affect the content of free fatty acids, moisture, on the other hand, exerts a strong influence. On closer examination, only a small increase can be noticed in the dried coffee. The content of free fatty acids stagnates at a low level, i.e even after 18 months the content of free fatty acids was found to be only at 1.9 to 2.3 $g.kg^{-1}$. The highest increase, however, was observed in the moisturised coffees. In these samples the content rose to almost 4.8 $g.kg^{-1}$.

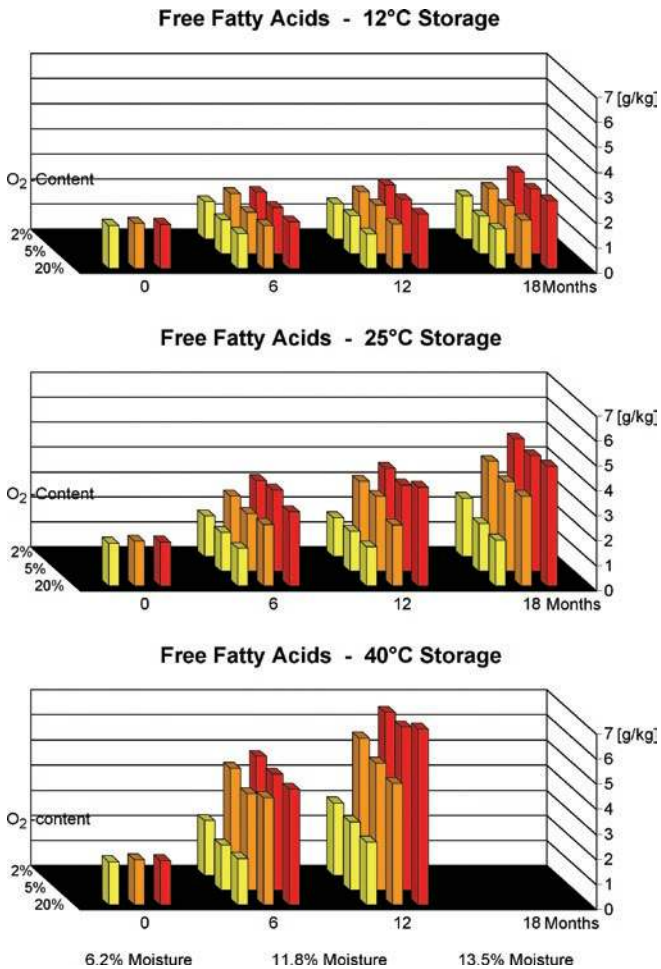

Figure 2. Contents of free fatty acids in relation to temperature, oxygen content and moisture.

Besides moisture, temperature also exerts a strong influence. The first graph in figure 2 shows the values determined for the raw coffees stored at 12°C. Here, as seen for the coffees stored at 25°C, the highest increase was observed in the moisturised coffees after 18 months. However, the content of free fatty acids was only at a maximum of 2.7 $g.kg^{-1}$ which is slightly, but not significant, higher, than in the dried coffee stored at 25°C.

Particular emphasis should be placed on the results from the raw coffees stored at 40°C. Except for the dried coffee, pronounced changes already took place after as little as three months. This especially refers to the moisturised coffees. After one year the content of free fatty acids in these coffees increased up to 7 $g.kg^{-1}$. However, no changes in the ratio of single fatty acids are detectable. Therefore, all the different fatty acid esters must be hydrolysed to the same degree.

The investigations at 40°C were stopped after one year because the brews produced from the roasted coffee were simply not worth discussing.

Diterpenes

Diterpenes in coffee are mainly pentacyclic diterpene alcohols based on the kauran skeleton. The structure of two of the coffee diterpenes, namely kahweol and cafestol, were elucidated by several work groups (Bengis and Anderson (1932), Chakravorty et al. (1943), Wettstein et al. (1945), Haworth and Johnstone (1957), Finnegan and Djerassi (1960)). Both are sensitive to acids, heat and light, and especially kahweol is unstable in purified form. In 1989, 16-O-methylcafestol (16-OMC) was isolated from Robusta coffee beans and its structure was elucidated by synthesis (Speer and Mischnick, 1989; Speer and Mischnick-Lübbecke, 1989). With 16-O-methylkahweol a further diterpene has been found in Robusta coffee beans by Kölling-Speer and Speer (2001). The structural formulae of these diterpenes are assembled in figure 3.

Arabica coffees contain cafestol and kahweol, and Robusta coffees contain cafestol, small amounts of kahweol and, additionally, 16-OMC (figures 4 and 5)(Speer

and Mischnick-Lübbecke, 1989; Speer and Montag, 1989; Speer et al., 1991b). The absence of 16-OMC in Arabica coffee beans was confirmed later by White (1995), Frega et al. (1994), Trouche et al. (1997) and by Kamm et al. (2002). Because of its stability even during the roasting process, 16-OMC has become the ideal quality characteristic for reliably detecting Robusta in Arabica coffee blends (Speer et al., 1991b; Kölling-Speer et al., 2001; Speer et al., 2005).

It should be mentioned here, that although 16-O-methylcafestol was not detectable in Arabica coffee beans, it has clearly been found in other parts of the Arabica coffee plant, for instance in the leaves (Kölling-Speer and Speer, 1997).

16-O-methylkahweol, clearly identified using different spectroscopic methods (Kölling-Speer and Speer, 2001), was detected in various Robusta coffees, in both, green and roasted beans (Kölling-Speer et al., 2001). These findings are in contrast to the statement by De Roos et al. (1997) who described this diterpene only tentatively as a 16-O-methyl derivative of kahweol and as being present exclusively in *Coffea stenophylla.*

In beans of Coffea Arabica, Wahlberg et al. (1975) isolated and identified ent-16-kauren-19-ol, a diterpene alcohol without the furan ring.

The three diterpenes cafestol, kahweol, and 16-OMC are mainly esterified with various fatty acids. In order to analyse the total amount of the individual diterpenes, coffee oil must be saponified and the diterpenes then determined in the unsaponifiable matter by means of GC (Speer and Mischnick-Lübbecke, 1989; Frega et al., 1994) or even faster by RP-HPLC with acetonitrile/water as eluent (Nackunstz and Maier, 1987; Speer, 1989; White, 1995; Trouche et al., 1997). Kamm et al. (2002) described an analysis of 16-O-methylcafestol by on-line LC-GC.

In Germany, a validated method of 16-OMC determination in roasted coffee was published as the DIN method No. 10779 (1999) of the German institute for standardization. This DIN method based on the method by Speer (1989) allows the detection of Robusta in parts smaller than two percent in mixtures with Arabica coffees.

Free diterpenes: In their free form, the diterpenes cafestol, kahweol, and 16-OMC occur only as minor components in coffee oil. Quantifying them requires an effective separation from the major compounds of the lipid fraction, namely diterpene esters and triglycerides which interfere with the analysis. Using the gel permeation chromatographic system described for the free fatty acids, the free diterpenes could

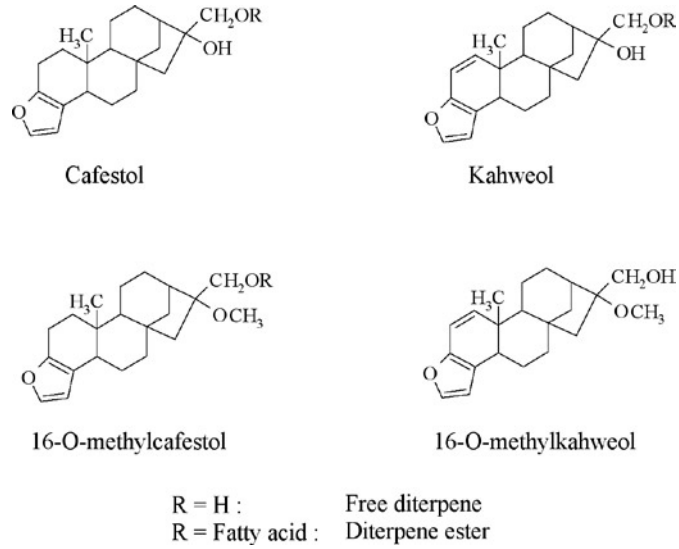

Figure 3. Structural formulae of the diterpenes.

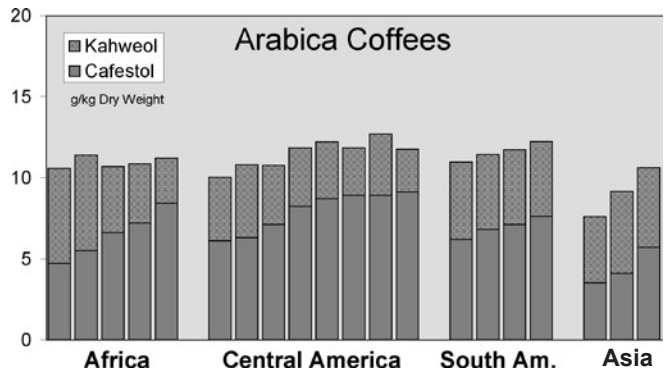

Figure 4. Contents of cafestol and kahweol in Arabica coffees.

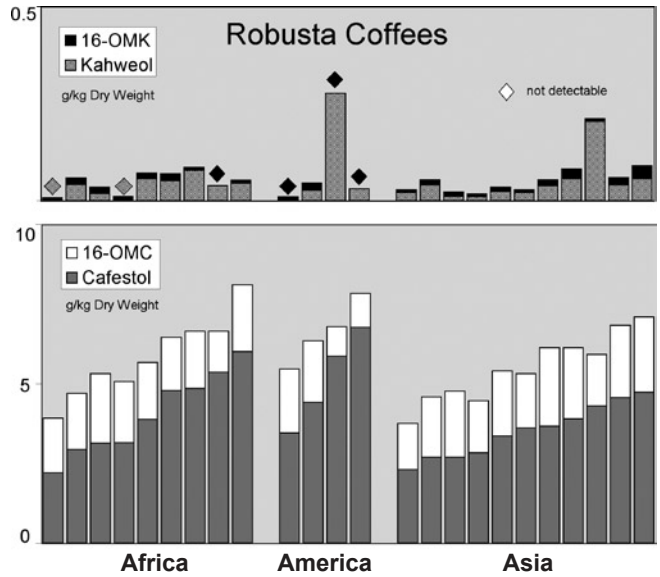

Figure 5. Contents of cafestol, 16-OMC, kahweol, and 16-OMK in Robusta coffees.

be analysed by subsequent RP-HPLC (Speer et al., 1991a; Kölling-Speer et al., 1999). In Arabica coffees, both, free cafestol and free kahweol, were determined in amounts of about 50-200 mg.kg^{-1} dry matter with mostly more cafestol than kahweol. In Robusta coffees, the free cafestol contents ranged from about 50-100 mg.kg^{-1} coffee, i.e. slightly higher than the 16-OMC contents with 10-50 mg. Only traces of kahweol could be detected in some of them.

The proportions of the free diterpenes with the total content of each are usually smaller than 3.5 %.

Influence of different storage conditions on the content of free diterpenes: As shown for the free fatty acids, the contents of free diterpenes were also influenced by the storage conditions of the green beans. Exemplarily, our results for cafestol are presented in figure 6. While during cold and dry storage of the green beans the content of the free cafestol increased only slightly, higher levels of up to 16 % of total cafestol-content were determined in wet coffees stored at 25°C or at 40°C.

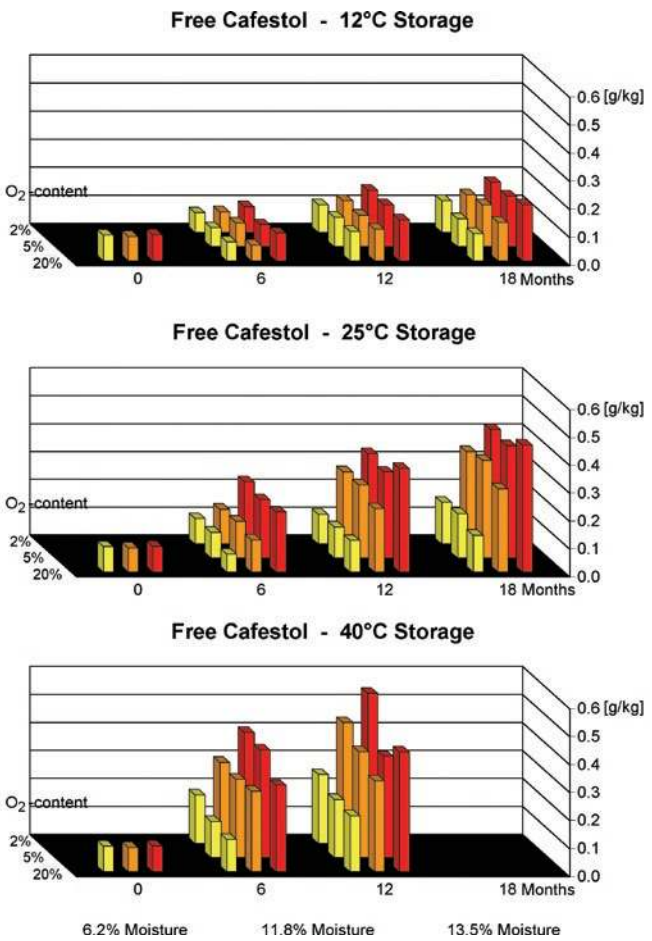

Figure 6. Contents of free cafestol in relation to temperature, oxygen content and moisture.

The reason for these different results is again the activity of the lipase. Cold temperatures and low water contents in the beans inhibit the enzyme reversibly (Kurzrock et al., 2005).

Influence of steaming on the content of free diterpenes: In order to encourage as many people as possible to drink coffee, the industry offers processed coffees besides the conventional coffees. These include decaffeinated coffees as well as caffeine-containing but steam-treated coffees. The latter are supposed to be particularly stomach-friendly. By steaming coffee beans prior to the roasting process the content of the individual free diterpenes can be altered, depending on the chosen steaming parameters (Speer and Kurt, 2001; Kurt and Speer, 2001). The concentrations of the roasting components cafestol, kahweol, dehydrokahweol and dehydrocafestol diminish with the time of treatment (figure 7). In the coffee steamed for 120 min at 2 bars the free kahweol content was below the detection limit with 0.01 mg.g^{-1} lipid. That is, the free kahweol was completely degraded by intensive steaming. Thus the lack of free kahweol is an objective indicator for steaming. Unfortunately, such a coffee steamed for 120 min at 2 bars is not accepted by the consumer. Therefore, it has proved to be difficult to assess steamed roasted coffees or particularly steamed roasted coffees with mixtures of Robusta if the untreated coffee is not available for comparative analysis.

Nevertheless if the steamed and the unsteamed coffee are available, the content of kahweol will be a helpful tool in the assessment of steamed coffees (figure 8).

Diterpene fatty acid esters: Until 1987 only a few esters with different fatty acids were reported (Kaufmann and Hamsagar, 1962a; Folstar et al, 1975; Folstar, 1985; Pettitt, 1987). The group working under Speer identified a number

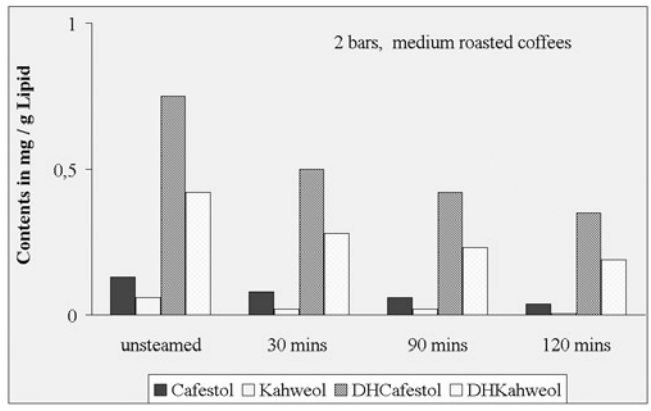

Figure 7. Contents of free diterpenes in untreated and treated roasted coffees.

of further esters of 16-OMC (Speer, 1991; Speer, 1995), of cafestol (Kurzrock and Speer, 1997a,b) and of kahweol (Kurzrock and Speer, 2001a,b). Using the gel chromatographic system described above (see the section Fatty acids), the diterpene esters were isolated together with sterol esters, which could be removed by using solid phase extraction on silica cartridges. For Arabicas, one fraction containing the cafestol and kahweol esters was achieved; a second fraction was achieved for Robustas containing the 16-O-methylcafestol esters. The subsequent analysis by RP-HPLC with acetonitrile/iso-propanol as eluent permitted the determination of the individual esters. Figure 9 shows the chromatogram of the cafestol esters of a Robusta coffee sample.

Cafestol esters with fatty acids such as C_{14}, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, C_{20}, C_{22}, C_{24} were identified as well as esters with the fatty acid $C_{20:1}$ and some odd-numbered fatty acids such as C_{17}, C_{19}, C_{21} and C_{23}. These data were proved for the fatty acids with 16-O-methylcafestol and kahweol (Kurzrock, 1998; Kurzrock and Speer., 2001a,b).

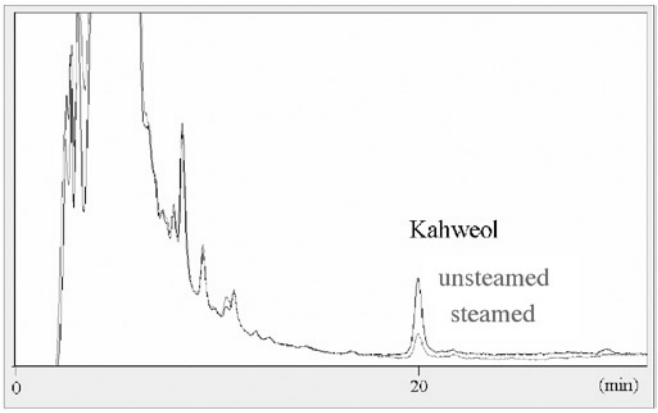

Figure 8. The kahweol signal of a steamed and of an unsteamed roasted coffee sample.

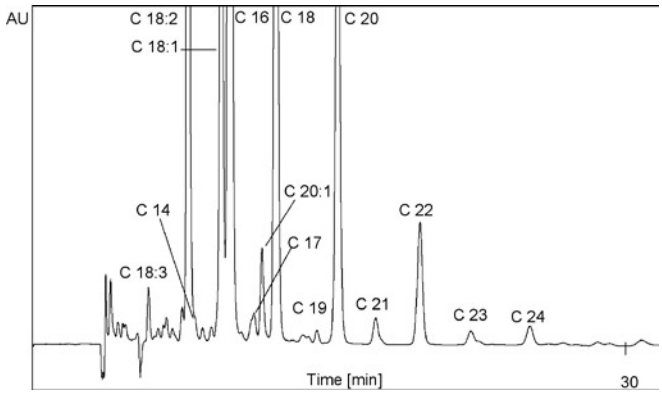

Figure 9. HPLC chromatogram of cafestol fatty acid esters. Conditions: column 250x4mm, Nucleosil 120-3 C_{18}, eluent: acetonitrile/iso-propanol (60:40), detection: UV 220 nm.

The individual diterpene esters were present in the coffee oil in irregular amounts. The odd-numbered fatty acid esters were minor components, whereas the diterpenes, esterified with palmitic, linoleic, oleic, stearic, arachidic, and behenic acid, existed in larger amounts (Speer, 1991; Speer, 1995; Kurzrock and Speer, 1997a). The focus was therefore placed on these six diterpene esters, which made up the sum of nearly 98 % of the respective diterpenes. In table 3 the distribution of the six esters are presented for Arabica coffees.

The total content of these six cafestol esters in sum ranged from 9.4-21.2 $g.kg^{-1}$ dry weight, corresponding to 5.2-11.8 $g.kg^{-1}$ cafestol in different Arabica coffees. In Robusta coffees, it was determined as between 2.2 and 7.6 $g.kg^{-1}$ dry weight, corresponding to 1.2-4.2 $g.kg^{-1}$ cafestol, notably less than in the Arabica coffees.

Diterpenes in the lipid fraction of roasted coffees: During the roasting process a number of new diterpene compounds are formed. With dehydrocafestol and dehydrokahweol two decomposition products from cafestol and kahweol were identified in roasted coffee (figure 10). The amounts of both compounds increase with raising roasting temperatures but also depend on the contents of cafestol and kahweol in the green coffee (Speer et al., 1991c; Tewis et al., 1993; Kölling-Speer et al., 1997). Nevertheless, using the ratio of cafestol and dehydrocafestol, the formation of this decomposition product is suitable as an objective characteristic for the roasting degree of coffees (Kölling-Speer et al., 1997). Thus, a ratio of 25-40 describes a well-roasted coffee, whereas a ratio of up to 15 describes a strongly roasted coffee. More strongly roasted espresso coffees, however, have a ratio of 10-15.

Cafestal and kahweal are two further degradation products of cafestol and kahweol which have been discovered

Table 3. Distribution (%) of diterpene esters in Arabica coffees.

	Cafestol Kurzrock and Speer (1997a) n = 10	Kahweol* Kurzrock (1998) n = 10
C_{16}	40 - 49	46 - 50
C_{18}	9 - 11	8 - 11
$C_{18:1}$	9 - 15	8 - 12
$C_{18:2}$	24 - 30	25- 29
C_{20}	3 - 6	3 - 6
C_{22}	0,6 - 1,2	0,7 - 1,3

* Kahweol esters calculated as cafestol esters

in the unsaponifiable matter of commercial roasted coffees in amounts of less than 0.6 mg.g^{-1} lipid for cafestal (Hruschka and Speer, 1997; Speer et al., 2000).

Recently, in commercial roasted coffees as well, with isokahweol and dehydroisokahweol (figure 11) two new diterpenes were discovered and elucidated by means of EI- and CI-high-resolution mass spectrometry and several NMR-spectroscopic methods (Kölling-Speer et al., 2005).

A typical HPLC chromatogram of a roasted coffee sample is presented in figure 12.

Surprisingly, Guerrero et al. (2005), using GC/MS, found several dehydroditerpenes and iso-components in green coffees previously found exclusively in roasted coffees. We suggest that these components may have been formed in the hot GC-injector (280°C). We had obtained similar results for green coffees when analysing diterpenes with GC/MS and split/splitless injector, and therefore to avoid such artefacts the latter was replaced by a cold-on-column injector.

In the roasted coffees, the main parts of cafestol, kahweol and 16-OMC are still esterified, although the stability behaviour of the fatty acid esters of the three diterpenes is quite different. Examination of the 16-OMC esters showed that they are clearly stable during roasting, and the proportional distribution for the individual diterpene esters remains nearly the same (Speer et al., 1993).

In contrast, the contents of the diterpene esters of cafestol and kahweol decrease depending on the roasting temperature with only little change in the distribution (Kurzrock and Speer, 1997).

Kurzrock et al. (1998) demonstrated that cafestol was dehydrated within the fatty acid esters as well. In model experiments by heating cafestol palmitate and cafestol linoleate, they obtained the corresponding dehydrocafestol esters, which have meanwhile been identified in roasted coffee, too.

Atractylosides: A further important group of diterpene derivatives found in coffee is the class of the atractylosides, which are mainly present as glycosides (Obermann and Spiteller, 1976; Maier and Wewetzer, 1978; Maier and Mätzel, 1982; Aeschbach et al., 1982; Bradbury and Balzer, 1999).

Diterpenes in coffee beverages and health aspects: Several studies have reported that through the drinking of specially prepared coffee the serum cholesterol level might increase. It was shown that this effect is caused by the lipids present in the coffee brew, which, although poorly soluble in water, could be incorporated in the brew depending on the method of infusion. Initially, triglycerides were said to be responsible for this effect but in more recent years, it has been established that it is the diterpenes, especially cafestol and kahweol, both in free form and as palmitate esters which influence the serum cholesterol level (Bak and Grobbee, 1989; Weusten-Van der Wouw et al., 1994; Mensink et al., 1995; De Roos and Katan, 1999; Terpstra et al., 2000; Boekschoten et al., 2005). Other diterpenes have not been tested yet.

Furthermore, a substantial number of scientific publications exist where the positive effects of diterpenes were

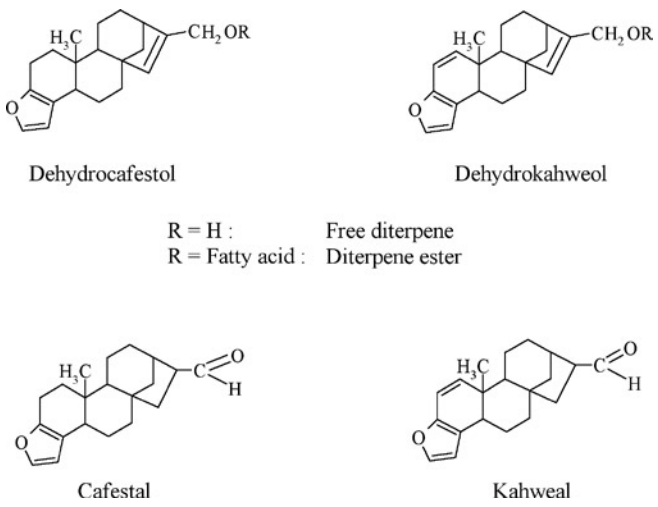

Figure 10. Structural formulae of decomposition products of cafestol and kahweol.

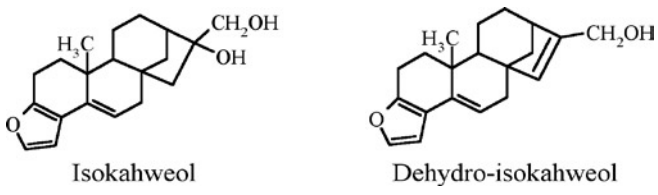

Figure 11. Structural formulae of isokahweol and of dehydro-isokahweol.

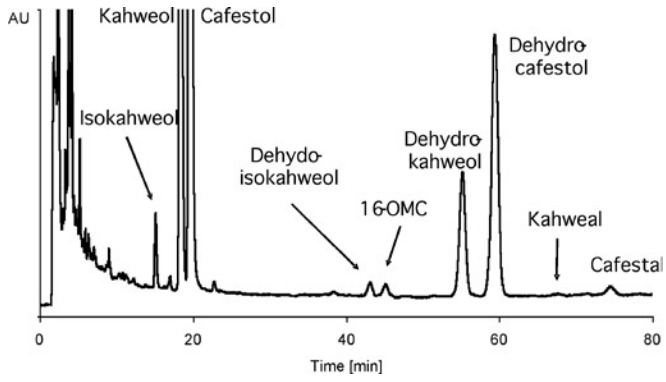

Figure 12. HPLC chromatogram of a strong-roasted Arabica coffee with 2 % Robusta.

reported. It was shown that cafestol stimulates the glutathion-S-transferase activity, through which the decomposition of xenobiotica is accelerated (Lam et al., 1982). Other authors reported that cafestol and kahweol protect against B1-induced genotoxicity (Miller et al., 1993; Cavin et al., 1998).

Therefore, investigations on the presence of diterpenes in differently prepared coffee beverages are of great interest (Ratnayake et al., 1993; Sehat et al., 1993; Urgert et al., 1995; Gross, et al., 1997).

Using the example of 16-O-methylcafestol esters, Sehat et al. (1993) were able to show that lipophile diterpene esters flow into the coffee infusion and are even detectable in instant coffee granules.

The amount in the drink is strongly dependent on the method of preparation and is directly related to the amount of total lipids in the brew. With filtered coffee prepared in a common household coffeemaker, the amount of lipids was less than 0.2 %. In contrast, when preparing an espresso, between 1-2 % of the lipids and thereby diterpenes as well, flow from the finely ground espresso coffee into the beverage.

When coffee was prepared Scandinavian style, it contained even up to 22 % of the coffee fat. The proportional distribution of diterpenes in the coffee beverage was nearly identical to the distribution in the roasted coffee powder.

In espresso prepared from Arabica coffee, a total amount of 1.3 mg cafestol fatty acid esters and 0.5 mg kahweol esters per 50 ml cup were determined by Kurzrock (1998), corresponding to approximately 1.5 % of cafestol esters and approximately 1.0 % of kahweol esters in the roasted ground coffee. These results confirm the findings for the 16-O-methylcafestol esters. In addition, the decomposition products dehydrokahweol, dehydrocafestol and cafestal as well as some esters from dehydrocafestol were identified in coffee beverages as well.

Sterols

Coffee contains a number of sterols that are also typical of other seed oils. In addition to 4-desmethylsterols, various 4-methyl- and 4,4-dimethylsterols have been identified (Nagasampagi et al., 1971; Itoh et al., 1973a,b, Tiscornia et al., 1973; Picard et al., 1984; Duplatre et al., 1984; Mariani and Fedeli, 1991; Frega et al., 1994; Speer and Kölling-Speer, 2001).

The sterols were found both in free and esterified form (Nagasampagi et al., 1971; Picard et al., 1984). The total amount is determined in the unsaponifiable matter of the coffee oil as TMS-derivatives by means of GC or GC/MS. Often, a fractionation containing desmethyl, 4-methyl- and 4,4-dimethylsterols using TLC, HPLC or silica gel cartridges was applied (Nagasampagi et al., 1971; Itoh et al., 1973a,b; Picard et al., 1984; Horstmann and Montag, 1986; Homberg and Bielefeld, 1989). The desmethylsterols represent 90 % of the total sterol fraction which ranged from 1.5 to 2.4 % of the lipids (Picard et al., 1984). Nagasampagi (1971) found higher portions with 5.4 %.

The distribution of the main desmethylsterols in different Robusta und Arabica coffee samples is presented in table 4. The main sterol is β-sitosterol with about 50 %, followed by stigmasterol and campesterol.

24-Methylenecholesterol and Δ5-avenasterol, occurring in much higher amounts in Robusta than in Arabica coffee beans, are suitable for coffee blend studies (Duplatre et al., 1984; Frega et al., 1994; Carrera et al., 1998; Valdene et al., 1999; Kamm, 2002) because the roasting process hardly effects the amounts and the distribution of the sterols (Duplatre et al., 1984; Speer and Kölling-Speer, 2001). However, because of their varying natural contents, their usefulness for determining Robusta portions in Arabica coffee mixtures is only valid from 20 % onward.

In 1984, Picard et al. studied the individual fatty acids of the sterol esters. Stearic acid, palmitic acid and oleic acid are the main compounds with a proportional distribution similar to that reported for triacylglycerols.

Tocopherols

The presence of tocopherols in coffee oil was first described by Folstar et al. (1977). α-tocopherol was clearly identified, while β- and γ-tocopherol, not separated by TLC

Table 4. Distribution (%) of desmethylsterols in Arabica and Robusta coffees (30 samples) (Mariani and Fedeli, 1991).

Sterols	Arabica	Robusta	Mean value	
			Arabica	Robusta
Cholesterol	0.2-0.4	0.1-0.3	0.3	0.2
Campesterol	14.7-17.0	15.5-18.8	15.8	16.9
Stigmasterol	20.5-23.8	20.0-26.7	21.9	23.1
β-Sitosterol	46.7-53.8	40.6-50.7	51.6	45.4
Δ_5-Avenasterol	1.6-4.1	5.1-12.6	2.7	9.1
Campestanol	0.2-0.6	0.1-0.3	0.4	0.2
24-Methylenecholesterol	0.0-0.4	1.5-2.4	0.2	1.9
Sitostanol	1.4-2.8	0.5-1.2	2.0	0.8
Δ_7-Stigmastenol	0.9-4.5	0.1-0.8	2.2	0.2
Δ_7-Avenasterol	1.2-2.1	0.2-0.6	1.5	0.4
Δ_7-Campesterol	0.4-1.2	0.1-0.6	0.6	0.2
$\Delta_{5,23}$-Stigmastadienol	0.2-0.5	0.1-2.0	0.3	0.5
$\Delta_{5,24}$-Stigmastadienol	0.0-0.4	0.0-0.3	0.1	0.0
Clerosterol	0.2-0.8	0.5-1.0	0.5	0.7

and GC, were considered as one group (figure 13). Cros et al. (1985) also determined β- and γ-tocopherol as a sum by HPLC. Folstar et al. (1977) found concentrations of α-tocopherol of 89-188 mg.kg^{-1} oil, and for β- + γ-tocopherol 252-530 mg.kg^{-1} oil.

In 1988, Aoyama et al. analysed α-, β- and γ-tocopherols in different varieties of coffee beans. They were present in approximately a 2:4:0.1 ratio, the total content being about 5.5-6.9 mg/100 g. The predominance of α-tocopherol is a prominent feature of coffee beans - in contrast to other vegetables and fruits.

Ogawa et al. (1989) determined the contents of tocopherols in 14 green coffee beans, their roasted beans and infusions, and in 38 instant coffees by HPLC. The maximum of total tocopherols in the green coffee beans was 15.7 mg.100 g^{-1} and the average was 11.9 mg.100 g^{-1}. The contents of α- and β-tocopherol were 2.3-4.5 and 3.2-11.4 mg.100 g^{-1}, respectively. γ-and δ- tocopherol were not found. Roasting diminishes the content of α-, β-tocopherol, and total tocopherols to 79-100, 84-100, and 83-99 %, respectively.

Using GC-MS, γ-tocopherol was detected in some Robusta coffees (Speer and Kölling-Speer, 2001). Incomprehensible are the results by González et al. (2001) as they found higher amounts of γ- tocopherol in roasted coffees than in green coffees.

Other compounds

Kaufmann and Sen Gupta (1964) identified squalene in the unsaponifiable matter of coffee oil. Furthermore, Folstar (1985) reported a number of both odd and even chain-length alkanes in wax-free coffee oil as well as in coffee wax.

In 1999, Kurt and Speer detected and isolated a new component with the molecular formula $C_{19}H_{30}O_2$. Its structure is similar to the known coffee diterpene cafestol. The most important differences are the absence of the furan ring and the location of one methyl group at the carbon atom C_{10}. The new component was named coffeadiol (figure 14).

With the molecular formula $C_{22}H_{28}O_2$ another substance (figure 15) was identified by Kölling-Speer et al. (2005) in a wet processed Colombian green Arabica coffee stored at 40°C. The structure is similar to that of the coffee diterpene kahweol, but instead of the furan group there is an aromatic ring. This component was named arabiol I.

Coffee wax

The surface of green coffee beans is covered by a thin waxy layer. Coffee wax is generally defined as the material obtained by extracting it from coffee beans using chlorinated organic solvents. The amount of the surface wax is about 0.2 - 0.3 % of the total bean weight. The main constituents of the petroleum ether insoluble part of the coffee wax are the so-called carboxylic acid-5-hydroxytryptamides (C-5HT). This substance group, amides of serotonine (5-hydroxytryptamine, 5HT) and fatty acids with different chain lengths, was first introduced by Wurziger and his co-workers (Dickhaut, 1966; Harms and Wurziger, 1968). They isolated and characterised three 5HT with arachidic, behenic and lignoceric acid (figure 16). Later on, Folstar described stearic acid-5HT as well as 20-hydroxy-arachidic- and 22-hydroxy-behenic acid-5HT (Folstar et al., 1979; 1980).

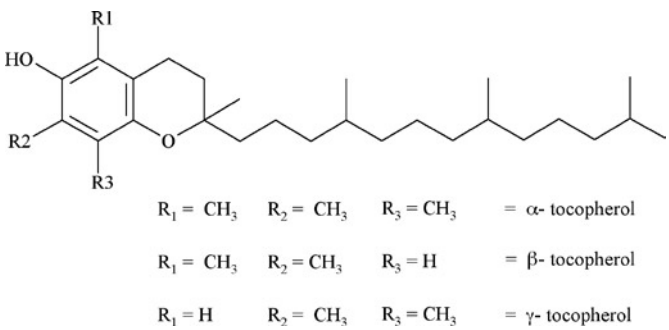

Figure 13. Structural formulae of tocopherols.

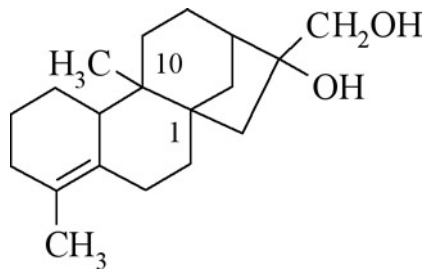

Figure 14. Structural formula of coffeadiol.

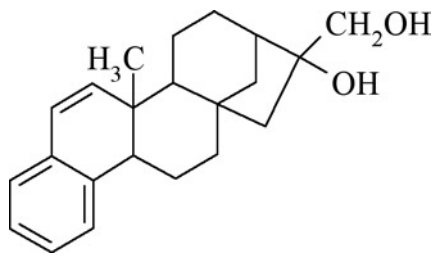

Figure 15. Structural formula of arabiol I.

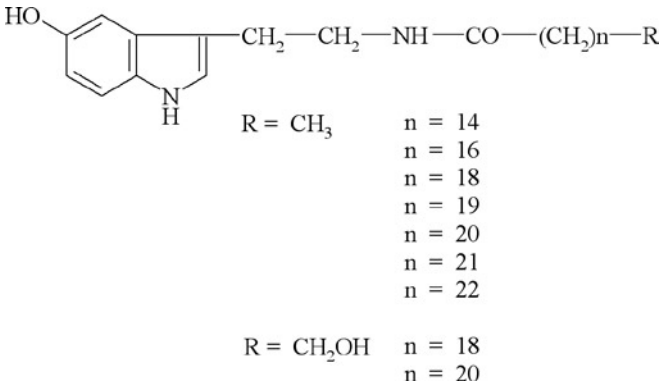

Figure 16. Structural formulae of carboxylic acid-5-hydroxytryptamides (C-5HT).

Kurzrock et al. introduced two carboxylic acid-5HT with the odd-numbered fatty acids henicosanoic and tricosanoic acid at the 20th Intern. Conference on Coffee Science, held in Bangalore, 11-15 October 2004 (Kurzrock et al., 2005). Later these results were confirmed by Lang and Hofmann (2005). Recently, apart from palmitic acid-5HT, eicosenoic acid-5-hydroxytryptamide and octadecadienoic acid-5-hydroxytryptamide were described by Hinkel and Speer (2005).

Several research groups developed analytical methods for determining the contents of C-5HT in green roasted and differently treated coffees. In the beginning, an analysis was carried out by thin layer chromatography with spectrophotometric or densitometric determination (Culmsee, 1975; Kummer and Bürgin, 1976; Hubert et al., 1977; van der Steegen and Noomen, 1977; Studer and Traitler, 1982), followed by liquid chromatography with UV detection at 278 nm (Hunziker and Miserez, 1979; Folstar et al., 1979; Battini et al., 1989; Kele and Ohmacht, 1996). In addition to the analysis by HPLC with fluorescence detection (Laganà et al., 1989; Kurzrock et al., 2005; Hinkel and Speer, 2005; Lang and Hofmann, 2005) at an excitation wavelength of 280 nm and an emission wavelength of 330 nm, the LC-MS/MS - methods were described as well (Kurzrock et al., 2005; Hinkel and Speer, 2005; Lang and Hofmann, 2005).

In figure 17 a typical HPLC chromatogram of carboxylic acid-5HT in a Robusta coffee is shown. The ground beans were extracted by using the accelerated solvent extraction (ASE) and before injection the extract was purified by solid phase extraction (SPE) (Hinkel and Speer, 2005).

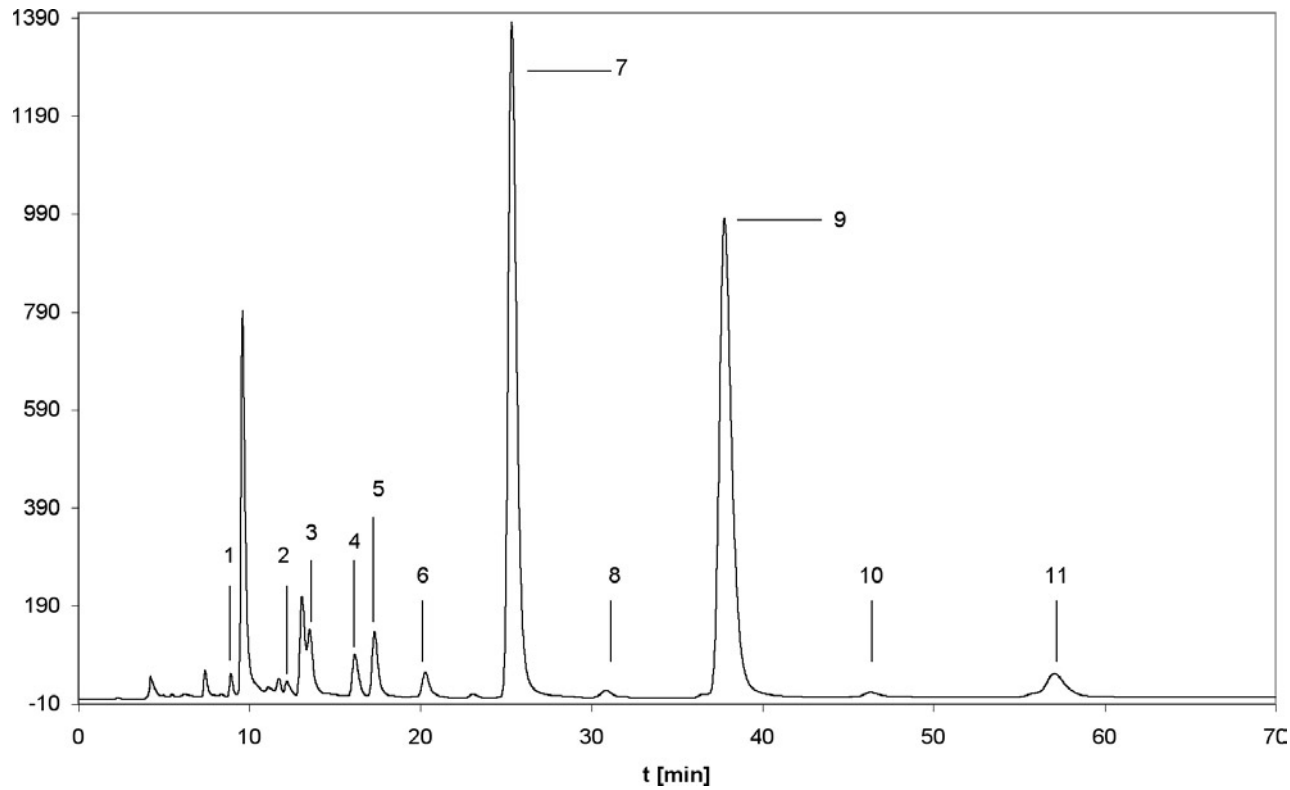

Figure 17. HPLC chromatogram of carboxylic acid-5-hydroxytryptamides (C-5HT). **1**: 18:2-5HT, **2**: 16-5HT, **3**: 20OH-5HT, **4**: 20:1-5HT, **5**: 18-5HT, **6**: 22OH-5HT, **7**: 20-5HT, **8**: 21-5HT, **9**: 22-5HT, **10**: 23-5HT, **11**: 24-5HT.

Whereas arachidic and behenic acid-5-hydroxytryptamides are dominant, the other amides are only minor components. Compared with the total C-5HT content in Robusta coffees (565 - 1120 $mg.kg^{-1}$), the overall amount in Arabica coffees is clearly higher (500 - 2370 $mg.kg^{-1}$) (Maier, 1981). Long-term storage periods of 30 years lead to low total contents of between 160 and 950 $mg.kg^{-1}$ (Wurziger, 1973).

In addition, C-5HTs are partially decomposed by roasting (Hunziker and Miserez, 1979; van der Steegen and Noomen, 1977; Nebesny and Budryn, 2002). For normal roasted coffees the contents ranged from 500 - 1000 $mg.kg^{-1}$. Viani and Horman (1975) proposed pathways for the thermal decomposition of carboxylic acid-5HT. They identified a number of alkylindoles and alkylindanes after pyrolysis of pure behenic acid-5HT.

The removal of the waxy layer by technological treatment like polishing, dewaxing, steaming or decaffeinating the coffee beans, besides the reduction of the total amount of C-5HT, results in a more digestible coffee brew (Behrens and Malorny, 1940; Wurziger, 1972; van der Steegen, 1979; Fintelmann and Haase, 1977; Hunziker and Miserez, 1979; Corinaldesi et al., 1989). Hence, in 1933, the first steaming-method was developed to minimize any irritating effects the coffee brew might have on certain coffee drinkers (Lendrich et al., 1933). In the course of time, this method was improved repeatedly (Kurz and Vahland, 1971; Roselius et al., 1971; Bürgin, 1975; Kurzhals and Sylla, 1978; Werkhoff, 1980; Seidlitz and Lack, 1987).

Even though the C-5HTs are the main constituents of the coffee wax, it is unlikely that they are solely responsible for the undesirable effects of untreated coffee. One reason for this assumption is their poor water solubility (2.3 $mg.l^{-1}$), another is their absence in the percolated coffee brew made from untreated beans (Wurziger, 1971; Rösner et al., 1971; van der Steegen, 1979). Fehlau and Netter (1990), studying the influence of coffee infusions on the gastric mucosa of rats, came to a similar conclusion.

The antioxidant effects of the C-5HT have led to a great interest in coffee wax as a natural antioxidizing agent to be used in food (Wurziger, 1973; Mohr, 1975; Bertholet, 1996; Okada and Hirazawa, 1995; Brimmer, 1997).

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