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Extraction of coffee diterpenes and coffee oil using supercritical carbon dioxide

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Abstract

Commercial green and roasted coffee beans were used to maximize oil extraction and conditions were studied to obtain the highest and lowest diterpene levels on green and roasted coffee oil, respectively. Thus, operational temperatures (60–90 °C) and pressure (235–380 bar) were optimized for coffee oil extraction. Oil content levels and diterpene oil concentration were compared to the results obtained with the extraction with Soxhlet apparatus, using hexane as solvent. In general, an inverse correlation was observed between the amount of extracted oil and diterpene concentration levels. As a result, different oil contents with different diterpene concentrations could be obtained. The HPLC analysis of cafestol and kahweol in the oil extracted from green coffee beans at 70 °C/253 bar resulted in the highest concentration (453.3 mg 100 g^{-1}), which was 48% lower than in the oil extracted with hexane while in the oil extracted from roasted coffee beans at 70 °C/371 bar, resulted in 71.2% reduction of diterpenes.

Keywords: Supercritical carbon dioxide extraction; Green coffee oil; Roasted coffee oil; Cafestol; Kahweol; HPLC

1. Introduction

The food and pharmaceutical industries have rapidly taken advantage of the possibilities of using carbon dioxide as a nontoxic, environmentally safe, cheap, and selective extraction solvent (Brunner, 1994; McHugh & Krukonis, 1994). Compared to liquid solvents, carbon dioxide has the advantage of displaying adjustable selectivity or solvent power that can be set to values ranging from gas to liquid-like. Carbon dioxide has a lower critical temperature (31.1 °C) and moderate critical pressure (73.8 bar), thus being an ideal solvent for compounds that may suffer thermal degradation (Palmer & Ting, 1995).

Global coffee production comes from two major species, *Coffea arabica* and *Coffea robusta*, with nearly three-quarters coming from the former, which contains cafestol (about 0.6%) and kahweol (0.3%) (Urget & Katan, 1996) (see Fig. 1). The latter, *C. robusta*, contains mostly cafestol (0.2%). Total diterpene content ranges from 1.3% to 1.9% (w/w) in *C. arabica* beans and 0.2–1.5% in *C. robusta* beans (Ratnayake, Hollywood, Ogrady, & Stravic, 1993).

Cafestol and kahweol, naturally occurring diterpenes found only in coffee, are present in the unsaponifiable lipid fraction (Kolling-Speer, Strohschneider, & Speer, 1999). Their content in a coffee drink is influenced by the brewing method (Gross, Jaccaud, & Huggett, 1997); brewing releases oil droplets containing cafestol and kahweol from the ground coffee beans. Boiled coffee, such as Scandinavian-style and Turkish-style, contains the highest concentrations, while instant, drip-filtered, and percolated coffees contain negligible amounts. The amount of cafestol and kahweol can be significantly reduced by roasting the green coffee (Bak & Grobee, 1989; Kolling-Speer et al., 1999).

Green coffee oil has been used in the cosmetics industry because of the emollient property provided by its fatty acids and its capacity to block sunlight harmful to human skin (Alvarez & Rodriguez, 2000; Grollier & Plessis, 1988;

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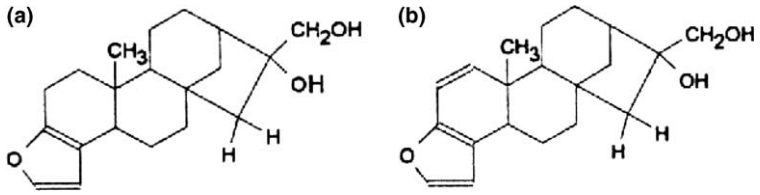

Fig. 1. Chemical structure of cafestol (a) and kahweol (b).

Pelle, 1999). Roasted coffee oil has also been widely used as a flavor source in food and cosmetics. Moreover, a reduction in the diterpene levels of roasted coffee oil significantly increases its stability and sensorial profile, decreasing its hypercholesterolemic effect (Bak & Grobee, 1989; Kolling-Speer et al., 1999).

The anticarcinogenic property of cafestol and kahweol has been hypothesized to be related to their ability to induce glutatione S-transferase (GST). In mice and rats, both substances were found to induce GST activity of the liver and intestinal mucosa. Studies with derivatives of cafestol and kahweol indicate that furan moiety is the active site for induction of enzyme activity (Aro, Kostiainen, Huttunen, & Sepalla, 1985; Cavin, Holzhaeuser, Scharf, Constable, & Hubber, 2002; Lam, Sparnins, & Wattenberg, 1982; Miller, McWhorter, Rivera-Hidalgo, & Wright, 1991; Scharf, Prutomersky, & Huber, 2001; Wattenberg, 1984).

The cholestrol-raising effect of boiled coffee in humans has been linked to these diterpenes (AL Kanhal, 1997; Burr, Limb, Sweetnan, & Fehily, 1995; Heckers, Gobel, & Kleppel, 1994; Mensink, Lebbink, Lobbezoo, & Katan, 1995). Paper-filtered coffee does not elevate cholesterol since the lipid content (including diterpenes) is negligible (Urget & Katan, 1996). Studies have shown that an intake of cafestol and kahweol causes an increase in total cholesterol as well as low-density lipoprotein (LDL) cholesterol, triglycerides, and alanine aminotransferase (ALT) activity in abnormal subjects (Grubben, Boers, & Blom, 2000; Halvorsen, Ranhein, Nenseter, Huggett, & Drevon, 1998; Roos, Caslake, & Stalenhoef, 2001; Terpstra, Katan, & Beynen, 2000; Van Rooij et al., 1995).

Common methods for extracting the oil from coffee beans include organic solvent extraction with hexane using soxhlet over several hours. However, this procedure has an important drawback, long extraction time, consuming large quantities of solvent and requiring additional concentration step. Some research has been published on supercritical extraction of oil from coffee beans as a source of aroma (Gopalakrishnan, 1990; Lopez-Fontal & Castano-Castrilon, 1999; Roselius, Vitzthum, & Hubert, 1982; Sarrazin, Le Quere, Gretsh, & Liardon, 2000). However, none studied the behavior of the diterpenes on extraction of oil in green and roasted coffee beans.

This work aimed to study the feasibility of applying carbon dioxide under supercritical conditions to maximize oil extraction to obtain the highest and lowest diterpenes levels on green and roasted oil, respectively. Operational temperature, carbon dioxide density, moisture content, granulometry and CO_2 flow rate were optimized for coffee oil extraction. A method using high performance liquid chromatography (HPLC) with detection at 220 nm has been used for diterpene identification.

2. Materials and methods

2.1. Materials

The standards used in this work for HPLC analysis were cafestol (LKT Labs. Inc., Saint Paul, MN and kahweol (Sigma, Saint Louis, MO). HPLC grade methanol and hexane (Merck) were used as solvents after filtration through a 0.45 μm pore size filter (Milipore, Bedford, MA). Two different types of carbon dioxide, (supercritical fluid and refrigerant fluid), were supplied by White Martins (Brazil).

2.2. Sample preparation

Commercial green and roasted coffee beans (*C. arabica*) containing 9.98% and 2.40% humidity, respectively, were used for supercritical extraction. The coffee beans were ground in a bench coffee grinder and sieved to obtain particles with diameters ranging from 0.297 to 0.35 mm; 0.35 to 0.42 mm; and 0.42 to 0.50 mm.

2.3. Extraction methods

2.3.1. Soxhlet method

Twenty grams of sample were weighted in filter paper and placed in a 500 ml soxhlet glass timble. The extraction was carried out using hexane as solvent (10 ml g^{-1} of sample) at the solvent boiling point for 16 h. After extraction, the solvent was evaporated by reduced pressure evaporation (30 °C; Fisaton model 802) and the extract was dried at 103 °C to remove residual solvent, cooled for 30 min in a dessicator, and weighted. This procedure was repeated until a constant extract weight was obtained. Table 1 summarizes the average percent extractives obtained by soxhlet extraction.

2.3.2. Supercritical fluid extraction method

The SFE experiments were performed on a Hewlett-Packard model 7680A SFE module. An experimental design of two factors (temperature and pressure) was per-

Table 1
Average percent extractives via soxhlet

	Humidity	Oil (g 100 g^{-1})		Diterpene (mg 100 g^{-1})	
		Wet base (%)	Dry base (%)	Cafestol	Kahweol
Green coffee	9.98	10.23	11.37	383.9 ± 2.3	476.2 ± 6.4
Roasted coffee	2.40	15.42	15.49	328.5 ± 3.9	397.8 ± 6.6

formed to define the best conditions. The extractions were performed in a 4 × 4 factorial scheme, with three replications. Two hundred milligrams of the sample were weighted in a filter paper and packed into a 7-ml thimble (green coffee with 9.98% humidity and 11.37% oil or roasted coffee with 2.4% humidity and 15.49% oil). The volume of the thimble was reduced to 5.46 ml with a glass stick to increase the ''thimble-volume-swept''. The thimble was closed with hand screw caps and system-pressurized with pressure ranging from 235 to 380 bar at temperatures from 60 to 90 °C. Extractions were carried out using a static period (5 min), followed by a dynamic period (20 min) to a total extraction time of 25 min. The flow of supercritical CO_2 was held constant at 1.5 ml min^{-1}. After extraction, the ODS trap was automatically rinsed three times with 1.5 ml of hexane at a flow rate of 0.5 ml min^{-1} and collected in three separate vials. The solvent was vaporized with nitrogen at 45 °C, and the oil was weighted and saponified through addition of KOH 0.5 mol L^{-1} in methanol (Gross et al., 1997; Hartman & Lago, 1973; Kolling-Speer et al., 1999). The unsaponifiable fraction was extracted with hexane, washed repeatedly with water, vaporized with nitrogen at 45 °C and dissolved in mobile phase. This solution was filtered through a 0.45 μm filter for diterpenes analysis by HPLC.

2.4. High performance liquid chromatography (HPLC)

Samples was carried out with HPLC equipped with a Reodyne 7125 injector linked to a 50 μl loop and variable-wavelength UV detector (Hewlett-Packard, 1050 series). The HPLC method and identification and quantification procedures used have been described in detail elsewhere (Gross et al., 1997; Hartman & Lago, 1973; Kolling-Speer et al., 1999). Some of the features are as follows: samples were analyzed on a reverse-phase column (HP-C_{18}, 200 mm × 4.6 mm insider diameter, particle size 5 μm) at ambient temperature. The mobile phase was a mixture of solvent methanol/water, 85/15 using a flow rate of 0.7 ml min^{-1} and detection wavelength set to 220 nm. For diterpene identification in the samples, stock solutions of cafestol and kahweol (1.0 mg ml^{-1}) were first prepared in a mobile phase (methanol/water, 85/15), and then diluted to a final concentration between 0.019–0.152 and 0.016–0.128 mg ml^{-1}, respectively. The identity of the separated diterpenes in the oil extracts was assigned by comparing the retention times and co-chromatography with authentic standards. The chromatogram obtained at wavelength of 220 nm was used for quantification in all cases. Response factors for each of the HPLC standards were obtained by linear regression of known concentrations versus peak areas. Response linearity was observed for a concentration range of 0.016–0.147 mg ml^{-1} with 1% confidence level. The calibration curves (correlation coefficient) for cafestol and kahweol were higher than 0,99. Each extract was analyzed until reaching reproducibility higher than 95%. The chromatogram of the extract obtained by SFE is presented in Fig. 2.

3. Results and discussion

Although several authors have reported methods (SFE, Destillation) for the extraction of coffee oil as aroma source (Lopez-Fontal & Castano-Castrilon, 1999; Roselius et al., 1982; Sarrazin et al., 2000), the SFE of the diterpenes in coffee oil has not been investigated to date. We had previously performed soxhlet extractions in duplicate on a green and roasted coffee sample for 16 h in hexane (Table 1). The crude extract was concentrated under reduced pressure evaporation and weighted. Roasting coffee beans decrease diterpene levels, which are dependent on the roasting temperature utilized (Kolling-Speer et al., 1999).

3.1. Direct SFE from coffee beans

This study attempted to directly extract oil from green coffee beans containing high diterpene levels and from roasted coffee beans with low diterpene levels using SC-CO_2. Efforts including changing the operating temperature and pressure were applied to improve the process. The experimental design focused on two variables, pressure and temperature, with four extraction conditions being selected for each temperature and pressure to obtain the pre-determined objectives. The extraction yields from different methods – SFE and Soxhlet – are shown in Table 2. In general, an inverse correlation was observed between the amount of extracted oil and the diterpene concentration levels. Different oil contents with different diterpene concentrations could be obtained via single-step direct SFE extraction from coffee beans, after selected the best temperature and pressure.

3.2. SFE extraction of green coffee beans

The initial development of the conditions for analytical SFE of diterpene in a green coffee beans, was performed in pressure range of 235–380 bar and temperatures between 60 and 90 °C. The oil content and diterpene were compared

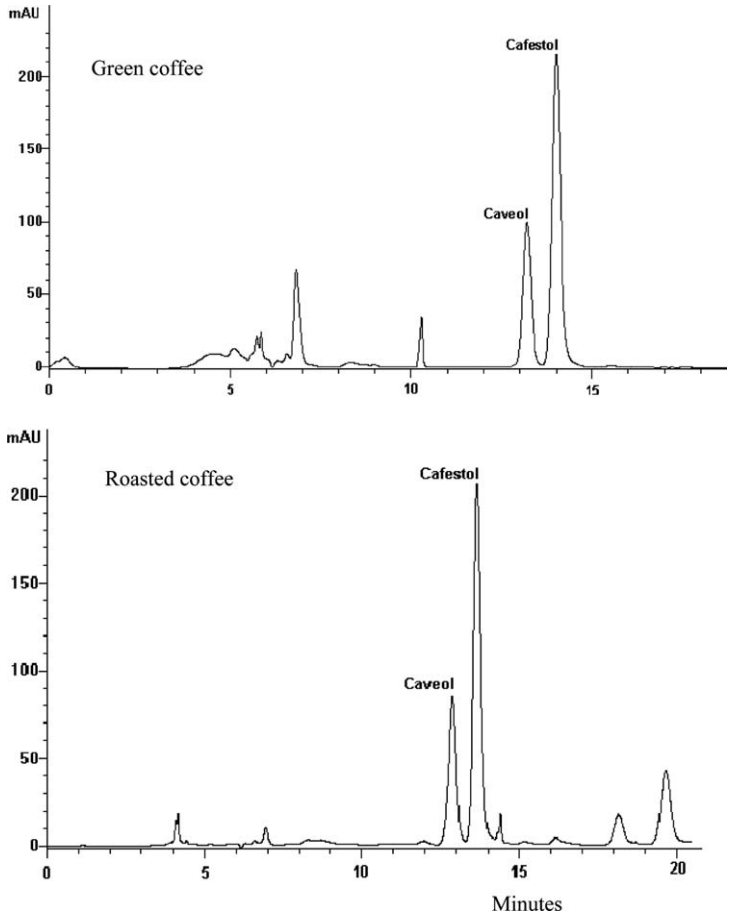

Fig. 2. Chromatograms of the unsaponified oil extracted by SFE.

Table 2
Mean value of the amount of diterpenes from green and roasted coffee extracted for each extraction method

Diterpenes (mg 100 g^{-1})	Extraction method							
	Soxhlet		CO_2-SC (°C/bar)					
	Green	Roasted	Green coffee			Roasted coffee		
			70/253	70/327	90/373	70/371	80/379	90/379
Cafestol	383.9 ± 2.3	328.5 ± 3.9	201.7 ± 1.82	230 ± 2.11	182.8 ± 1.87	94.4 ± 0.67	122.2 ± 0.87	113.3 ± 0.51
Kahweol	476.2 ± 6.4	397.8 ± 6.6	251.6 ± 2.32	261.1 ± 2.57	230.9 ± 2.22	114.7 ± 0.87	165.7 ± 1.55	139.8 ± 0.92
Total	860.1	726.3	453.3	491.1	413.7	209.1	287.9	253.1

to the results obtained with the extraction with soxhlet apparatus. Best results were obtained with the following raw material characteristics and extraction conditions: moisture content (9.98%), granulometry (0.297–0.35 mm.), sample weight (200 mg), CO_2 flow (1.5 ml min^{-1}), non-static extraction and dynamic extraction (20 min), kept constant.

The solubility of fat in supercritical carbon dioxide increases with density and temperature (Sthal, Schutz, & Mangold, 1980). In a natural matrix, the distribution of the solutes in the solid substrate and the interactions among them have a high influence on the course of the extraction. Thus, fat extraction in a fat matrix should preferably be performed at the highest possible temperature and density. As coffee oil has a high unsaponifiable content as compared to that normally found in other plant oils (AL Kanhal, 1997; Lopez-Fontal & Castano-Castrilon, 1999), coffee oil solubility in SC-CO_2 might differ from that commonly observed. The most efficient extraction (Fig. 3) was obtained with temperature of 90 °C/373 bar/0.77 g ml^{-1}. These conditions completely promoted oil extraction within 20 min, using 28.6 g of CO_2 but the concentration of diterpenes was low (Table 2). A strong interaction between temperature and density (pressure) was observed. The predominant effect of the temperature in the amount of oil extracted is due to an increase in the extract steam pressure in detriment of fluid density and a higher kinetics of desorption of the oil from the sample matrix. As the temperature increases, desorption is faster and more solute is available for extraction.

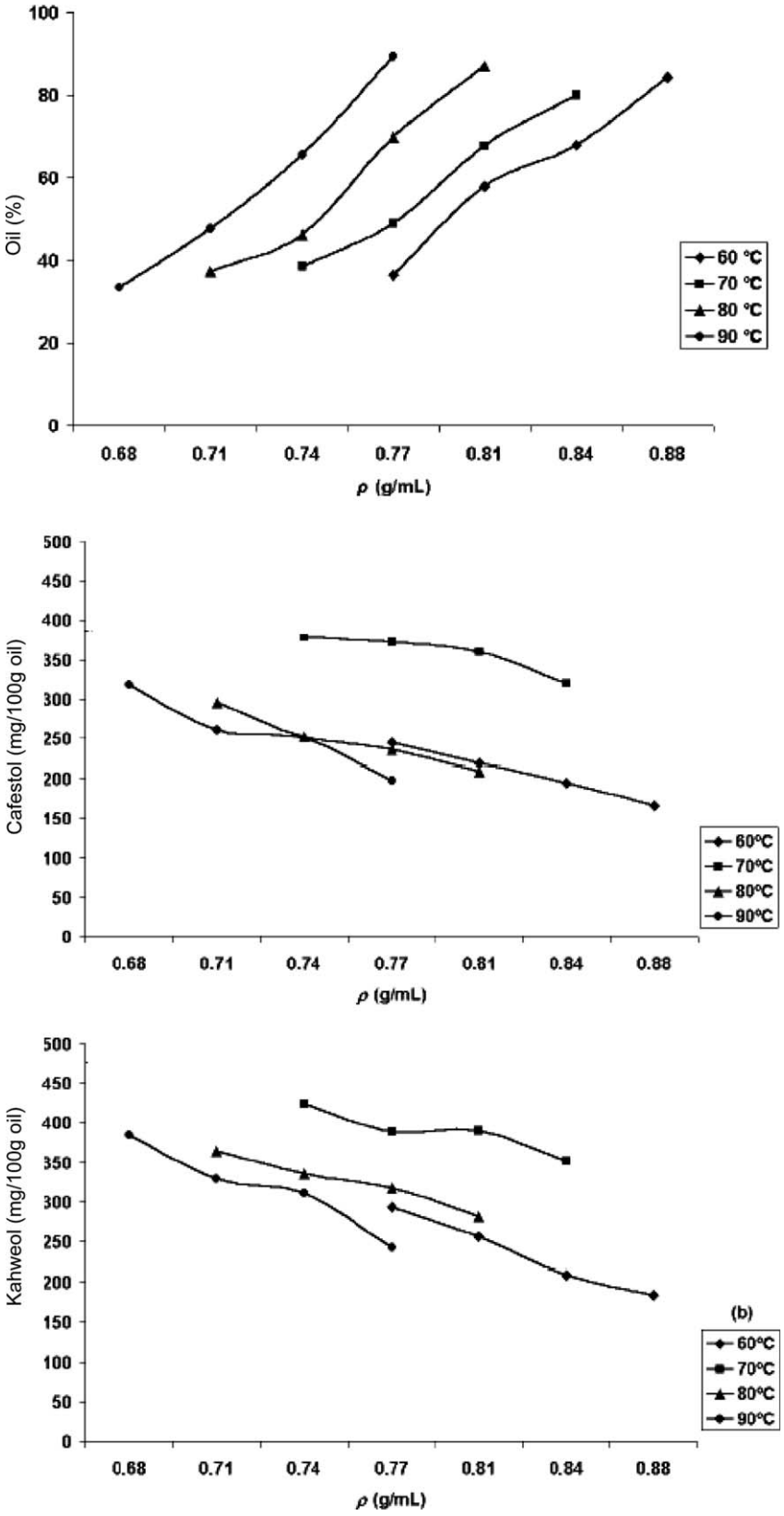

Fig. 3. Oil content and diterpene (cafestol and kahweol) in green coffee beans extracted by SFE.

Table 2 shows that the highest concentration of diterpenes was achieved at 70 °C/327 bar/0.81 g ml^{-1} with cafestol and kahweol values being 230 and 261.1 mg 100 g^{-1}, respectively, i.e., 43% lower when compared to oil obtained with hexane extraction. Increasing density significantly decreases diterpenes yield in oil for all isotherms.

3.3. SFE extraction of roasted coffee beans

The initial development of the conditions for analytical SFE of diterpene in roasted coffee oil with 2.4% humidity, to obtain the lowest diterpene levels was performed in pressure range of 235–380 bar and temperatures between 60 and 90 °C. Dynamic extraction time (20 min), granulometry (0.297–0.35 mm), extraction sample weight (200 mg), CO_2 flow (2.0 ml min^{-1}), and 5 min static extraction were kept constant, and the exper-

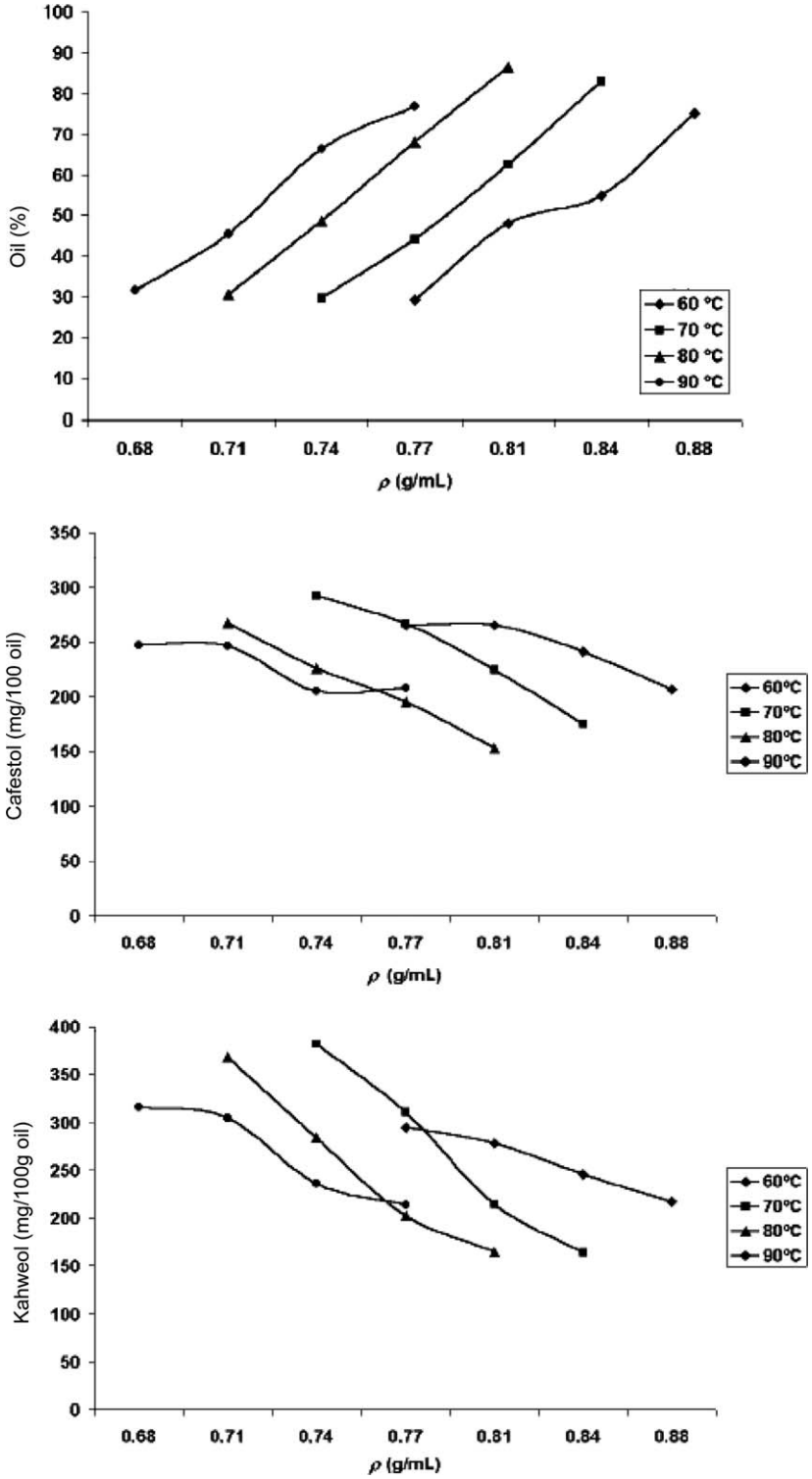

Fig. 4. Oil content and diterpene (cafestol and kahweol) in roasted coffee beans extracted by SFE.

imental design was the same as described above. The amount of cafestol and kahweol was significantly reduced by roasting the green coffee (Table 2), which was 14.4% and 16.5% less for cafestol and kahweol, respectively. Fig. 4 shows that roasted coffee oil was completely extracted in 20 min at 70 °C/371 bar/ 0.84 g ml^{-1} using 36.8 g of CO_2. This condition was the most efficient in significantly reducing the oil diterpene concentrations, with the values 94.4 and 114.7 mg 100 g^{-1} being obtained for cafestol and kahweol, respectively (Table 2). These values represent 28.8% of the total of diterpenes compared to levels obtained in oil extraction using hexane. One can observe (Fig. 4) that from a same isotherm, as density increase, the inclination of the isotherm is more pronounced for the roasted coffee oil. It means that oil concentration in the CO_2 became higher for roasted coffee oil than for the green coffee oil, and significantly decreases diterpenes yield in oil for all isotherms.

4. Conclusion

Classical solvent extraction of coffee oil rich in diterpenes is well known and several solvents have been assayed. SFE is a very useful method for extracting valuable oil from green coffee beans with high levels of diterpenes for cosmetics, and a healthy coffee oil with low levels of diterpenes from roasted coffee beans for food industry, without any remaining organic solvent such as hexane or petroleum benzene.

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