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ORIGINAL ARTICLE

Variability of some diterpene esters in coffee beverages as influenced by brewing procedures

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Abstract Several coffee brews, including classical and commercial beverages, were analyzed for their diterpene esters content (cafestol and kahweol linoleate, oleate, palmitate and stearate) by high performance liquid chromatography with diode array detector (HPLC–DAD) combined with spectral deconvolution. Due to the coelution of cafestol and kahweol esters at 225 nm, HPLC–DAD did not give accurate quantification of cafestol esters. Accordingly, spectral deconvolution was used to deconvolve the co-migrating profiles. Total cafestol and kahweol esters content of classical coffee brews ranged from 5–232 to 2–1016 mg/L, respectively. Commercial blends contained 1–54 mg/L of total cafestol esters and 2–403 mg/L of total kahweol esters. Boiled coffee had the highest diterpene esters content, while filtered and instant brews showed the lowest concentrations. However, individual diterpene esters content was not affected by brewing procedure as in terms of kahweol esters, kahweol palmitate was the main compound in all samples, followed by kahweol linoleate, oleate and stearate. Higher amounts of cafestol palmitate and stearate were also observed compared to cafestol linoleate and cafestol oleate. The ratio of diterpene esters esterified with unsaturated fatty acids to total diterpene esters was considered as measure of their unsaturation in analyzed samples which varied from 47 to 52%. Providing new information regarding the diterpene esters content and their distribution in coffee brews will allow a better use of coffee as a functional beverage.

Introduction

The coffee plant belongs to the family of *Rubiaceae* which has more than 70 species. Global coffee production comes from *Coffea arabica* (Arabica) and *Coffea canephora* var. *robusta* (Robusta) with nearly 75% originating from the former and 25% from the latter (Mussatto et al. 2011). In terms of chemical composition, coffee oil consists predominantly of triglycerides (75–80 wt%), although it is source of other valuable products such as sterols (2–5.4 wt%) and diterpene esters (7–20 wt%) (Novaes et al. 2015). The latter include cafestol, kahweol and 16-*O*-methylcafestol (16-OMC) that can be thermally degraded during the roasting process to form decomposed derivatives such as dehydrocafestol, dehydrokahweol, cafestal and kahweal (Speer and Kölling-Speer 2006). The occurrence of cafestol as the main cholesterol-raising factor has been reported in all commercial coffee species, while kahweol is mainly present in Arabica coffee and it is only found as minor component in Robusta beans (Kitzberger et al. 2013). Robusta beans contain 16-OMC which is absent in Arabica and is described as a chemical marker of Robusta coffee (Kurzrock and Speer 2007). The pentacyclic diterpenes exist in coffee oil as monoesters on C_{17} by saturated or unsaturated fatty acids (Novaes et al. 2015) as 14 and 12 different fatty acid esters has been detected for cafestol and kahweol, respectively. Also, 12 fatty acid esters were identified for 16-OMC. Nevertheless, from quantitative point of view, C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, C_{20}, and C_{22} esters of fatty acids predominate as the total content of these six cafestol esters in sum ranged from 9.4 to 21.2 g/kg dry

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weight in different Arabica coffees (Speer and Kölling-Speer 2006). The chemical structure of the main eight diterpene esters of Arabica coffee are presented in Fig. 1. Recently, more attention has been dedicated to desirable effect of diterpenes and diterpene esters against cancer and diabetes. Other beneficial properties like induction of the degradation of toxic substances as well as protection against aflatoxin B1 had been also reported for diterpenes (Cavin et al. 2002).

Free diterpenes (either originally present or after cleavage of the esters) can be analyzed using liquid or gas chromatography (Erny et al. 2015) as well as spectropho-tometric method (Dias et al. 2013). However, to provide information on individual esters, diterpene esters should be analyzed through HPLC only after gel permeation chro-matography or solid phase extraction (Kurzrock and Speer 2007). To the best of our knowledge, baseline separation of diterpene esters where both cafestol and kahweol esters are present has never been achieved. In this case a MS detector should be used to adequately monitor the target species. However those instruments are costly and are not always available. Spectral deconvolution (SD) is a mathematical tool for analysis of dataset. Through analysis of samples with HPLC–DAD, a two dimensional matrixes are obtained with chromatographic profile (retention time dimension) in one dimension and spectrometric profile (the wavelength dimension) in the other. Deconvolution aims to extract from the original data chromatographic and spec-troscopic profiles of each individual component (Van Hoeylandt et al. 2014). Deconvolution has been success-fully implemented in order to identifying or quantifying several compounds (Erny et al. 2015; Namara et al. 2007; Van Hoeylandt et al. 2014). This approach designated as

HPLC–DAD with spectral deconvolution (HPLC–DAD/SD) which allowed quantifying each diterpene esters despite an important co-elution (Erny et al. 2015).

The chemical composition of roasted coffee, including diterpenes and diterpene esters exhibit different extraction rates according to coffee species (Bicho et al. 2011), genetic variability (Kitzberger et al. 2014) as well as cli-matic conditions and post-harvest techniques (Kitzberger et al. 2013). However, they may also be influenced by the choice of brewing technique and conditions (home brewing or industrial extraction) as diterpene extraction greatly varies among the different brewing methods (Gross et al. 1997; Moeenfard et al. 2015). Despite the high occurrence of diterpene esters in coffee, few reports are found in the literature regarding diterpene esters as they mainly focus on total diterpenes like total cafestol and kahweol. How-ever, the transition of the cafestol esters into the espresso coffee was tested by other author (Kurzrock and Speer 2007). The content of 16-OMC esters in few coffee brews was also studied previously (Sehat et al. 1993). To the best of our knowledge, few reports exist on diterpene esters profile (Kurzrock and Speer 2007; Sehat et al. 1993) of commercial coffee available in retail outlets which may overshadowed by brewing procedures. Application of deconvolution for this purpose has not been reported elsewhere, as well.

Considering that diterpene esters have biological importance, investigation of their content in a wide range of coffee brews through a simple and inexpensive tech-nique is relevant. Thus, it could be possible to identified not only coffee brews with higher diterpene esters content, but also evaluate the distribution of individual esters as well as unsaturation of coffee brews that may affect the health
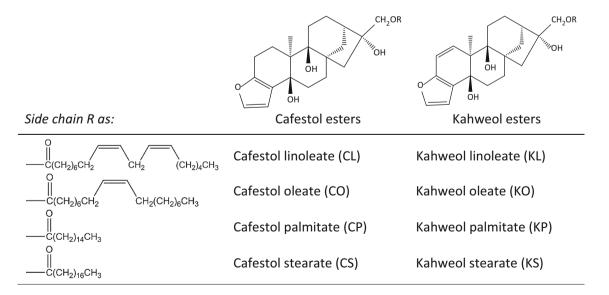

Fig. 1 Chemical structure of the main eight diterpene esters present in Arabica coffee

properties of final beverage. For these reasons, the aim of this study was to investigate diterpene esters profile of a range of classical and commercial coffee brews, in particular cafestol esters as the main diterpene present in coffee species, via HPLC–DAD/SD, which have been subjected to different preparation methods in order to understand the potential variation in the amount of diterpene esters consumed by coffee drinkers.

Materials and methods

Chemicals and reagents

Individual standards of cafestol and kahweol linoleate, oleate, palmitate and stearate were acquired from LKT lab (MN, USA). HPLC grade acetonitrile and methanol along with diethyl ether (purity of 99%) were purchased from VWR (BDH Prolabo, Belgium). Sodium chloride was supplied by Panreac Quimica (Spain). Filtered distilled water used for HPLC–DAD analysis was prepared by vacuum purification through 0.45 μm filter ionic exchange membranes.

Coffee samples

Roasted Arabica (100% *C. arabica*, 2.34% water content) packaged in protective atmosphere was kindly supplied by a local company in Porto, Portugal. Roasted beans were ground by means of a home grinder (Braun KSM 2 model 4041, Mexico) as the particle size distribution of roasted and ground Arabica was as follows: 51% > 500; 24%, 300–500; 13%, 212–300; 11% < 212 μm). These ground coffee was used to prepare classical brews (boiled, French, filtered and mocha). Various brands were also purchased

randomly from local commerce in Porto, Portugal. Table 1 describes the coffee beans used for commercial coffee brew preparation.

Classical coffee brews preparation

Four different coffee samples were obtained using Arabica coffee with coffee/water ratio of 7.5 g/100 mL to uniformize the comparison of brewing techniques. The preparation modes were performed in duplicate and were as follows:

Boiled coffee

It was prepared by boiling 11.25 g roasted and ground coffee with 150 mL of distilled water for 10 min, followed by 2 min of settling time, followed by decanting the liquid. Individual cup size was 150 mL.

French press coffee

It was brewed by pouring 150 mL of boiled distilled water onto 11.25 g of roasted and ground coffee in glass French press pot, followed by stirring. After 2.5 min, the coffee brew was separated from ground coffee by pressing the plunger. Individual cup size was 150 mL.

Mocha coffee

It was brewed using an aluminum mocha pot. Around 11.25 g roasted and ground coffee was placed in filter cup. Mocha pot was filled with 150 mL of cold distilled water. The pot was heated until the water reservoir was empty. Individual cup size was 60 mL.

Table 1 General description of ground coffees used for preparation of commercial coffee brews

Type of coffee	Type of coffee beans	Roasting conditions
Capsule A—type 1	Blend of Arabica and Robusta	Roasted slowly and fine grinding
Capsule A—type 2	Blend of Arabica and Robusta	Light roasted and fine grinding
Capsule A—type 3	Blend of Arabica and Robusta	Light roasted
Capsule A—type 4	Blend of Arabica	Light roasted
Capsule A—type 5	Blend of Arabica	Long roasting at low temperatures
Capsule B	100% Arabica	NA[a]
Vending coffee	NA	NA
Pod espresso	Blend of Arabica and Robusta	NA
Espresso-bar	NA	NA
Instant natural	Soluble coffee natural	NA
Instant decaffeinated	Soluble coffee decaffeinated	NA
Instant espresso	Blend of Arabica	NA

All information was obtained from the label of coffee products

[a] *NA* not available

Filtered coffee

22.5 g of roasted and ground coffee were put in a paper filter bag (N°2) and extracted with 300 mL of boiled distilled water by means of conventional percolation coffee machine KRUPS Aroma Café 5 (Germany). The brew dripped into a heated pot within 2–3 min. The individual cup size was 150 mL.

Commercial coffee brews preparation

Commercial coffee samples were brewed using various brands of different types of coffee purchased randomly from local commerce in Porto, Portugal. Thirteen coffee brews were prepared according to the manufacturers' instructions. Coffee brews (three replicates for each sample) were prepared and stored at −22 °C until analysis, made in duplicates.

Capsule coffee

Extraction of each capsule was performed using an automatic coffee maker (KRUPS, XN2100, Germany) at a pressure of 19 bar by hot water (93 ± 2 °C). Amount of coffee in each capsule was as follows: A-type 1 (6.01 ± 0.01 g/capsule), A-type 2 (5.01 ± 0.06 g/capsule), A-type 3 (5.01 ± 0.03 g/capsule), A-type 4 (5.14 ± 0.02 g/capsule), A-type 5 (6.13 ± 0.11 g/capsule), B (5.71 ± 0.02 g/capsule). Each cup contained 40 mL of coffee brew.

Pod espresso

It was brewed using the SGL coffee machine, designed for pod. The size of a single serving was 40 mL derived from the brewing of a coffee pod (7.08 ± 0.15 g/pod).

Instant coffee

For this purpose, 2 g of commercial instant coffee powder was extracted with 150 mL of boiled distilled water. Regarding instant espresso, one pack contain 1.8 g of soluble coffee was dissolved in 50 mL of boiled distilled water.

Vending coffee

It was obtained from Necta Coffee Vending Machine (Necta Astro Double Brew) to draw a cup of coffee (30 mL).

Diterpene esters extraction

The compounds were extracted in duplicate according to a previously developed methodology (Moeenfard et al. 2015). Briefly, 2.5 mL of coffee brew was extracted using 5.0 mL of diethyl ether along with 0.5 mL of methanol in order to break the emulsion and create a neat interface between the aqueous and organic phases. Extraction procedure was repeated two times. The combined ether phase was washed with 5.0 mL of 2 M NaCl solution and after centrifugation (4000 rpm, 10 min), the cleaned ether phase was brought to dryness under N_2 stream and stored at −22 °C prior to HPLC–DAD analysis.

HPLC–DAD analysis

Analysis was carried out in a Merck Hitachi Elite LaChrom (Tokyo, Japan) system equipped with a quaternary pump (L-2130), an L-2200 autosampler. Separation was achieved using a Purospher STAR LichroCART RP 18 end-capped (250 × 4 mm, 5 μm) column attached to a guard column (4 × 4 mm, 5 μm) of the same kind. The dried extract was dissolved in 0.5–5.0 mL of acetonitrile depending on diterpene esters content in coffee sample. Reconstituted samples were filtered through 0.45 μm filter membrane (PTFE, VWR, USA) and 20 μL was injected and analyzed using the isocratic elution of acetonitrile/isopropanol (70:30, v/v) at 0.4 mL/min. The detection was made using an L-2455 (Merck Hitachi) UV/vis spectrophotometry diode array detector in the range of 200–400 nm. Two detection wavelengths were also set, namely, 225 and 290 nm for cafestol esters and kahweol esters, respectively. EZChrom Elite 3.1.6 software was used for data acquisition and analysis. Target compounds were identified by comparing spectra and retention times with reference standard solutions. Diterpene esters contents were reported as mg/L ± standard deviation after applying the dilution or concentration factors.

Because kahweol esters can be adequately quantified using the chromatogram obtained at 290 nm or using deconvoluted chromatograms, these compounds were used to compare the analytical performance of deconvolution approach over the classical technique. Calibration curves were prepared by plotting the peak area against the corresponding concentrations by duplicate injections of 20 μL of standard solutions. Validation parameters, including linearity (expressed as coefficient of determination, R^2), precision (expressed as coefficient of variation, %CV), accuracy (expressed as percentage of recovery), sensitivity (expressed as slope of calibration

curve), limit of detection (LOD) and limit of quantification (LOQ) were determined.

Deconvolution

After each run, data were exported as coma-separated values format by the acquisition software (EZChrom Elite 3.1.6). Those files were opened using Matlab R2013b and the chromatographic data loaded into a two-dimensional (2D) array. The Matlab function was programmed using Matlab R2013b and can be found in previous published study (Erny et al. 2015). Through applying spectral deconvolution to every spectrum, deconvoluted chromatograms were obtained that are specific to the cafestol and kahweol esters.

Statistical analysis

A one-way ANOVA analysis with a level of significance of 95% was performed to evaluate the differences among coffee brews in terms of cafestol and kahweol esters content. Differences were considered significant when $p < 0.05$. Data were reported as means ± standard deviations of samples submitted to duplicate extractions and injections. Statistical analysis was done by Matlab R2013b or Minitab 16 (2009) software. Graphs were plotted by means of Excel (2010) and Matlab R2013b.

Results and discussion

Method validation

Before validation, individual standard of each diterpene esters (100 mg/L) was injected for HPLC–DAD analysis in order to obtain reliable spectra. Spectra of analytes of interest and background are illustrated in Fig. 2a–c. Through applying spectral deconvolution to every spectrum using Matlab R2013b, deconvoluted chromatograms were obtained (Fig. 2d). Although interfering substances were existed in the matrix, acceptable separation was achieved after deconvolution.

Six standard solutions contain all diterpene esters were prepared in the range of 5–150 mg/L. Deconvoluted chromatograms and subsequently calibration curves were obtained. Satisfactory results were obtained within the linearity range (5–150 mg/L) for both analytical approaches (HPLC–DAD and HPLC–DAD/SD).

The calibration curves were linear and exhibited the coefficient of determination (R^2) higher than 0.99 (Table 2). The limits of detection (LOD) and of quantification (LOQ) were calculated based on a signal to noise ratio of three (S/N = 3) and ten (S/N = 10), respectively. Considering the

deconvolution approach the LOD of cafestol and kahweol esters were in the range of 0.1–0.9 and 0.3–1.2 mg/L, respectively. Direct analysis of kahweol esters through classical approach by setting the wavelength at 290 nm, exhibited the LOD of 0.3–1.3 mg/L which was similar to those obtained by deconvolution technique. Regarding the LOQ, the results ranged from 0.3 to 2.9 mg/L for cafestol esters and 0.9–3.9 mg/L for kahweol esters analyzed by deconvolution method. Analysis of kahweol esters with classical approach revealed LOQ of 0.9–4.0 mg/L.

Method and instrumental precisions were obtained through the coefficient of variation of repeated analysis at intra-day and inter-day conditions, respectively. Intra-day precision (repeatability) was evaluated through six replicate analysis in the same day. In the inter-day variation studies (reproducibility), an extract of the same sample was analyzed three times on three consecutive days. Considering deconvolution, the average coefficient of variation of kahweol esters was 3.0 (min 0.9, max 6.3) and 1.8% (min 0.4, max 4.8%) for intra and inter-day precision, respectively. Regarding cafestol esters, intra-day precision of 1.6% (min 0.3, max 2.9%) and inter-day precision of 1.5% (min 0.8, max 4.6%) were obtained. Average intra-day precision of 1.5% (min 0.6, max 2.0%) and inter-day precision of 0.5% (min 0.1, max 1.7%) were obtained for kahweol esters in classical approach, as well.

The accuracy of both analytical methods was studied through recovery test. For this purpose, coffee sample (capsule B) was spiked with known quantity of each diterpene esters at two spiking levels (50 and 100 mg/L). The average recovery (%) was reported as the mean ratio between the obtained and expected concentration of each diterpene esters in spiked sample. In terms of deconvolution approach, the mean recovery ranged 87–103% and 91–97% for cafestol esters and kahweol esters, respectively. Regarding classical approach, the mean recovery ranged from 92 to 101% for kahweol esters which was almost similar to those obtained by deconvolution technique.

Comparison of deconvolution and classical approach

Looking at both analytical approaches (deconvolution and classical), Fig. 3 demonstrated the applicability of deconvolution for quantification of diterpene esters in variety of coffee brews. Setting the wavelength at 290 nm allowed baseline separation of kahweol esters while such wavelength does not exist for measuring cafestol esters. Therefore, the following wavelength (290 nm) was used to compare deconvolution approach with classical separation in HPLC–DAD. Considering all coffee brews in the present study, no significant differences ($p \geq 0.05$) were observed between deconvolution and classical approach and perfect agreement
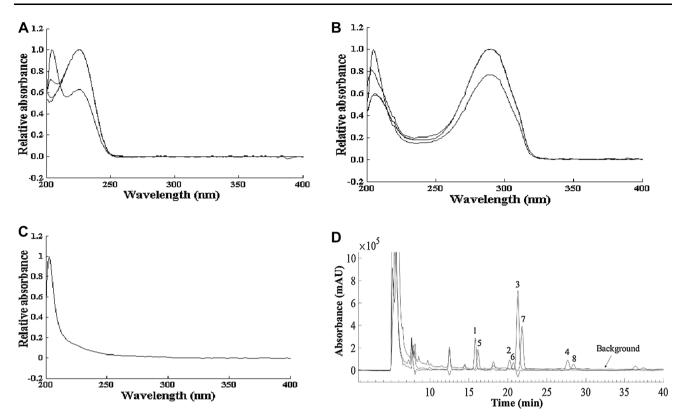
Fig. 2 Spectra of **a** cafestol esters, **b** kahweol esters, **c** background, **d** deconvoluted chromatogram obtained using HPLC–DAD/SD analysis of a real coffee sample (*espresso-bar*) (*1* kahweol linoleate, *2* kahweol oleate, *3* kahweol palmitate, *4* kahweol stearate, *5* cafestol linoleate, *6* cafestol oleate, *7* cafestol palmitate, *8* cafestol stearate). The chromatogram was recorded at 225 nm to allow the observation of all compounds of interest

was achieved for all kahweol esters among these two analytical techniques. Accordingly, deconvolution method was then successfully extended to quantify cafestol esters in coffee brews. These results demonstrated the advantageous of using this low-cost and accurate analytical technique over other methods like LC–MS for quantification of cafestol and kahweol esters in variety of coffee samples.

Diterpene esters content in coffee brews

Even potentially small health benefits or risks associated with coffee intake may have important public health implications due to its widespread consumption. Therefore, the aim of this part of the study was to provide information about some diterpene esters of coffee brews that are directly available to the consumer. For this purpose, the average concentrations of four cafestol esters and four kahweol esters were quantified in some traditional and commercial coffee brews through an inexpensive and accurate method and the results were summarized in Tables 3 and 4, respectively.

A large variability was found among diterpene esters content in coffee brews. In general, boiled coffee was the most concentrated brew in terms of total cafestol esters

(232 ± 9 mg/L) and total kahweol esters (1016 ± 29 mg/L) content followed by French press coffee. While, mocha and espresso brews had an intermediate contribution to cafestol esters intake with values of 36 ± 3 and 10–54 mg/L, respectively. They also were consider as an intermediate source of kahweol esters (123 ± 2 mg/L of mocha and 40–403 mg/L of espresso brews).

The most widespread espresso brews obtain from bar machines and single-dose capsules, designed in large numbers because of their commercial popularity (Parenti et al. 2014). Over the last few decades, capsule and pod received substantial attention in market share as 14 billion units of capsule and pods has been sold in 2005 (Parenti et al. 2014). According to our results, among espresso brews, espresso-bar was the most concentrated one in terms of total cafestol esters (54 ± 2 mg/L), followed by vending (43 ± 4 mg/L) and capsule B (41 ± 4 mg/L). Capsule B which composed of 100% Arabica coffee, presented the highest total kahweol esters concentration (403 ± 11 mg/L), as well.

Despite the similarity of the coffee/water ratio (7.5 g/100 mL) of boiled coffee in comparison to other classical brews, the prolonged contact time of ground coffee with the surrounding water (10 min for boiled and around

Table 2 Validation parameters for some diterpene esters analyzed by HPLC–DAD and HPLC–DAD/SD

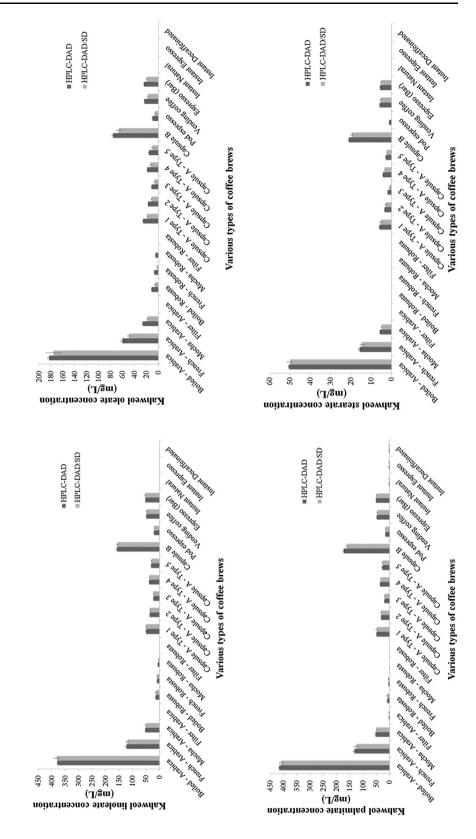| Diterpene esters	R^2 (N = 6)[a]	Sensitivity[b]	LOD (mg/L)[c]	LOQ (mg/L)[c]	Recovery (%)[d]		Precision		
					50 (mg/L)	100 (mg/L)	Standards (mg/L)	Intra-day (%CV)[a]	Inter-day (%CV)[a]
Deconvolution approach (HPLC–DAD/SD): linearity range of 5–150 mg/L									
Cafestol linoleate	0.999	334,945	0.1	0.3	93.0	90.9	10	0.5	0.9
							75	1.1	0.8
							150	1.0	0.8
Cafestol oleate	0.998	277,090	0.1	0.5	102.8	94.5	10	0.7	0.8
							75	2.3	1.0
							150	2.1	1.3
Cafestol palmitate	0.999	349,985	0.1	0.4	102.6	95.5	10	0.3	1.2
							75	2.0	1.0
							150	2.3	1.5
Cafestol stearate	0.994	133,808	0.9	2.9	87.2	94.5	10	2.9	4.6
							75	1.9	1.1
							150	1.9	3.1
Kahweol linoleate	0.996	66,446	0.8	2.6	95.4	92.2	10	0.9	0.4
							75	2.6	0.4
							150	1.3	1.1
Kahweol oleate	0.999	62,642	1.2	3.9	95.1	97.2	10	3.8	3.3
							75	4.4	1.5
							150	6.3	4.8
Kahweol palmitate	0.999	200,594	0.3	0.9	97.5	95.2	10	3.7	2.4
							75	2.8	1.2
							150	3.8	3.3
Kahweol stearate	0.999	265,300	0.3	0.9	94.1	91.1	10	1.3	1.0
							75	2.1	0.8
							150	2.9	1.8
Classical approach (HPLC–DAD): linearity range of 5–150 mg/L									
Kahweol linoleate	0.995	26,168	0.9	2.6	96.2	93.8	10	1.5	1.2
							75	0.6	0.3
							150	1.7	0.2
Kahweol oleate	0.997	24,226	1.3	4.0	94.2	94.0	10	2.0	1.7
							75	1.8	0.4
							150	1.9	0.3
Kahweol palmitate	0.999	76,415	0.3	0.9	101.0	97.8	10	1.0	0.4
							75	1.5	0.2
							150	1.8	0.1
Kahweol stearate	0.999	102,582	0.3	0.9	96.3	92.4	10	0.7	0.7
							75	1.5	0.2
							150	1.8	0.1

[a] R^2 coefficient of determination, N number of calibration curve standards, CV coefficient of variation

[b] Sensitivity was expressed as the slope of the calibration curves

[c] Calculated from the signal to noise ratio of 3 for limit of detection (LOD) and 10 for limit of quantification (LOQ)

[d] Obtained from the ratio of measured diterpene esters content to the expected one from spiked sample (capsule B) at two different spiking levels (50 and 100 mg/L) for all diterpene esters

Fig. 3 Concentration of
kahweol esters in various types
of coffee brews obtained from
HPLC–DAD and HPLC–DAD/
SD measurement at 290 nm.
One-way ANOVA indicated
that for each coffee sample,
there was no significant
differences ($p \geq 0.05$) among
values obtained from classical
(HPLC–DAD) or deconvolution
(HPLC–DAD/SD) approach.
Since, in some samples the
diterpene esters concentrations
were below the LOQ, their *bar*
were not shown in the
respective figure

2.5–3.0 min for French press coffee) may justify the higher
concentration of diterpene esters in boiled and French press
coffee compared with other brews. Besides that, as other
authors stated (Gross et al. 1997) the higher content of
diterpenes in boiled and French press may be attributed to
the presence of high amount of fine coffee particles as the

Table 3 Cafestol esters concentration (mg/L ± standard deviation) in various types of coffee brews analyzed by deconvolution approach (HPLC–DAD/SD)

Coffee sample	Cafestol linoleate	Cafestol oleate	cafestol palmitate	cafestol stearate	Total cafestol esters
Classical brews prepared using Arabica beans					
Boiled	46.5 ± 0.3[a]	19.6 ± 0.5[a]	103.5 ± 3.5[a]	62.0 ± 3.9[a]	232 ± 9[a]
French	13.7 ± 0.6[b]	5.2 ± 2.1[b]	32.8 ± 4.0[b]	26.9 ± 2.5[b]	79 ± 10[b]
Mocha	4.9 ± 0.2[c]	3.6 ± 0.8[b]	18.5 ± 1.3[c]	8.6 ± 1.1[c]	36 ± 3[c]
Filter	0.30 ± 0.03[d]	≤LOQ	1.07 ± 0.04[d]	3.2 ± 0.7[d]	5 ± 1[d]
Commercial coffees					
Espresso type-brews					
Capsule A—type 1	3.5 ± 0.1[b]	0.5 ± 0.1[c]	16.3 ± 0.2[c]	2.5 ± 0.2[cd]	23 ± 1[c]
Capsule A—type 2	2.2 ± 0.1[c]	≤LOQ	12.3 ± 0.3[d]	7.2 ± 1.1[bc]	22 ± 2[c]
Capsule A—type 3	0.7 ± 0.1[d]	≤LOQ	9.5 ± 0.1[e]	≤LOQ	10.2 ± 0.2[d]
Capsule A—type 4	2.28 ± 0.01[c]	≤LOQ	12.3 ± 0.3[d]	5.8 ± 1.4[bcd]	20 ± 2[c]
Capsule A—type 5	1.9 ± 0.5[c]	0.6 ± 0.3[c]	11.5 ± 0.6[de]	4.7 ± 1.9[bc]	19 ± 5[c]
Capsule B	6.7 ± 0.6[a]	3.2 ± 0.7[b]	21.4 ± 0.6[b]	9.8 ± 1.8[ab]	41 ± 4[b]
Pod espresso	1.4 ± 0.2[cd]	0.52 ± 0.04[c]	11.0 ± 0.1[de]	≤LOQ	13 ± 1[d]
Vending	7.2 ± 0.2[a]	3.8 ± 1.3[b]	22.4 ± 1.1[b]	10.2 ± 1.6[ab]	43 ± 4[b]
Espresso (bar)	8.1 ± 0.1[a]	6.2 ± 0.4[a]	24.8 ± 0.2[a]	14.6 ± 0.9[a]	54 ± 2[a]
Instant coffees					
Instant natural	0.33 ± 0.03[a]	≤LOQ	1.3 ± 0.1[a]	≤LOQ	1.7 ± 0.1[a]
Instant espresso	≤LOQ	≤LOQ	1.3 ± 0.1[a]	≤LOQ	1.3 ± 0.1[b]
Instant decaffeinated	0.33 ± 0.02[a]	≤LOQ	1.48 ± 0.01[a]	≤LOQ	1.81 ± 0.02[a]

In each class of brew, values of individual ester with similar superscripts in a column (a–f) did not differ significantly ($p \geq 0.05$)

main source of diterpene esters, in the respective brews. Sehat et al. (1993) reported the highest concentration of 16-OMC esters as one of the main esters existing in Robusta beans in Scandinavian boiled coffee and espresso coffee.

One the other hand, brews that were made by filtration or instant coffees showed significantly ($p < 0.05$) lower diterpene esters content than other samples. Concerning filtered coffee, probably diterpene esters were retained on filter paper (Silva et al. 2012). The low concentration of diterpene esters in instant coffees may be attributed to additional thermal extraction treatment at high temperature after roasting (Vignoli et al. 2011). The presence of negligible amount of 16-OMC esters in filtered coffee was also reported previously (Sehat et al. 1993).

The volume of coffee brew per cup varies from person to person, depending on the cultural and personal preferences of each consumer. A cup of boiled or mocha coffee may range from 150–190 mL to 40–120 mL, respectively (Alves et al. 2010). Accordingly, the big cup size of boiled and French press makes them a rich source of these compounds. On the other hand, espresso coffees are very small beverages ranging between 20 and 35 mL in Italy and up to 70 mL in Portugal (Alves et al. 2010). Therefore, the

concentration of diterpene esters in espresso coffees are balanced with the limited volume of a serving cup, which resulted in a moderate diterpene esters intake in comparison to French press or boiled coffee. On the other hand, instant and filtered coffees are light brews and despite the high volume of consumption they might not be an important contributor to diterpene esters consumption.

One the other hand, it seems that individual diterpene esters were not affected by brewing procedure as among kahweol esters, the kahweol palmitate (2–409 mg/L) was the main compound in all samples, followed by kahweol linoleate (20–381 mg/L), oleate (5–176 mg/L) and stearate (1–50 mg/L). The order was in agreement with other report (Kurzrock and Speer 2007). Higher amounts of cafestol palmitate (1–103 mg/L) and stearate (2–62 mg/L) were also observed compared to cafestol linoleate (0.5–46 mg/L) and cafestol oleate (0.5–20 mg/L). The average percentage of cafestol esters distribution in coffee samples were slightly different from those that were previously reported (Kurzrock and Speer 2007; Speer and Kölling-Speer 2006) as in the present work, the highest percentage of individual cafestol esters has been attributed to cafestol palmitate, whereas cafestol stearate, linoleate and oleate were in the next order. This discrepancy could probably be

Table 4 Kahweol esters concentration (mg/L $\pm$ standard deviation) in various types of coffee brews analyzed by deconvolution approach (HPLC–DAD/SD)

Coffee sample	Kahweol linoleate	kahweol oleate	kahweol palmitate	kahweol stearate	Total kahweol esters
Classical brews prepared using Arabica beans					
Boiled	381.5 $\pm$ 8.9[a]	175.7 $\pm$ 12.3[a]	409.2 $\pm$ 6.9[a]	50.0 $\pm$ 1.2[a]	1016 $\pm$ 29[a]
French	119.3 $\pm$ 4.7[b]	49.1 $\pm$ 2.0[b]	125.7 $\pm$ 7.2[b]	14.6 $\pm$ 0.8[b]	309 $\pm$ 15[b]
Mocha	50.9 $\pm$ 0.8[c]	18.3 $\pm$ 0.3[c]	49.3 $\pm$ 0.7[c]	4.8 $\pm$ 0.1[c]	123 $\pm$ 2[c]
Filter	$\leq$LOQ	$\leq$LOQ	1.8 $\pm$ 0.2[d]	$\leq$LOQ	1.8 $\pm$ 0.3[d]
Commercial coffees					
Espresso type-brews					
Capsule A—type 1	49.2 $\pm$ 2.0[b]	18.3 $\pm$ 0.9[bc]	47.7 $\pm$ 3.5[b]	5.3 $\pm$ 0.5[b]	121 $\pm$ 7[b]
Capsule A—type 2	34.9 $\pm$ 0.5 [cd]	11.1 $\pm$ 0.6[def]	30.9 $\pm$ 1.3 [cd]	2.8 $\pm$ 0.3 [cd]	80 $\pm$ 3 [cd]
Capsule A—type 3	22.2 $\pm$ 0.4[e]	6.1 $\pm$ 0.7[f]	18.5 $\pm$ 0.4[ef]	0.9 $\pm$ 0.1[e]	48 $\pm$ 2[e]
Capsule A—type 4	36.3 $\pm$ 0.6[c]	12.9 $\pm$ 0.2[cde]	33.3 $\pm$ 0.1[c]	3.4 $\pm$ 0.0[c]	86 $\pm$ 1[c]
Capsule A—type 5	29.7 $\pm$ 1.1[d]	9.9 $\pm$ 1.2[ef]	25.9 $\pm$ 1.6[de]	2.3 $\pm$ 0.1[d]	68 $\pm$ 4[d]
Capsule B	156.5 $\pm$ 3.4[a]	66.5 $\pm$ 3.5[a]	161.0 $\pm$ 3.7[a]	19.5 $\pm$ 0.4[a]	403 $\pm$ 11[a]
Pod espresso	19.7 $\pm$ 0.1[e]	5.4 $\pm$ 0.9[f]	15.2 $\pm$ 0.01[f]	$\leq$LOQ	40 $\pm$ 1[e]
Vending	48.0 $\pm$ 1.9[b]	17.6 $\pm$ 0.4[bcd]	46.0 $\pm$ 1.7[b]	5.3 $\pm$ 0.4[b]	117 $\pm$ 4[b]
Espresso (bar)	53.1 $\pm$ 0.2[b]	19.7 $\pm$ 0.1[b]	49.4 $\pm$ 0.4[b]	5.2 $\pm$ 0.1[b]	127 $\pm$ 1[b]
Instant coffees					
Instant natural	$\leq$LOQ	$\leq$LOQ	2.1 $\pm$ 0.03[a]	$\leq$LOQ	2.1 $\pm$ 0.03[a]
Instant espresso	$\leq$LOQ	$\leq$LOQ	2.0 $\pm$ 0.2[a]	$\leq$LOQ	2.0 $\pm$ 0.2[a]
Instant decaffeinated	$\leq$LOQ	$\leq$LOQ	1.92 $\pm$ 0.01[a]	$\leq$LOQ	1.92 $\pm$ 0.01[a]

In each class of brew, values of individual ester with similar superscripts in a column (a–f) did not differ significantly ($p \geq 0.05$)

due to the different numbers of diterpene esters quantified. It should be noted that, despite the variety of diterpene esters reported for coffee, few of them are considered key esters (Speer and Kölling-Speer 2006). Among them, 16-OMC are dedicated only to Robusta coffee. Others, like dehydrocafestol or dehydrokahweol esters are formed during coffee bean roasting. However, cafestol and kahweol esters, mainly palmitate, linoleate, oleate and stearate are the most abundant diterpene esters in particular in Arabica coffee beans (Speer and Kölling-Speer 2006) which gained also the main proportion in coffee blends.
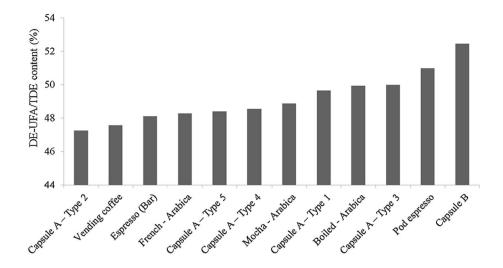
Comparison of coffee brews according to the fatty acid composition of their diterpene esters

Even though individual cafestol and kahweol esters vary significantly between samples, differences among the ratio of total unsaturated diterpene esters to total diterpene esters were small. The values for the ratio of diterpene esters esterified with unsaturated fatty acids (DE-UFA) to total diterpene esters (TDE) content as a measure of their unsaturation ranged from 47 to 52%, with the lowest value for capsule A-type 2 and the highest value for the capsule B (Fig. 4). Generally, coffee brews sample presented

approximately 49 $\pm$ 2% unsaturated cafestol and kahweol esters content (esters of oleic acid, 18:1 *cis*-9 and linoleic acid, 18:2 *cis*, *cis*-9,12). Fatty acids of coffee lipid extract may present either free or esterified with glycerol or diterpenes alcohols. It is noteworthy to mention that these results were based on the determination of the eight diterpene esters of Arabica coffee so they include only four esters of cafestol and another four for kahweol. Accordingly, it seems that brewing method does not modify the distribution of diterpene esters as both saturated and unsaturated diterpene esters presented the similar extraction rate.

The fatty acids are found to distributed differently among various lipid classes of coffee oil and the only common feature being that palmitic (16:0) and linoleic acids (*cis* 9,12–18:2) are the dominant fatty acids components in all classes (Nikolova-Damyanova et al. 1998). As reported by Nikolova-Damyanova et al. (1998), in lipid classes of crude beans of Catuaí Vermalho (*C. arabica* L.), terpenes and sterol esters were the most saturated and free fatty acids and triacylglycerol were the most unsaturated classes (Nikolova-Damyanova et al. 1998).

Several epidemiological studies suggested the controlled intake of saturated fatty acids in particular lauric, myristic

Fig. 4 The ratio of diterpene esters esterified with unsaturated fatty acids (DE-UFA) to total diterpene esters (TDE) content as a measure of their unsaturation

and palmitic acids due to their effects on increasing the blood cholesterol concentration (Williams 2000). Considering the various effects of diterpenes on human health, in order to reinforce the beneficial properties, we should favor coffee with moderate content of diterpenes and high proportion of unsaturated fatty acid. Although, more experiments require, probably selection of type of bean or roasting degree may allowed that. Besides that, provide information about the content of saturated or unsaturated fatty acids may be used for study the effect of coffee oil on quality of final product.

Conclusion

Diterpene intake through coffee brews is substantially dependent on the method of brew preparation. HPLC–DAD measurement combined with spectral deconvolution allowed us to determine four cafestol esters and four kahweol esters in several coffee brews, simultaneously.

This method can be applied routinely for determination of these compounds in a large monitoring scheme of coffee brews allowing a high sample throughput. With the results obtained, in general, boiled and French press were the most concentrated brews in terms of diterpene esters content, whereas mocha and espresso had an intermediate contributions to diterpene esters intake. Only negligible amount of diterpene esters were effectively extracted to the filtered and instant coffee. Regarding kahweol esters, the palmitate ester was the main compound in all samples, followed by linoleate, oleate and stearate. Higher amounts of cafestol palmitate and stearate were observed compare to linoleate and oleate. Among these eight diterpene esters studied, diterpenes esterified with unsaturated fatty acids were included almost half (47–52%) of the total diterpene esters content. Considering the significant consumption of coffees

in the world, they could be account as important source for natural biological active compounds such as diterpene esters. As a consequence, although various effects of coffee consumption on human health has been raised, these data provide new information regarding the diterpene esters that are directly available for consumption which may have a large influence on the potential health of coffee intake.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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