J. of Supercritical Fluids 95 (2014) 137–145

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Enrichment of diterpenes in green coffee oil using supercritical fluid extraction – Characterization and comparison with green coffee oil from pressing

Paola Maressa Aparecida de Oliveira[a], Rafael Henrique de Almeida[a], Naila Albertina de Oliveira[a], Stephane Bostyn[b], Cintia Bernardo Gonçalves[a], Alessandra Lopes de Oliveira[a,*]

[a] Departamento de Engenharia de Alimentos, Universidade de São Paulo, Av Duaue de Caxias Norte 225, Caixa Postal 23, CEP 13635-900 Pirassununga, Sao Paulo, Brazil
[b] Institut de Combustion, Aérothermique, Réactivité, et Environnement (ICARE) 1C, Avenue de la recherche scientifique, 45071 Orléans cedex 2, France

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 12 August 2014
Accepted 15 August 2014
Available online 23 August 2014

Keywords:
Cafestol
Kahweol
Supercritical fluid extraction
Coffea arabica
Yellow Catuaí
Fatty acids

ABSTRACT

Supercritical fluid extraction (SFE) was used to obtain green coffee oil (*Coffea arabica*, cv. Yellow Catuaí) enriched in the diterpenes, cafestol and kahweol. To obtain diterpenes-enriched green coffee oil relevant for pharmaceuticals, a central composite rotational design (CCRD) was used to optimize the extraction process. In this study, pressure and temperature did not have influences on cafestol and kahweol concentrations, but did affect the total phenolic content (TPC), which ranged from 0.62 to 2.62 mg GAE/g of the oil. The analysis and quantification of diterpenes according to gas chromatography indicated that green coffee oil from SFE presented a cafestol content of 50.2 and a kahweol content of 63.8 g/kg green coffee oil under optimal conditions. The green coffee oil produced from conventional pressing methods presented lower diterpenes content of 7.5 and 12.8 g/kg green coffee oil for cafestol and kahweol, respectively. When SFE was used, the content of the diterpenes in the same green coffee beans was relatively higher, approximately 85% for cafestol and 80% for kahweol. Green coffee oil from SFE also presented fatty acids, such as palmitic acid (9.3 mg MAE/g green coffee oil), the polyunsaturated linoleic acid (ω-6; 11 mg MAE/g green coffee oil) and oleic acid (ω-9; 3.8 mg MAE/g green coffee oil). The physical properties for green coffee oil produced from SFE and conventional pressings showed that densities and viscosities decreased with temperature. For oils produced from both extractions, the density behaviors were similar with values ranging from 0.9419 g/cm^3 (25 °C) to 0.9214 g/cm^3 (55 °C) from pressings and 0.9365 g/cm^3 (25 °C) to 0.9157 g/cm^3 (55 °C) for the oil obtained by the SFE. The dynamic viscosity for the pressed oil ranged from 127.8798 mPa × s (25 °C) to 35.0510 mPa × s (55 °C); for the oil from SFE, these lower values ranged from 84.0411 mPa × s (25 °C) and 24.2555 mPa × s (55 °C).

1. Introduction

Brazil produces coffee arabica and robusta. Arabica coffee represents approximately 75% of the national production of coffee. The renovation of coffee plantations in the 1970s and 1980s in Brazil focused on two Arabica cultivars (cv.), the New World and the Catuaí, which dominate the coffee crop with nearly 100% of the planted area. The cv. Yellow Catuaí originated from the artificial crossing of Yellow Caturra and New World cultivars, and grows to a low height allowing for easy manual and mechanical harvesting and high productivity [1].

Coffee is traditionally used as a beverage, but in recent times, green or roasted coffee oil have been commercially exploited. Green coffee beans contain 7–17% lipid in mass. Its oil is composed of triacylglycerols (75%) and free fatty acids, similar to other vegetable oil compositions [2]. The unsaponifiable fraction of green coffee oil consists of sterols, tocopherols, phosphatides, diterpenes, ceramides and other minor components whose biological activities have been demonstrated.

Industrially, coffee oil is obtained by pressing beans with an expeller [3]. However, in recent years, new techniques, such as supercritical fluid extraction (SFE), have been evaluated at the

* Corresponding author. Tel.: +55 19 3565 4268; fax: +55 19 3565 4284.
E-mail address: alelopes@usp.br (A.L. de Oliveira).

http://dx.doi.org/10.1016/j.supflu.2014.08.016
0896-8446/

laboratory scale to increase the fraction of compounds of interest and to increase the process yield [4–7].

Green coffee oil is normally used as emollient in the cosmetics industry due to its fatty acid content and its ability to block harmful sunlight [8–10]. Green coffee oil is also marketed as a raw material for pharmaceutical industries because of its antioxidant activity. It is an ingredient for creams and ointments used in aging skin treatment, dry and cracked skin, eczema, psoriasis and other skin-related diseases [11].

Phenolic compounds in green coffee oil have also become important due to their antioxidant properties. Hydroxycinnamic acids are one of the main classes of phenolic compounds, which include caffeic, ferulic and p-coumaric acids, found in plants. In soluble or conjugated forms, these groups are known as chlorogenic acids in green coffee beans [12,13].

Compounds found in green coffee oil act as chemoprotector agents, which have been shown to be effective in cancer treatment in protecting healthy tissue from the toxic effects of anticancer drugs. These compounds include caffeine, polyphenols and chlorogenic acids [14]. However, particular antitumor effects are associated with diterpenes compounds that can be attributed to an inhibition of bio-activation and stimulation of cellular detoxification [15].

Kahweol and its derivative cafestol are two main diterpenes present in green coffee beans, oils and drinks [16]. In animal models and in human cells, diterpenes cafestol and kahweol have produced a wide range of biochemical effects that reduced the genotoxicity of various carcinomas [17] and have been shown to offer some degree of protection against carcinogenic effects [18,19]. Recently, attention has been focused on the biological effects of kahweol and cafestol because the mechanisms responsible for chemoprotector effects are not yet completely known.

The cafestol and kahweol plus chemoprotector present antioxidant, anti-inflammatory and hepatoprotective action. Moreover, epidemiological evidence suggests that coffee consumption with a high level of kahweol is associated with a lower rate of colon cancer, one of the most common worldwide cancers [20].

These diterpenes are only produced by Coffea genus plants at species-dependent amounts. Cafestol is found in both *C. arabica* and *C. canephora*, and kahweol is more specific to *C. arabica* [21–23]. It is difficult to individually isolate these diterpenes because kahweol is highly unstable when purified. Thus, their biological properties have traditionally been studied together [17].

Kahweol and cafestol are classified as pentacyclic diterpenes, which are alcohols based on the fusion of isoprene units (C5) to form a kaurane structure of 20 carbons. Karurane and kahweol differ by a single double bond in kahweol between carbons 1 and 2 [24].

Although Brazil is the largest producer and exporter of green coffee beans in the world [25], the oil from green coffee is fairly rudimentarily obtained by simple mechanical pressing.

In this work, the parameters for a supercritical fluid extraction (SFE) of green coffee oil rich in diterpenes (i.e., kahweol and cafestol) were studied. The process variables were optimized to determine the extraction process yields of green coffee oil. Finally, diterpenes content extracted via SFE was compared with the oil content obtained by the conventional method (i.e., pressing).

The supercritical fluid extraction (SFE) is a powerful technique used in separation processes to obtain extracts from natural products in various segments of industry and research. Our studies have focused on industrial applications for this technology. SFE has been applied in industrial production of coffee and tea, flavors, nicotine, extractions of essential oils and fatty acids and the purification of pharmaceuticals, which demonstrates the wide applicability of this technology [26].

Supercritical CO_2 has been used in oil extractions from coffee, roasted coffee [3,4] and green coffee [5,6]. The extraction conditions from Araújo and Sandi [5] were used in this study as the starting point for the extraction process optimization of green coffee oil rich in cafestol and kahweol.

2. Materials and methods

2.1. Raw material preparation

Arabica green coffee beans (*Coffea arabica*), cultivar (cv.) Yellow Catuaí were produced in the Jau, Torrinha and Dois Córregos regions of São Paulo, Brazil (22°25′34″ S and 48°10′09″ W) 802 m above sea level at an average temperature of 22 °C.

Coffee beans were harvested in June 2012, washed and naturally sun-dried by producers. For comparison, green beans from the same harvest were used for both SFE and conventional pressing. Samples of agroproducer-pressed oils produced in 2010 (PCO-2010) and in 2012 (PCO-2012) were obtained. The pressing process consisted of feeding green coffee beans into an oil extractor by steam injection. The resultant oils were filtered via filter press to obtain the green coffee oil [3].

Sun-dried beans supplied by producers were dried in an oven with forced air circulation at 50 °C for 48 h. The grain humidity ($4.73\% \pm 0.10$) was determined by gravimetric method. Beans were ground in a hammer-type mill (Buhler–Miag, Braunschweig, GE) and the apparent density of the crushed grains (0.30 ± 0.01 g/cm^3) was determined empirically by the relationship between the mass of crushed green coffee beans packaged in a fixed bed with a volume of 300 cm^3. A fixed mass of green coffee beans packed in the extractor (≈90 g) was used in all experiments. The real density of crushed beans (1.26 ± 0.01 g/cm^3) was measured by a helium gas pycnometer (Quantachrome 1200e Ultrapyc, Florida, USA).

An important characteristic of the porous medium is the voidspace, which may have complex geometric shapes and different dimensions. To characterize the structure of dry materials used in SFE, the porosity ($\varepsilon = 0.76$) was determined by the ratio between the apparent and real density.

To determine the particle size of crushed green coffee beans, 90 g was wrapped in a set of six sieves (Tyler standard series) and stirred for 5 min. The average diameter (0.92 mm) was calculated by the following relation (Eq. (1)):

$$d_{mg} = \log^{-1}\left[\frac{\sum_{j=1}^{n}(w_i \log \overline{d_i})}{\sum_{j=1}^{n} w_i}\right], \quad (1)$$

where $d_i = (d_i \cdot d_{i+1})^{0.5}$; d_i is the nominal gap of the ith sieve (mm); $d_i + 1$ is the nominal gap of the sieve greater than ith sieve (mm); and w_i is the mass of the material retained on the ith sieve.

2.2. Supercritical extraction

For supercritical fluid extraction (SFE), the crushed beans were placed in a fixed bed extractor of a TharSCF system (Waters, Milford, USA). Although this equipment is capable of using a co-solvent in the extraction process, this study only used supercritical CO_2 as the solvent.

The supercritical extraction process promotes contact between the vegetal matrix and supercritical CO_2 in the extractor at constant pressure (P) and temperature (T) conditions. The P is controlled by a high-pressure pump and a micrometric regulation back pressure control. The extract, separated from the gas was continuously collected in a smaller collection chamber on ice. The volume of CO_2 was metered by the pump flow meter. The extraction system was automatic, and the operation and variables (i.e., T, P, and CO_2 flowrate) were controlled via software (Process Suit for SFE, Thar, Waters, Milford, USA).

Table 1
Matrix of the central composite rotational design (CCRD) for 2 variables in the study of temperature (T) and pressure (P) on the yield of green coffee oil from SFE, cafestol and kahweol concentration and TPC – real variables.

Essay	P (bar)	T (°C)	ρ CO_2 (kg/cm^3)	Y (g/100 g)	Oil (g/100 g)	Caffeine (g/100 g)	Cafestol (g/kg green coffee oil)	Kahweol (g/kg green coffee oil)	Ratio (c/k)	CFT	
										mg GAE/100 g green coffee beans	mg GAE/g green coffee oil
1	300	90	695.42	8.08	6.50	1.60	21.5	26.5	0.81	8.79	1.09
2	200	90	524.65	6.34	5.15	1.21	28.5	22.8	1.25	16.59	2.62
3	300	70	788.67	4.20	3.17	1.03	46.9	60.4	0.77	3.75	0.89
4	200	70	661.10	2.72	1.70	1.02	50.2	63.8	0.79	1.68	0.62
5*	250	80	687.42	6.76	5.64	1.10	18.9	18.0	1.05	5.66	0.84
6*	250	80	687.42	7.26	5.61	1.65	17.6	17.4	1.01	8.71	1.20
7*	250	80	687.42	6.82	5.87	0.94	17.1	15.2	1.12	6.29	0.92
8	250	94.1	618.10	2.95	2.40	0.55	25.6	30.7	0.83	7.06	2.39
9	250	65.9	758.10	0.26	0.01	0.26	0.00	0.00	0.00	0.00	0.00
10	325.5	80	764.78	4.56	3.76	0.81	8.8	5.3	1.65	0.28	1.06
11	174.5	80	509.42	1.62	0.41	1.21	19.1	20.4	0.94	4.96	1.09

* Central points; GAE, Gallic acid equivalent. $\alpha = 1.41$.

Supercritical CO_2 extractions were performed from 179 to 325 bar and 66–94 °C. Prior to dynamic extractions, for all experiments, CO_2 under the pre-established conditions of P and T remained in contact with crushed green coffee beans during a static period of 20 min. Both the dynamic extraction time (6 h) and the static contact time were defined in preliminary tests. The CO_2 flow rate was maintained at 5 g/min. By varying the extraction experimental conditions (i.e., P and T), the extraction yields, the diterpenes content in the green coffee oil, the total phenolic content (TPC), the fatty acids profile and other physical properties were measured.

In the optimization of SFEs of diterpenes from green coffee oil, variations in P and T were studied according to a central composite rotational design (CCRD) with 4 axial points and triplicate in the central points, for a total of 11 assays. The levels of the P and T studied are presented in the CCRD matrix with real values (Table 1).

The results were statistically analyzed using Statistica program v.7 [27] and presented as analyses of response surfaces.

2.3. Chemical characterization of green coffee oil

Caffeine is highly soluble in supercritical CO_2 and is therefore, extracted with the oil and other compounds. To separate the caffeine from the oil, extracts were centrifuged at 3000 rpm for 10 min (ThermoJouan BR4i Thermo FisherScientific, Waltham, USA). After centrifugation, the oil was separated and caffeine was precipitated by the addition of 3 mL of methyl tert butyl ether (MTBE; Sigma–Aldrich, St. Louis, USA); the solution was subsequently centrifuged again to remove residual coffee oil. The white solid (caffeine) was dried with N_2 at ambient temperature. The precipitate and separated green coffee oil were weighed.

Caffeine was diluted in methanol (Carlo Erba, Val de Reuil, FRA) at 24 mg/g and analyzed by gas chromatography coupled with a mass spectrometer (GC/MS) for identification.

Green coffee oils extracted via SFE and pressing were submitted to physical and chemical analyses. The focus of the chemical analyses was to quantify the diterpenes and other compounds and to verify any physical differences of the oils obtained by conventional and SFE methods.

2.3.1. Preparation of green coffee oil for diterpenes quantification

The diterpenes content in green coffee oils from conventional pressing and SFE were determined according to the methodology described earlier [28]. The oil was directly saponified with a subsequent separation of the unsaponifiable fraction. The organic phase rich in unsaponifiables was separated and washed to eliminate sodium and water-soluble compounds and dried with N_2 at room temperature.

To volatilize diterpenes for analysis in GC/MS, the unsaponifiable fraction was silanized. The dry unsaponifiable fraction was dissolved in 3 mL hexane, 9 mL pyridine (Merck, Darmstadt, GE) and 1 mL mixture of hexamethylsilazane (Sigma–Aldrich, St. Louis, USA) and trimethylchlorosilane (Sigma–Aldrich, St. Louis, USA) at a 9:3:1 proportion. This mixture of hexamethylsilazane and trimethylchlorosilane was functionalized with silanized hydroxyl groups [29]. After 30 min at room temperature, excess pyridine was removed, the sample was centrifuged and 1 μL of the supernatant was used for GC/MS.

2.3.2. Quantification of diterpenes

The quantification of cafestol and kahweol was performed using external standards; for each substance present in the unsaponifiable fraction, this method compares the integrated area of the peak with the areas of external standards at known concentrations. Different concentrations of pure cafestol and kahweol (Santa Cruz Biotechnology, California, USA) were used to construct the standard curve. These diterpenes were diluted in MTBE at 300, 500, 800, 1000, 1200, 1400, 1600 and 1800 μg/g.

The diluted silanized and non-silanized unsaponifiable fractions (1000 and 500 μg/g) and standard solutions were injected (1 μL) in a GC/MS (QP 2010 Plus, Shimadzu, Kyoto, JP) with automatic injection (AOC-5000, Shimadzu, Kyoto, JP) and a RTX®-5MS capillary column (30 m × 0.25 mm id × 0.25 microns composed of 5% diphenyl/95% dimethylpolysiloxane, Restek, USA). The analysis conditions were based on those suggested by Roos [23] and Lercker [30]. The injection was made in splitless mode with an injector temperature at 300 °C. The oven temperature started at an initial temperature of 70 °C/2.5 min, increased at 40 °C/min up to 200 °C/10 min, followed by an increase of 6 °C/min up to 235 °C/0 min and an increase of 30 °C/min to a final temperature of 330 °C/7 min, totaling 31.75 min. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the GC/MS interface was 290 °C and the ionization temperature in the source was 250 °C. The ionization energy was 70 eV and the mass scan range was 40–800 m/z.

2.3.3. Determination of total phenolic content (TPC)

The total phenolic content (TPC) was determined using the method described by Singleton and Rossi [31]. However, extracts from SFE were insoluble in methanol; therefore, the extracts were diluted in petroleum ether (10 mg/g; Synth, Diadema, BRA), unlike the method presented by Singleton and Rossi [31]. To 1 mL of diluted extract, 5 mL of Folin-Ciocalteu (Haloquímica, São Paulo,

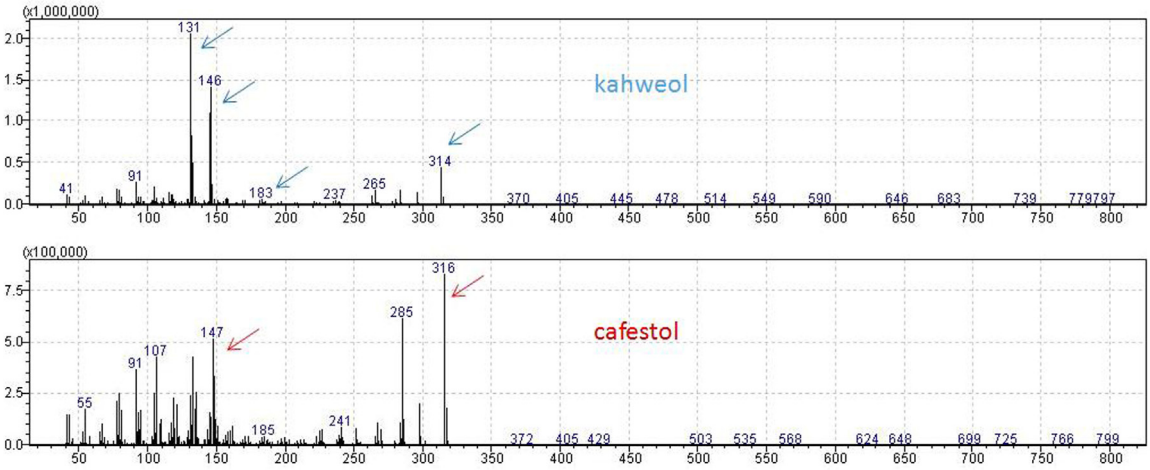

Fig. 1. Ion mass spectra of kahweol and cafestol present in green coffee oil.

BRA) solution in demineralized water (1:10, v:v; MilliQ, Direct Q3 – Billerica, Massachusetts, USA) was added. After 10 min, 4 mL of anhydrous sodium carbonate (Synth, Diadema, BRA) solution (75 g Na_2CO_3/1 L H_2O) was added. Following two hours at ambient temperature in the absence of light and ultrasound homogenization (Unique, UltraCleaner 1400s, Indaiatuba, BRA), samples were analyzed in a spectrophotometer (Biospectro SP-22, Curitiba, BRA) at 765 nm. Specifically, for these nonpolar extracts diluted in petroleum ether, constant ultrasound excitation was necessary to initiate the reduction reaction of the Folin-Ciocalteu reagent. The standard curve was made using 1–30 μg/g gallic acid (Vetel Química Fina, Rio de Janeiro, BRA) diluted in methanol.

The total phenolic content was calculated and expressed as mg of gallic acid equivalent/100 g of green coffee beans (mg GAE/100 g).

2.3.4. Determination of fatty acids composition

The determination of the fatty acids composition in the green coffee oil was carried out using the methodology developed by AOAC [32], using KOH (0.5 M) as a saponification reagent and boron trifluoride (BF3) in methanol as an esterification reagent.

The chromatographic analysis to determine the fatty acids profile was performed on a GC/MS (QP-2010 plus, Shimadzu, Kyoto, JP) with the automatic injection of 1 μL of unsaponifiable solution diluted in MTBE. An SP-2560 column (100 m × 0.20 μm × 25 mm; Supelco, Bellefonte, EUA) with an injector temperature of 250 °C and an initial over temperature at 100 °C, which increased at 5 °C/min up to 195 °C, followed by an increased rate of 2 °C/min up to 250 °C for a total time of 47.50 min. Hydrogen was used as a carrier gas at a flow rate of 1 mL/min. The temperature of the GC/MS interface was 290 °C and the ionization temperature in the source was 250 °C. The ionization energy was 70 eV, and the mass scan range was 40–800 *m*/*z*.

Quantification of fatty acids was performed using external standards to create a calibration curve of methyl myristate (Sigma–Aldrich, St. Louis, USA) diluted in MTBE. The fatty acids content was expressed as myristic acid equivalents (μg MAE/g green coffee oil).

2.4. Physical characterization of green coffee oil

Physical properties are important in a processes study. For example, the system scale-up and transport phenomena in an industrial plant are related to the characteristics of viscosity and density in each stage of the process. Based on these considerations and to compare the physical properties of green coffee oil from SFE and pressing, the viscosity, the density and the refractive index were determined.

Viscosities were determined in an automatic falling ball microviscometer (AMVn, Anton Paar, Österreich, AUT) with temperature and tilt angle controls. Measurements were performed in quadruplicate to provide accurate and reliable results.

The viscosity determination requires a prior knowledge of the densities. The density of green coffee oil was determined employing a digital densitometer (DMA 4500, Anton Paar, Österreich, AUT). This apparatus provides density data with an accuracy of 0.00005 g/cm^3 at a standard deviation of 0.00001 g/cm^3. It also measures the refractive index (RI). For density measurements, samples were directly injected into the equipment, which after temperature stabilization provided a direct measurement. The low standard deviation of the measurements in this equipment is due the analysis performed on static samples inside a U-shaped tube.

For viscosity measurements, samples were injected into a capillary in the equipment. Samples were measured at three different angles (i.e., 50°, 60°, and 70°) for a total of 12 measurements per sample.

3. Results and discussion

3.1. Kahweol and cafestol quantification

To accurately quantify the diterpenes, initial chromatographic tests were conducted to assess if silanization was necessary. The unsaponifiable fraction was silanized and analyzed by GC/MS. These analyzes were performed to identify the two major diterpenes present in green coffee oil (i.e., kahweol and cafestol).

The ion mass spectra of kahweol and cafestol from unsaponifiable fractions from silanized green coffee oil are shown in Fig. 1. The ions *m*/*z* 131, *m*/*z* 146, *m*/*z* 183, *m*/*z* 223 and *m*/*z* 314 are characteristic of kahweol ion fragmentation, with *m*/*z* 314 as the molecular ion of this diterpene. Cafestol was identified by the relatively large intensity of ions *m*/*z* 117, *m*/*z* 147, *m*/*z* 223, and with ion *m*/*z* 316 as its molecular ion [30].

During the methodological study [28], silanization was not required of the unsaponifiable fraction from green coffee oil for GC/MS analyses. Although the silanization of low volatile compounds is appropriate when using gas chromatography, in this work it was verified that an unsaponifiable fraction can be directly injected into the GC resulting in more defined peaks (approximately 25 and 27 min) for the diterpenes, kahweol and cafestol (Fig. 2).

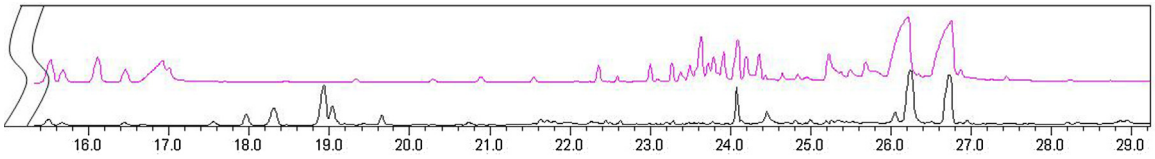

Fig. 2. Unsaponifiable fractions of green coffee oil silanized ( ) and non-silanized ( ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Diterpenes concentration, TPC and refractive index (RI) of green coffee oil obtained by pressing.

Oil	Cafestol (g/kg green coffee oil)	Kahweol (g/kg green coffee oil)	Ratio *c*/*k*	RI		TPC
				25 °C	40 °C	g GAE/g
PCO-2010	3.5	8.6	0.41	1.4787	1.4737	2.90
PCO-2012	7.5	12.8	0.59	1.4800	1.4750	18.31

GAE is Gallic acid equivalent.

The quantification of the two diterpenes in non-silanized unsaponifiable fractions of green coffee oil from pressings (PCO-2012 and PCO-2010) and from SFE were made to compare diterpenes content when different extraction methods or operational conditions were used to extract the oil. Diterpenes content in green coffee oil obtained by SFE were higher than those in green coffee oil obtained from pressings (Tables 1 and 2).

3.2. Process optimization of supercritical fluid extraction (SFE)

In the SFE of green coffee oil using supercritical CO_2, according to the CCRD, 2^2 samples were studied to determine the total yield of the green coffee oil, the caffeine and the oil concentration. Additionally, the influences of process variables (i.e., *P* and *T*) on concentrations of cafestol and kahweol and the total phenolic compound (TPC) were calculated. This study aimed to optimize the extraction process of obtaining diterpenes-enriched oil from green coffee beans.

Crude oil obtained by pressing and SFE presented as white solid precipitates after extractions. The solid material, separated from oil, was analyzed under the same chromatographic conditions used for diterpenes analysis. White precipitates were confirmed to be caffeine, which was performed by comparing retention times with pure caffeine and by the mass spectrum that showed the molecular ion *m/z* 194 as the main ion, followed by ion fragments *m/z* 109, *m/z* 67 and *m/z* 55.

The SFE performance was evaluated for various *P* and *T* values, corresponding to the first order model of the CCRD 2^2 (i.e., assays 1 to 7, Table 1), and showed no *P* or *T* influence ($p > 0.05$) on the total yield (*Y*) and concentrations of green coffee oil and caffeine as represented by Pareto diagrams (Fig. 3).

Individual analysis of the experiments presented good total and green coffee oil yields for the central point conditions. The failure to determine optimized conditions (*P* and *T*) that offers the best yield of green coffee oil may be due to the choice of the operating conditions to form the CCRD, which were chosen according to high-quality results presented by Araujo and Sandi [5] and Azevedo et al. [6].

In addition to the center points, the first axial point (assay 1, Table 1) also presented high total and green coffee oil yields, showing that higher yields were obtained for high *T* and *P*. In assay 3 (Table 1), *P* was high, but *T* was low resulting in a lower yield of the total extract. For temperatures of 80 and 90 °C and pressures of 250 and 300 bar, optimal oil yields from green coffee beans were obtained.

Caffeine is highly soluble in supercritical CO_2 and the influences of *P* and *T* in its extraction from green coffee oil were also evaluated. The caffeine concentration influenced the total yield in some conditions; however, in the ranges studied, these variables had no detectable influence (Fig. 3). High concentrations were obtained at elevated temperatures (80–90 °C) regardless of the *P* value (Table 1). However, a condition allowed for the separation of nearly pure caffeine in an extract (assay 9, Table 1); in that test, the caffeine amount was relatively low compared with tests at high temperatures. Caffeine extraction using supercritical CO_2 is used in industrial processes in Germany; the solubility of this compound is high at 63 °C and 230 bar. Park et al. [33] and Kim et al. [34] showed that high solubilities of this compound could be achieved at high pressures and low temperatures (e.g., 40 °C), confirming the results obtained in our study.

The cafestol and kahweol diterpenes contents were determined in green coffee oil from SFE. For different *T* and *P*, distinct concentrations were obtained. High diterpenes content was archived at *P* between 200 and 300 bar and *T* of 70 °C (Table 1).

Although the statistical analysis of main effects (i.e., assays 1–7, Table 1) presented on the Pareto diagram (Fig. 4) indicated that *P* and *T* did not affect cafestol and kahweol contents ($p > 0.05$) according to the CCRD 2^2, the experimental results revealed an

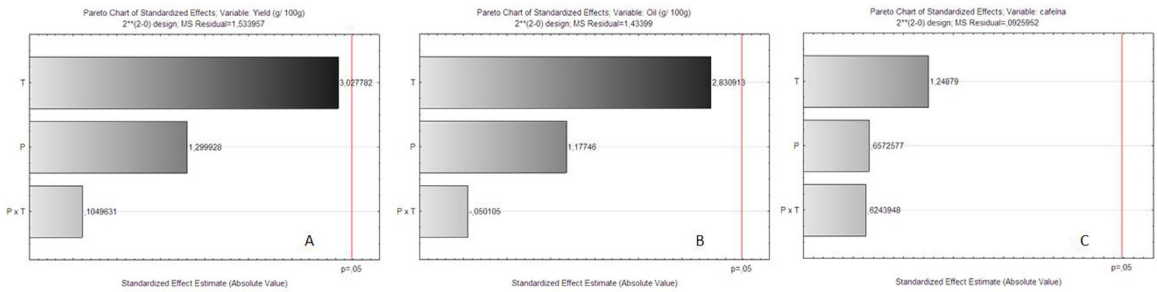

Fig. 3. Pareto diagrams that shows effects of *P* and *T* and their interaction $P \times T$ in total yield (A), green coffee oil (B) and caffeine concentration (C).

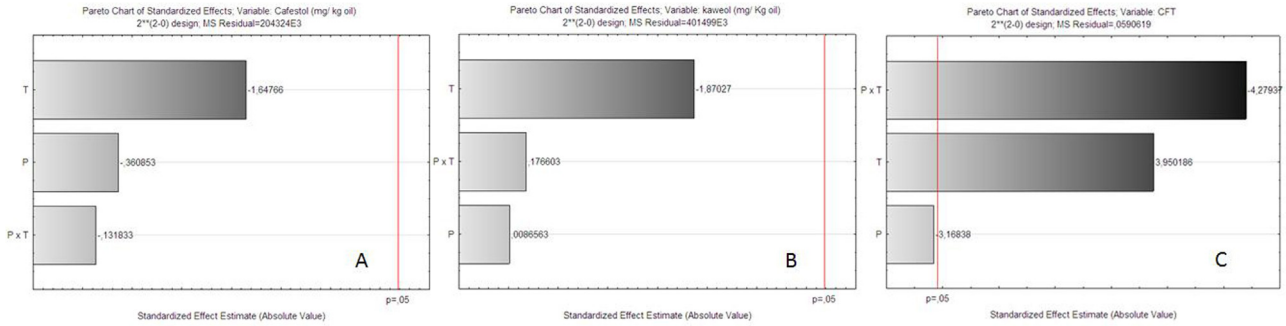

Fig. 4. Pareto diagrams showing *P* and *T* effects and their interaction $P \times T$ in cafestol (A) and kahweol (B) concentrations (mg/kg of green coffee oil) and TPC (g GAE/100 kg of green coffee oil) (C).

enrichment of diterpenes in green coffee oil from SFE. The total yield and the yield of green coffee oil were increased at high temperatures. For cafestol and kahweol, higher concentrations were identified at 70 °C and were independent of the pressure (200–300 bar; Table 1), which indicates that the optimum condition to extract diterpenes-rich green coffee oil using SFE is 70 °C. These results showed that the ability for fractionation in supercritical CO_2 extraction. Another important factor is that when the temperature was lowered from 70 to 65.9 °C and the density of CO_2 from 788.67 to 758.10 kg/m^3, no diterpenes were able to be extracted.

The ratio of cafestol to kahweol (C/K) extracted from green coffee oil, as measured in assays 3 and 4 (Table 1), indicated a higher concentration of kahweol in the oil (0.77 and 0.79, respectively). Other assays, such as those from the center point (i.e., 5, 6 and 7; Table 1), showed ratios near unity (i.e., 1.05, 1.01 and 1.65, respectively). This ratio was dependent on the operating conditions (*P* and *T*), which would increase the solubility of one diterpene to the other. This property indicates the potential for fractionation in this technology.

SFE permitted the enrichment of green coffee bean oil with cafestol and kahweol compared with the oil from the same beans using conventional pressing (Table 2). Conventional pressing of coffee oil presented cafestol contents of 3.5 g/kg oil for the oil processed in 2010 (PCO-2010) and 7.5 g cafestol/kg oil for the oil processed in 2012 (PCO-2012). For kahweol, contents were 8.6 g/kg oil (PCO-2010) and 12.8 g/kg oil (PCO-2012). Compared with oils from pressings from different years, there was considerable diterpenes degradation over time. Specifically, for Arabica coffee produced in the state of São Paulo, considerably higher concentrations of diterpenes, compared with conventional pressings, were obtained via supercritical CO_2 extraction. A higher content of cafestol in green coffee oil was produced: 50.2 g/kg oil, equivalent to 137 mg/100 g of green coffee beans; a higher kahweol concentration in green coffee oil was produced: 63.8 g/kg oil or 174 mg/100 g of green coffee beans (Table 1).

The diterpenes contents widely varies for different green or roasted coffee species; for cafestol, the content may vary from 200 to 900 mg/100 g of coffee beans and for kahweol, the concentration varies from 5 to 1000 mg/100 g of coffee beans [5,22–24,28,30]. Most of these authors used organic solvents for oil extraction and achieved higher extraction yields. More recently, Barbosa et al. [7] performed an extraction with supercritical CO_2 with and without polarity modifiers to extract cafestol and kahweol from spent coffee grounds. They obtained a maximum diterpenes concentration of 102 mg/g oil using only supercritical CO_2 in the extraction, similar to the 114 mg/g oil found in our work.

The total phenolic content (TPC) of green coffee oil from SFE (Table 1), ranged from 3.1 to 13.1 μg GAE/g of oil diluted in methanol (5000 μg green coffee oil in 1 g methanol) or from 1.68 to 16.59 mg GAE/100 g green coffee beans, with the highest value achieved at 200 bar and 90 °C. For green coffee oil from conventional pressing (Table 2), the TPC was 2.9 μg GAE/g for PCO-2010 and 18.31 μg GAE/g for PCO-2012. Coffee oil pressings (PCO-2012) presented a higher TPC when compared with green coffee oil from SFE. A lower TPC for PCO-2010 could be explained by the fact that phenolic compounds degrade during storage due to light and oxygen exposure.

In the optimization of SFE, the analysis of the main effects of *P* and *T* in TPC (mg GAE/100 g of green coffee beans), i.e., in assays 1–7 (Table 1), shows that both *T* and $P \times T$ had significant influence (Fig. 4). $P \times T$ presented a negative effect at lower *P* values because *T* promoted higher TPC in green coffee oil. The coefficients of the first order model (Eq. (2)) and their significance, as indicated by ANOVA (Table 3), indicates that the model is significant and

Table 3
Analysis of variance (ANOVA) for the first and second-order models for TPC in the green coffee oil from SFE.

Source of variation	Sum square (SS)	Degree of freedom (DF)	MS	*F*	*p*
Fist-order model					
P	0.592900	1	0.592900	10.03862	0.050544
T	0.921600	1	0.921600	15.60397	0.028941
1 by 2	1.081600	1	1.081600	18.31299	0.023438
Error	0.177186	3	0.059062		
Total SS	2.773286	6			
Second-order model					
P (L)	0.313893	1	0.313893	2.45311	0.178071
P (Q)	0.027100	1	0.027100	0.21179	0.664688
T (L)	3.508317	1	3.508317	27.41795	0.003364
T (Q)	0.094544	1	0.094544	0.73887	0.429300
1 by 2	1.081600	1	1.081600	8.45284	0.033502
Error	0.639785	5	0.127957		
Total SS	5.644291	10			

Coefficient of determination (R^2) = SQR/SQT; significant regression coefficients are those with $p < 0.05$.

Table 4
Fatty acids profile of green coffee oil from SFE.

Peak	RT (min)	Fatty acid	%	mg MAE/g green coffee oil	mg MAE/100 g coffee beans
1	22.55	Palmitic acid (C16:0)	32.4	9.3	10.5
2	25.37	Stearic acid (C18:0)	9.7	2.8	3.1
3	26.37	Oleic acid (C18:1)	12.8	3.7	4.2
4	27.98	Linoleic acid (C18:2)	38.3	11	12.4
5	28.39	Arachidic acid (C20:0)	4.6	1.3	1.5
6	29.72	Linolenic acid (C18:3)	1.4	0.4	0.4
7	31.77	Behenic acid (C22:0)	0.8	0.2	0.2

RT is the retention time; MAE is Myristic acid equivalent.

predictive because the regression coefficient (R^2) was 0.94. The ANOVA of the second-order model, corresponds to assays 1–11 (Table 1) shows that only the linear coefficients of T and $T \times P$ were significant ($p < 0.05$; Table 3). Consequently, no second-order coefficient was included in this model (Eq. (3)). The regression coefficient (R^2) was 0.88, lower than that of the linear model.

$$\text{TPC} = 1.1285 + 0.48T - 0.52P \times T \quad (2)$$

$$\text{TPC} = 0.9863 + 0.6632T - 0.52P \times T \quad (3)$$

In the response surface analysis (RSA) generated for first-order model (Eqs. (2)), the highest TPC were obtained at high T (80, 90 and 94.1 °C) and P values (from 200 to 250 bar; Fig. 5). High temperatures and densities of supercritical CO_2 positively influenced the TFC in green coffee oil. A high TPC was also obtained for higher temperature conditions (e.g., 94.1 °C) and a lower density; in this case, the high temperature had a positive influence on the TPC.

Arabica coffee beans are rich in phenolic compounds, for example, from 20 to 30 g GAE/100 g coffee beans [35]. Extracts from SFE have a low concentration of these compounds; the minimum TPC was 0.28 mg GAE/100 g of green coffee beans and the maximum TPC was 16.59 mg GAE/100 g (Table 1). This behavior was expected because the phenolic compounds present in green coffee beans are mainly caffeic, chlorogenic and ferulic acids [12,35], which are highly polar compounds and are slightly soluble in supercritical CO_2.

The green coffee oil obtained by pressing PCO-2012, when compared with the oil from SFE, presents a greater TPC (Table 2),

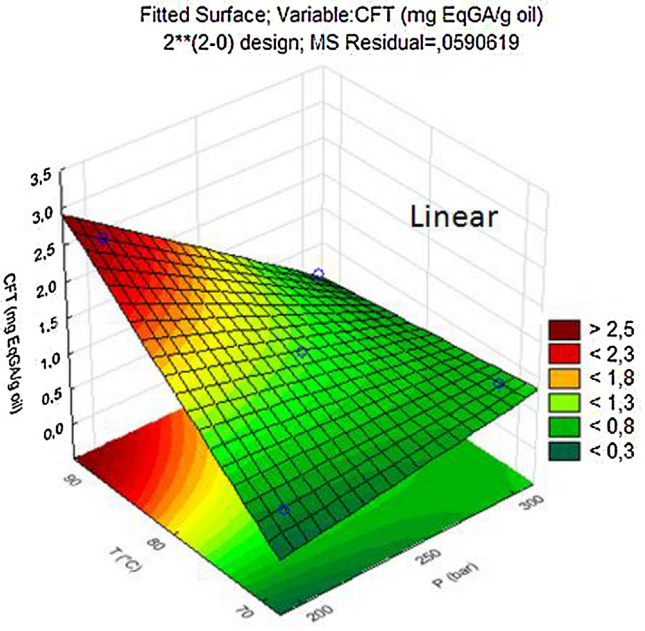

Fig. 5. RSA of the TPC (g GAE/100 kg of green coffee oil) as a function of P and T for first-order model from CCRD 22.

which is dependent on the extraction process. The green coffee oil from pressings is a result of a mechanical separation process and, therefore, was not fractionated. In this way, the oil obtained by pressing has polar and nonpolar compounds. Oils from SFE are rich in nonpolar compounds because of the polar properties of the CO_2 solvent. Because phenolic compounds are polar, they were not efficiently extracted with the oil. This is an important parameter for this process because the coffee oil extraction using supercritical CO_2 promotes the enrichment of these compounds. If green coffee beans were subjected to subsequent extraction processes using polar solvents, extracts rich in phenolic compounds without oil may be obtained.

3.3. Fatty acids profile of green coffee oil by SFE

The fatty acids composition and their proportions (Table 4) are in agreement with values reported in the literature. In coffee oil, the percentage of palmitic acid ranges from 32.1% to 33.2%; stearic acid from 7.5% to 8.2%; oleic acid from 8.2% to 12.5%; linoleic acid from 42.6% to 46.2%; linolenic acid from 0.9% to 1.4%; arachidic acid from 2.6% to 3.3% and behenic acid from 0.5% to 0.7% [36]. It is remarkable that the coffee oil composition is inherent to the species and that cultivation conditions.

The content of each specific fatty acid is expressed as myristic acid equivalents. Fatty acids at higher concentrations in the green coffee oil from SFE were palmitic, linoleic, oleic, stearic and arachidic acids. Over half of the fatty acids concentrations present in green coffee oil are polyunsaturated acids, linoleic acid (ω-6) and oleic acid (ω-9), which are essential to the human diet and are not synthesized by the body. Oils rich in these fatty acids, such as green coffee oil could be used as a source of supplemental nutrients.

3.4. Physical properties of green coffee oil from SFE

Densities and viscosities of crude green coffee oil from pressings supplied by the producers of São Paulo, Brazil and that obtained by SFE at 300 bar and 80 °C were determined. The analyses (Table 5) showed that green coffee oil from pressings presented a higher density and dynamic viscosity. These properties indicate differences

Table 5
Densities (ρ) and dynamic viscosity (η) of green coffee oil from SFE and obtained by pressing.

Temperature (°C)	Density (g/cm^3)	Dynamic viscosity (mPa × s)
Green coffee oil from pressing (conventional process)		
25	0.9419 (±0.0001)	127.8798 (±2.4733)
35	0.9350 (±0.0001)	78.0818 (±1.7090)
45	0.9282 (±0.0001)	50.8540 (±1.1662)
55	0.9214 (±0.0001)	35.0510 (±0.6927)
Green coffee oil from SFE		
25	0.9365 (±0.0001)	84.0411 (±1.7233)
35	0.9295 (±0.0001)	52.6311 (± 1.1405)
45	0.9226 (±0.0001)	34.9692 (± 0.6947)
55	0.9157 (±0.0001)	24.2555 (± 0.4692)

that provide essential data to characterize these oils. These results demonstrate a significant influence of the extraction process, which are fundamental to understanding transport phenomena in industrial processes.

Density data were correlated by linear regressions as a function of temperature (T, K) for the oil from SFE (Eq. (4)) and from pressings (Eq. (5)). The viscosities as a function of temperature were correlated to a logarithmic fit for green coffee oil from SFE (Eq. (6)) and from pressings (Eq. (7)). These equations presented regression coefficients (R^2) of 0.99.

$$\rho = -0.007 \times T + 1.439 \quad (4)$$

$$\rho = -0.007 \times T + 1.427 \quad (5)$$

$$\ln\ \eta = 4223.4 \times \frac{1}{T} - 9.3365 \quad (6)$$

$$\ln\ \eta = 4051.3 \times \frac{1}{T} - 9.1693 \quad (7)$$

Both the density and the viscosity decreases with increasing temperature. In fact, the higher the temperature is, the greater the kinetic energy of molecules. Therefore, there is an increase in the volume of the oil, resulting in a decrease in density. Regarding the viscosity, it is known that this property is a result of attraction forces between molecules when they are relatively close to one another. Consequently, when the kinetic energy increases due to temperature increases, molecules tend to remain together for a short time, making the intermolecular forces less effective in decreasing the viscosity. Density and viscosity values are higher for the green coffee oil from pressings (Table 5).

Densities, viscosities and colors of green coffee oils from these two separation methods were different. However, the refractive indices (RI) did not exhibit any significant differences. The refractive index of green coffee oil from SFE ranged from 1.4755 to 1.4810 at 25 °C and from 1.4715 to 1.4767 at 40 °C. For green coffee oil from pressings, the RI showed similar values at 25 and 40 °C (Table 2).

For SFE, refractive indices for all assays (CCRD 2^2, Table 1) were not influenced by the P and T process variables, which showed that, despite different extract colors for different P and T in SFE, these variables did not affect the main composition of the oil or the RI.

4. Conclusions

The supercritical fluid extraction (SFE) of green coffee oil produced oils enriched in cafestol and kahweol under different P and T. Although these conditions also favored the extraction of caffeine, this alkaloid was easily separated from the oil by centrifugation; the oil remained enriched in diterpenes even after separation.

The optimal yield of the green coffee oil from SFE occurred at a high temperature and pressure (300 bar and 90 °C). This condition does not provide optimal diterpene concentrations; higher concentrations in the extracts (50.2 g cafestol/kg oil and 63.8 g kahweol/kg oil) were obtained at 200 bar and 70 °C. The supercritical fluid technology allowed the enrichment of the green coffee oil in these diterpenes. The oil obtained by conventional pressing of the same beans presented lower contents for cafestol (3.5 g/kg oil) and kahweol (7.5 g/kg oil) for PCO-2010 and 8.6 g/kg oil of cafestol and 12.8 g/kg of kahweol for the oil extracted from beans from the same period (PCO-2012).

Cafestol concentrations for different species of green coffee beans depended on the species, region and growing conditions, among other processing factors. For Arabica coffee produced in São Paulo, Brazil, cafestol contents in oil obtained by supercritical CO_2 extraction ranged from 30 to 197 g/100 g of green coffee beans, and concentrations of kahweol ranged from 24 to 253 g/100 g. In this study, we found that the use of SFE made it possible to obtain diterpenes-enriched oils of green coffee beans. Additionally, physical properties of green coffee oils from SFE were determined, such as density, which ranged from 0.91570 to 0.93645 g/cm^3; and dynamic viscosity, from 24.5555 to 84.0411 mPa × s. Both properties were dependent on the temperature and refractive index (from 1.4755 to 1.4810 at 25 °C and from 1.4715 to 1.4767 at 40 °C).

The TPCs were low in green coffee oils from SFE, ranging from 0.28 to 16.59 mg GAE/100 g of green coffee beans. This behavior is advantageous in obtaining diterpenes-enriched coffee oil because other phenolic compounds present in green coffee beans are highly polar and are only slightly soluble in supercritical CO_2.

Green coffee oils contained fatty acids, such as palmitic acid (9.3 mg/g green coffee oil), and the polyunsaturated acids, such as linoleic acid (ω-6; 11 mg MAE/g green coffee oil) and oleic acid (ω-9; 3.8 mg MAE/g green coffee oil).

With these results that concern the characterization of green coffee oil from SFE and pressing, it was possible to identify that supercritical fluid operational conditions could promote conditions that provide greater diterpenes content in green coffee oil or phenolic compounds. Considering the promotion of supercritical CO_2 extraction as technological innovation against conventional methods, the results indicate the real advantages of this process.

Acknowledgments

The authors are grateful to FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) for their support of research projects 2010/16665-3. Paola Maressa Aparecida de Oliveira thanks CAPES/DS for a master's fellowship.

References

[1] J.B. Matiello, R. Santinato, A.W.R. Garcia, S.R. Almeida, D.R. Fernandes, Cultura do café no Brasil: Novo manual de recomendações, 2rd Ed., MAPA/Prócafé, Rio de Janeiro, 2005, 434 pp.
[2] M. Ferrari, F. Ravera, E. De Angelis, F. Suggiliverani, L. Navarini, Interfacial properties of coffee oil, Colloids and Surfaces A: Physicochemical and Engineering Aspects 365 (2010) 79–82.
[3] A.L. de Oliveira, P.M. Cruz, M.N. Eberlin, F.A. Cabral, Brazilian roasted coffee oil obtained by mechanical expelling: compositional analysis by GC–MS, Ciência e Tecnologia de Alimentos 25 (2005) 677–768.
[4] A.L. de Oliveira, S.S. Silva, M.A.P. Silva, M.N. Eberlin, F.A. Cabral, Sensory and yield response surface analysis of supercritical CO_2 extracted aromatic oil from roasted coffee, Journal of Food Science and Technology 38 (2001) 38–42.
[5] M.A. Araújo, D. Sandi, Extraction of coffee diterpenes and coffee oil using supercritical carbon dioxide, Food Chemistry 101 (2006) 1087–1094.
[6] A.B.A. Azevedo, T.G. Kieckbush, A.K. Tashima, R.S. Mohamed, P. Mazzafera, S.A.B. Vieira de Melo, Extraction of green coffee oil using supercritical carbon dioxide, Journal of Supercritical Fluids 44 (2008) 86–192.
[7] H.M.A. Barbosa, M.M.R. Melo, M.A. Coimbra, C.P. Passos, C.M. Silva, Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology, Journal of Supercritical Fluids 85 (2014) 165–172.
[8] A.M.R. Alvarez, M.L.G. Rodriguez, Lipids in pharmaceutical and cosmetic preparations, Grassas y Aceites 51 (2000) 74–79.
[9] J.F. Grollier, S. Plessis, Use of coffee bean oil as a sun filter. United States Patent no 4,793,990. US Patent & Trademaker Office, 1988.
[10] E. Pelle, Topical composition and method for enhancing lipid barrier synthesis. United States Patent n o 5,855,897. US Patent & Trademaker Office, 1999.
[11] From Nature with Love, Marketing site of natural extracts 2010. Available from: http://www.fromnaturewithlove.com/product.asp?product_id=oilcoffeegreen&track=nbw100608
[12] A. Farah, C.M. Donangelo, Phenolic compounds in coffee, Brazilian Journal Plant Physiology 18 (2006) 23–36.
[13] D. Perrone, A. Farah, C.M. Donangelo, T. Paulis, P.R. Martin, Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars, Food Chemistry 106 (2008) 859–867.
[14] G.F. Ferrazzano, I. Amato, A. Ingenito, A. De Natale, A. Pollio, Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea), Fitoterapia 80 (2009) 255–262.
[15] P. Muriel, J. Arauz, Review: coffee and liver diseases, Fitoterapia 81 (2010) 297–305.
[16] G. Gross, E. Jaccaud, A.C. Huggett, Analysis of the content of the diterpenos cafestol and kahweol in coffee brews, Food and Chemical Toxicology 35 (1997) 547–554.

[17] C. Cavin, D. Holzhaeuses, G. Scharf, A. Constable, W.W. Huber, B. Schlter, Cafestol and kahweol, two coffee specific diterpenos with anticarcinogenic activity, Food and Chemical Toxicology 40 (2002) 1155–1163.
[18] W.W. Huber, L.P. McDaniel, K.R. Kaderlik, C.H. Teitel, N.P. Lang, F.F. Kadlubar, Chemioprotection against the formation of colon DNA adducts from the food-borne carcinogen 2amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (PhIP) in the rat, Mutation Research 376 (1997) 115–122.
[19] C. Cavin, K. Mace, E.A. Offord, B. Schilter, Protective effects of coffee diterpenes against aflatoxin Bl-induced genotoxicity: mechanisms in rat and human cells, Food and Chemical Toxicology 39 (2001) 549–556.
[20] E. Giovannucci, Meta-analysis of coffee consumption and risk of colorectal cancer, American Journal of Epidemiology 147 (1998) 1043–1052.
[21] K. Speer, I. Kölling-Speer, The lipid fraction of the coffee bean, Brazilian Journal Plant Physiologie 18 (2006) 2010–2016.
[22] R. Urgert, G. van Der Weg, T.G. Kosmeijer-Schuil, P. van Der Bovenkamp, R. Hovenier, M.B. Katan, Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews, Journal of Agricultural and Food Chemistry 43 (1995) 2167–2172.
[23] B. Roos, G. van Der Weg, R. Urgert, P. van Der Bovenkamp, A. Charrierand, M.B. Katan, Levels of cafestol, kahweol, and related diterpenoids in wild species of the coffee plant coffea, Journal of Agricultural and Food Chemistry 45 (1997) 3065–3069.
[24] R.C.E. Dias, F.G. Campanha, L.G.E. Vieira, L.P. Pereira, D. Pot, P. Marraccini, M.T. Benassi, Evaluation of kahweol and cafestol in coffee tissues and roasted coffee by a new high-performance liquid chromatography methodology, Journal of Agricultural and Food Chemistry 58 (2010) 88–93.
[25] T. Halstead, Coffee: World Markets and Trade, USDA – United States Department of Agriculture, 2013, Available from: http://www.fas.usda.gov/data/coffee-world-markets-and-trade
[26] L.E.D.N. Sutter, M.F.F. Silva, E. Cassel, Extração supercrítica, uma nova tecnologia industrial, Ciência e Tecnologia de Alimentos 14 (1994) 3–10.
[27] STATISTICA (data analysis software system), v.7, StatSoft, Inc., 2004, Available from: www.statsoft.com
[28] A. Chartier, M. Beaumesnil, A.L. de Oliveira, C. Elfakir, S. Bostyn, Optimization of the isolation and quantitation of kahweol and cafestol in green coffee oil, Talanta 117 (2013) 102–111.
[29] C.C. Sweeley, R. Bentley, M. Makita, W.W. Wells, Gas–liquid chromatography of trimethylsilyl derivatives of sugars and related substances, Journal of the American Chemical Society 85 (1963) 2497.
[30] G. Lercker, N. Frega, F. Bocci, M.T. Rodriguez-Estrada, High resolution gas chromatographic determination of diterpenic alcohols and sterols in coffee lipids, Chromatographia 41 (1995) 29–33.
[31] V.L. Singleton, J.A. Rossi Jr., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture 14 (1965) 4–15, 8.
[32] AOAC, Official Methods of Analysis of AOAC International, 18th Ed., AOAC International, Gaithersburg, Official Method, 2005, pp. 969.33.
[33] H.S. Park, N.G. Im, K.H. Kim, Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves, Food Science and Technologies (2012) 73–78.
[34] W.J. Kim, J.D. Kim, J. Kim, S.G. Oh, Y.W. Lee, Selective caffeine removal from green tea using supercritical carbon dioxide extraction, Journal of Food Engineering 89 (2008) 303–309.
[35] M.M. Naidu, G. Sulochanamma, S.R. Sampathu, P. Srinivas, Studies on extraction and antioxidant potential of green coffee, Food Chemistry 107 (2008) 377–384.
[36] R.C.A. Lago, Lipídios em grãos de café, Boletim do CEPPA 19 (2001) 319–340.