

## CONTENTS OF DITERPENES IN ESPRESSO COFFEE BREWS PREPARED FROM COMMERCIAL CAPSULES

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**ABSTRACT:** The objective of this work was to quantify kahweol and cafestol diterpenes in coffee brews prepared from commercial capsules for espresso in the Brazilian market. Four types of brews, with five preparation replications, were evaluated. The capsules had differences in the amount and type of roasted and ground coffees used (blends of arabica and robusta coffee or 100% arabica coffee), and in the conditions of time and volume of extraction (dose) recommended by the manufacturer. The coffee brews presented 1.42 and 4.88 g of solids/100 mL. Concentration of solids decreased with the increase in time/volume extraction. Contents of 0.47 to 1.04 mg of kahweol and 0.38 to 0.92 mg of cafestol by dose (ranging from 35 to 120 mL) were observed. These contents corresponded to a range of 0.40 to 2.96 mg of kahweol/100 mL and 0.32 to 2.62 mg of cafestol/100 mL. The fraction of diterpenes extracted varied from 1.85 to 4.27 % for kahweol and 1.87 to 4.16 % for cafestol. Considering the contents of cafestol, there is no indication of a hypercholesterolemic effect due to a moderate consumption of coffee brews prepared from these commercial capsules.

**Index terms:** Kahweol, cafestol, blends, single-dose, UPLC.

## TEORES DE DITERPENOS EM BEBIDAS DE CAFÉ ESPRESSO PREPARADAS COM CÁPSULAS COMERCIAIS

**RESUMO:** Objetivou-se, neste trabalho, quantificar os diterpenos caveol e cafestol em bebidas de café preparadas a partir de cápsulas comerciais para espresso do mercado brasileiro. Foram avaliadas bebidas de quatro tipos de cápsulas com 5 repetições de preparo. As cápsulas apresentavam diferenças na quantidade e tipo de café torrado e moído empregado (blends de café arábica e robusta ou cafés 100% arábica), e nas condições de tempo e volume de extração (doses), preconizadas pelo fabricante. As bebidas apresentaram de 1,42 a 4,88 g de sólidos/100 mL. A concentração de sólidos diminuiu com o aumento no tempo/volume de extração. Observaram-se teores de 0,47 a 1,04 mg de caveol e 0,38 a 0,92 mg de cafestol por dose (variando de 35 a 120 mL), correspondentes à faixa de 0,40 a 2,96 mg de caveol/100 mL e 0,32 a 2,62 mg de cafestol/100 mL. A porcentagem de diterpenos extraídos variou de 1,85 a 4,27 % para caveol e 1,87 a 4,16 % para cafestol. Considerando-se os teores de cafestol, há indicação que o consumo moderado de café preparado a partir de cápsulas comerciais não implicaria em efeito hipercolesterolêmico.

**Termos para indexação:** Caveol, cafestol, blends, monodoses, CLUE.

### 1 INTRODUCTION

Coffee is one of the most consumed beverages in the world and Brazil is the world leader in the production and exportation of coffee and the second largest consumer of the brew (INTERNATIONAL COFFEE ORGANIZATION - ICO, 2014). Although the product has consolidated its place in the market, new options in coffee brewing are important strategies to increase consumption. A change in the coffee consumers' profile has been observed, which has intensified the consumption of brews with differentiated and high quality standard products (FIGUEIRÓ, 2014).

Coffee brews are prepared in different ways around the world. Espresso coffee is a well known way of brewing, prepared by forcing boiling water under pressure through ground coffee,

and consumed at the moment of the extraction. In Brazil, espresso coffee is more consumed out of home (ASSOCIAÇÃO BRASILEIRA DA INDÚSTRIA DE CAFÉ - ABIC, 2010).

Espresso coffee single-dose capsules have become a growing trend in coffee marketing worldwide. In Brazil, the consumption of this product is recent and low (about 1%), compared with other traditional preparations such as filtered coffee. However, an increase in the European and in US markets has been observed. In France, Portugal and in US, the consumption of coffee capsules is around 30%. In Brazil, the marketing of coffee capsules increased 52.4% between 2013 and 2014. Two coffee capsules production plants in Brazil were announced for 2015, with a production forecast of 360 million capsules per year (BUREAU DE INTELIGÊNCIA COMPETITIVA DE CAFÉ, 2015; FIGUEIRÓ,

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2014; INOVAÇÕES..., 2014; SANTOS; SILVA; CASTRO JUNIOR, 2012).

Moderate coffee consumption brings many health benefits due to the presence of several bioactive compounds in this type of beverage (ESQUIVEL; JIMENEZ, 2012; FREEDMAN et al., 2012; HIGDON; FREI, 2006; MURIEL; ARAUZ, 2010). Kahweol and cafestol diterpenes, present in the lipid fraction of coffee, are associated with desirable effects such as protection against chronic degenerative diseases and toxins and anticarcinogenic, antioxidant and anti-inflammatory actions. Undesirable effects as the increase of serum cholesterol, notably associated with the consumption of the cafestol, were also observed (CÁRDENAS; QUESADA; MEDINA, 2015; CAVIN et al., 2002; KIM et al., 2006; LEE; JEONG, 2007; TAO et al., 2008; URGERT et al., 1995).

A wide variation in contents of diterpenes in coffee brews is described. Contents in the range of zero to 13.2 mg/100 mL of kahweol and zero to 10.0 mg/100 mL of cafestol were reported for espresso coffee brews (GROSS; JACCAUD; HUGGETT, 1997; SRIDEVI; GIRIDHAR; RAVISHANKAR, 2011; URGERT et al., 1995). It is difficult to compare literature results due to the lack of important information as the concentration of solids in brews and the coffee species used. With the exception of the study realized by Gross, Jaccaud and Hugget (1997), contents of diterpenes in espresso brews prepared with commercial capsules have not been reported.

Considering the effects of diterpenes intake on consumer health and the increasing consumption of roasted and ground coffee capsules, the objective of this work was to quantify kahweol and cafestol in brews prepared with four types of commercial capsules available in the Brazilian market.

## 2 MATERIAL AND METHODS

**Coffee capsules:** Four types of capsules of roasted and ground coffee (samples A, B, C and D), from the same manufacturer, were evaluated. They were acquired in the Brazilian market, from January to July of the 2014. The products were marketed in paper packages containing 16 capsules. The capsules had differences in the weight (6 to 8 g) and type of roasted and ground coffee used (blends of the arabica coffee and robusta coffee or 100% arabica coffee) (Table 1).

Capsules A, B, C e D originated brews with the same denomination. During the preparation of the brews, an espresso machine, compatible with the capsules, was used, which operated at a 15 bar pressure. Coffee brews were prepared at doses ranging from 35 to 120 mL, as recommended by the manufacturer. Five preparation replications were made for each brew. After preparation, coffee brews were frozen for 24 h at -18 °C for subsequent freeze-drying.

Coffee brews were characterized by the amount of solids extracted (determined by the difference between brew weight ready for consumption and freeze-dried brew). Results were used to express diterpenes concentrations in mg/g of solids.

Kahweol and cafestol were quantified in the material used for the preparation of brews (roasted and ground coffee in the capsules) and in brews ready for consumption. The percentage of diterpenes extracted was calculated considering the relationship between the concentration of diterpene in the brew (mg by dose) and the concentration of diterpene in the capsule (mg by capsule).

### Reagents, standards and equipments:

The following reagents were used for samples extraction and preparation of the mobile phase: analytical grade potassium hydroxide KOH (Synth, Brazil), ethanol 96% (Impex, Brazil), analytical grade methyl tert-butyl ether (Vetec, Brazil) and HPLC grade acetonitrile (Fisher Scientific, USA). The water used in the preparation of standards and solutions was obtained from a purification and filtration system (Elga, Purelab option-Q, USA). The mobile phase and the samples were filtered in a membrane 0.22 µm (Millipore, USA). Kahweol and cafestol standards (Axxora, USA) with 98% of purity and certified by Alexis Biochemicals (Switzerland) were used. Lightness was analyzed by a Konica Minolta CR400 colorimeter (Japan), with 45/0 geometry and D65 illuminant. A freeze-dryer (Christ Alpha 2-4 LD plus, Germany) was used to dry the brews, operating at a temperature of -32°C, for approximately 72 hours until constant weight. The chromatographic analysis used the ultra-performance liquid chromatograph Acquity UPLC®System (Waters, USA), with an automatic injector, a quaternary pump, an oven and a diode array detector controlled by the software Empower 3.

### Extraction and quantification of kahweol and cafestol:

The kahweol and cafestol extraction was performed as described by Dias et al. (2010). Direct saponification with subsequent separation of the unsaponifiable fraction was applied. A solution of 2.5 M potassium hydroxide in ethanol was used for saponification. Saponification was followed by extraction with methyl tert-butyl ether and a clean up with purified water, as detailed in Figure 1. The ether extract was diluted in the mobile phase, filtered and injected into the chromatograph.

The chromatographic analysis was based on the method of Dias et al. (2010), originally developed for high-performance liquid chromatography (HPLC), but adapted to the chromatographic conditions for ultra-performance

liquid chromatography (UPLC) (Table 2).

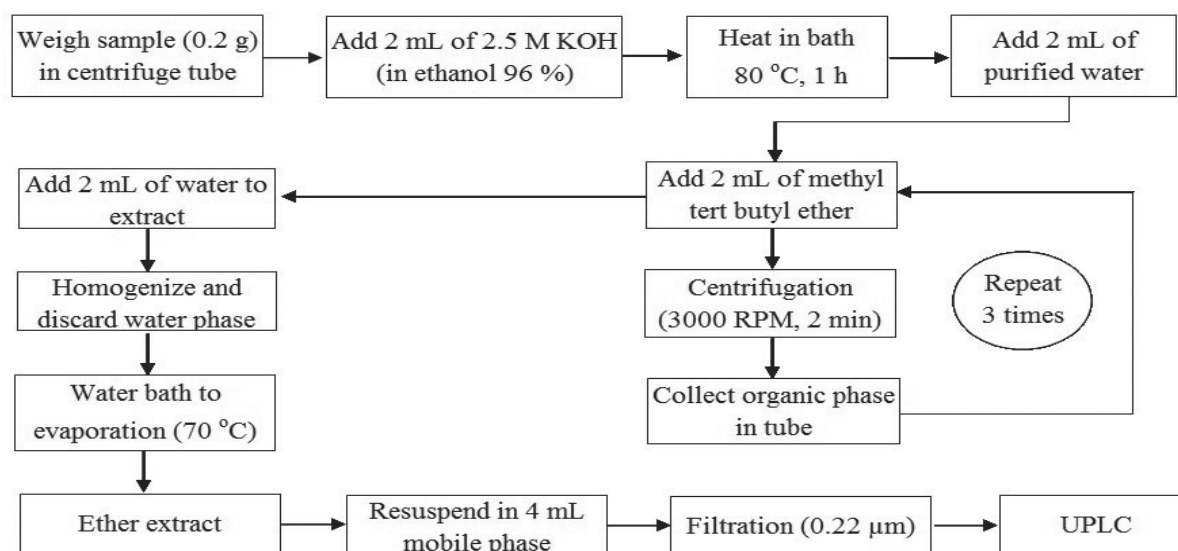
Compounds identification was made by comparing retention times and UV spectra with standards. Quantification was carried out by external standardization, generating calibration curves with cafestol and kahweol concentrations between 2 and 160  $\mu\text{g}/\text{mL}$  for both compounds, with six different concentrations in duplicate ( $r^2 \square 0.999$ ,  $p < 0.001$ ).

**Data analysis:** Data were analyzed by ANOVA, considering the brew (from different capsules and with differences in volume and preparation time) as the source of variation, and the Tukey test ( $p \leq 0.05$ ) using STATISTICA 7.0 software (Statsoft, Tulsa, EUA).

**TABLE 1** - Characterization of the capsules.

Capsules	Weight of coffee(g)	Packaging Information*
A	7.5	Arabica and robusta
B	8	Arabica and robusta
C	6	100 % arabica
D	7	100 % arabica

\* Coffee species.



**FIGURE 1** - Flowchart of diterpenes extraction.

**FONTE:** Adapted from Dias et al. (2010).

**TABLE 2** - Chromatography conditions for kahweol and cafestol analysis.

Chromatography conditions	
Stationary phase	Kinetex 2.6 $\mu$ m C18 Phenomenex, 150 x 4.6 mm
Mobile phase	Acetonitrile: Water (55:45), 1.2 mL / min, isocratic
Detection	UV, 230 nm (cafestol) and 290 nm (kahweol)
Time	4 min
Temperature	25 °C

### 3 RESULTS AND DISCUSSION

Roasted and ground coffees, matching each type of capsule, were characterized by lightness and kahweol and cafestol contents (Table 3).

For lightness, the values varied between 24.64 and 27.37 (Table 3). The results were in the same range as those reported by Campanha, Dias and Benassi (2010) for arabica and robusta coffees of medium roast degree (between 20.1 and 28.9) and were higher than those reported by Souza et al. (2010) (between 19.3 and 21.3) for Brazilian commercial roasted and ground coffees of medium to dark roast degree.

Contents of diterpenes varied from 3.25 to 4.14 mg/g for kahweol and from 2.81 to 3.66 mg/g for cafestol (Table 3). For Brazilian commercial coffees, Souza et al. (2010) reported levels of kahweol between 1.3 and 8.0 mg/g and of cafestol between 2.5 and 5.5 mg/g.

Solids content varied from 1.42 to 4.88 g/100 mL (Table 4). There was a decrease in the concentration of solids with the increase in extraction time/volume, also observed by Caprioli et al. (2012) for traditional espresso coffee brews. Considering the consumption doses of each brew recommended by the manufacturer (from 35 to 120 mL), the solids content (g/dose) was maintained in the range of 1.43 to 1.74 (Table 4).

In general, information on solids content in coffee brews is not described in the literature. Parenti et al. (2014) reported from 6.61 to 6.97 g of solids/100 mL of espresso brews prepared with capsules, which are higher than those obtained with the same coffee using the conventional espresso coffee system (5.94 g/100 mL). Contents from 1.4 to 32.6 g/100 mL were reported for coffee brews prepared in traditional espresso machines (CAPORASO et al., 2014; CAPRIOLI et al., 2012; MARTINS et al., 2005; PETRACCO, 2001; VIGNOLI, 2009; VITTORI et al., 2015).

The freeze-drying of coffee brews enabled to calculate the concentration of diterpenes in mg/g of solids. Contents of 0.20 to 0.76 mg of kahweol and 0.17 to 0.68 mg of cafestol by g of solids were observed (Tables 5 and 6).

Contents from 0.47 to 1.04 mg of kahweol and 0.38 to 0.92 mg of cafestol by coffee dose, corresponding to the range of 0.40 to 2.96 mg of kahweol by 100 mL and 0.32 to 2.62 mg of cafestol by 100 mL (Tables 5 and 6), were observed under the conditions recommended by the manufacturer.

To a better comparison with literature data, the values were informed and, whenever possible, converted to mg/100 mL. For espresso coffees sold in European countries, Urgert et al. (1995) described contents of diterpenes close to the values reported in this study: contents of kahweol varied from zero to 2.6 mg/100 mL and cafestol from zero to 2.1 mg/100 mL. For espresso prepared with arabica coffee, the authors reported higher levels: kahweol from 6.4 to 13.2 mg/100 mL and cafestol from 5.2 to 9.6 mg/100 mL. Sridevi, Giridhar and Ravishankar (2011) also reported higher content of diterpenes in espresso brews: 8.5 mg of kahweol and 10 mg of cafestol by 100 mL. Buchmann et al. (2010) reported contents of cafestol from 1 to 3.3 mg/100 mL of espresso brews. Gross, Jaccoud and Hugget (1997) reported contents of 1,7 mg/100 mL in espresso coffees for both cafestol and kahweol. For espresso coffees prepared with commercial capsules, the same authors observed contents of diterpenes lower than the values reported in this study, varying from 0.18 to 0.36 mg of kahweol and 0.12 to 0.34 mg of cafestol/100 mL. Silva et al. (2012) observed contents of kahweol from 0.06 to 0.4 mg/dose and cafestol from 0.2 to 0.8 mg/dose (volume of dose not informed) in espresso coffees.

Variations around 14 times in the contents of kahweol and cafestol/100 mL of espresso brews (Tables 5 and 6) were detected in the coffee brews studied, considering the variation range values. Comparing with other brewing methods,



variations, around twice in the contents of kahweol and cafestol for filtered brews (WUERGES, 2015) and in the contents of cafestol for french press and mocha brews (ZHANG; LINFORTH; FISK, 2012) were reported. Urgert et al. (1995) described variations around 13 times in the contents of kahweol and 15 times in the contents of cafestol in Scandinavian coffee, and higher variations for Turkish coffee (around 100 times for kahweol and

20 times for cafestol).

In general, a smaller concentration of both diterpenes (up to twice) is observed when comparing brews with greater time and volume of extraction (C and D) (Tables 5 and 6) with those with a smaller time and volume of extraction (A and B) and greater coffee weight in the capsule (Table 1).

**TABLE 3** - Characteristics of the roast and ground coffee in the capsules.

Capsules	Lightness*	Kahweol (mg/g)**	Cafestol (mg/g)**
A	25.1 <sup>bc</sup> ± 0.5	3.25 <sup>c</sup> ± 0.06	2.95 <sup>bc</sup> ± 0.03
B	25.9 <sup>b</sup> ± 0.3	3.63 <sup>b</sup> ± 0.05	3.22 <sup>b</sup> ± 0.06
C	27.3 <sup>a</sup> ± 0.1	4.14 <sup>a</sup> ± 0.05	3.66 <sup>a</sup> ± 0.06
D	24.9 <sup>c</sup> ± 0.2	3.49 <sup>bc</sup> ± 0.12	2.81 <sup>c</sup> ± 0.09

\* Mean ± standard-deviation (triplicate).

\*\* Mean ± standard-deviation (duplicate).

Mean values in column followed by the same letters are not significantly different ( $p \leq 0.05$ ).

**TABLE 4** - Extraction conditions (time and volume) and solids content in the coffee brews prepared with espresso capsules.

Brews	Extraction time (s)	Dose / Volume extracted (mL)	Solids (g/dose)*	Solids (g/100 mL)*
A	8	35	1.71 <sup>a</sup> ± 0.06	4.88 <sup>a</sup> ± 0.09
B	11	50	1.74 <sup>a</sup> ± 0.03	3.48 <sup>b</sup> ± 0.04
C	16	60	1.43 <sup>b</sup> ± 0.01	2.42 <sup>c</sup> ± 0.03
D	22	120	1.69 <sup>a</sup> ± 0.03	1.42 <sup>d</sup> ± 0.04

\* Mean ± standard-deviation (five repetitions of preparation).

Mean values in column followed by the same letters are not significantly different ( $p \leq 0.05$ ).

**TABLE 5** - Contents of kahweol in espresso coffee brews from capsules.

Brews	Kahweol			
	mg/g of solids*	mg/100 mL*	mg/dose*	CV (%)**
A	0.61 <sup>a</sup> (0.38-0.76)	2.96 <sup>a</sup> (1.95-3.70)	1.04 <sup>a</sup> (0.65-1.29)	26.17
B	0.54 <sup>a</sup> (0.44-0.60)	1.89 <sup>b</sup> (1.52-2.10)	0.95 <sup>a</sup> (0.76-1.05)	14.18
C	0.32 <sup>b</sup> (0.28-0.37)	0.77 <sup>c</sup> (0.69-0.89)	0.46 <sup>b</sup> (0.41-0.53)	9.52
D	0.28 <sup>b</sup> (0.20-0.34)	0.40 <sup>c</sup> (0.28-0.48)	0.47 <sup>b</sup> (0.34-0.57)	20.97

\* Mean of five repetitions of preparation and variation range. \*\*CV: coefficient of variation.

Description of coffee brews in Table 4; Doses: A (35 mL), B (50 mL), C (60 mL) and D (120 mL).

Mean values in column followed by the same letters are not significantly different ( $p \leq 0.05$ ).

**TABLE 6** - Contents of cafestol in espresso coffee brews from capsules.

Brews	Cafestol			
	mg/g of solids*	(mg/100 mL)*	(mg/dose)*	CV (%)**
A	0.54 <sup>a</sup> (0.34-0.68)	2.62 <sup>a</sup> (1.64-3.30)	0.92 <sup>a</sup> (0.57-1.15)	27.02
B	0.47 <sup>a</sup> (0.37-0.53)	1.64 <sup>b</sup> (1.29-1.83)	0.82 <sup>a</sup> (0.65-0.91)	15.12
C	0.28 <sup>b</sup> (0.26-0.32)	0.69 <sup>c</sup> (0.63-0.78)	0.41 <sup>b</sup> (0.38-0.47)	7.93
D	0.22 <sup>b</sup> (0.17-0.27)	0.32 <sup>c</sup> (0.24-0.38)	0.38 <sup>b</sup> (0.33-0.46)	19.38

\* Mean of five repetitions of preparation and variation range. \*\*CV: coefficient of variation.

Description of coffee brews in Table 4; Doses: A (35 mL), B (50 mL), C (60 mL) and D (120 mL).

Mean values in column followed by the same letters are not significantly different ( $p \leq 0.05$ ).

The fraction of diterpenes extracted is shown in Figure 2. The percentage of extracted diterpenes varied from 1.85 to 4.27 % for kahweol and from 1.87 to 4.16 % for cafestol. It is interesting to observe that extraction was less than 5 % for both compounds (Figure 2). Coffee brews A and B, prepared with small doses and volume of extraction, showed higher extractions of diterpenes. For coffee brews prepared by the Turkish style, Scandinavian style, French press and mocha, extractions of cafestol from 2.5 to 9.0 % were reported (ZHANG; LINFORTH; FISK, 2012).

Wuerges (2015) reported lower contents of kahweol and cafestol (0.28 mg/100 mL for both compounds) for traditional espresso coffees originated from arabica coffee with contents of diterpenes (4.04 mg of kahweol and 3.18 mg of cafestol by g of coffee) and solids (2.46 g/100 mL) in the same range adopted for the brews analyzed in this study (Tables 4, 5 and 6). This difference can be due to the variability in the grinding process. Sehat, Montag and Speer (1993) observed that espresso coffees with thinner grind showed less lipid compounds extraction.

Coefficient of variation (CV) values showed that variation in the contents of diterpenes was less than 30%. Brew A showed higher variability (CV 26.17% and 27.02% for kahweol and cafestol) and CVs from 7.93% to 20.97% were observed in other coffee brews (Tables 5 and 6).

The consumption of espresso coffees with high contents of diterpenes (especially cafestol) can bring a hypercholesterolemic effect (HIGDON; FREI, 2006; URGERT et al., 1995; WEUSTEN-VAN DER WOUW et al., 1994). Brew A, which showed the highest extraction and content of diterpenes values (Tables 5 and 6, Figure 2), has an average concentration of cafestol of 0.92 mg/dose. Considering a variation of 30%, the consumption of around 9 daily doses of brew A would be necessary to increase cholesterol by 5 mg/dL (URGERT et al., 1995). For brew D, which showed the lowest extraction and an average concentration of cafestol of 0.38 mg by dose (Table 6 and Figure 2), the increase in cholesterol levels would only happen with the consumption of around 20 daily doses.

According to a marketing research conducted by ABIC (2010), Brazilians consume an average of 5.5 cups of espresso coffee daily, which may not result in an increase in cholesterol levels. It is worth pointing out that, in coffee brews with higher extraction of diterpenes (Figure 2), as coffee brew A, a higher extraction of other solids also occurs (Tables 5 and 6). Therefore, a moderate consumption of this coffee brew will provide an amount of diterpenes that can bring beneficial effects and provide an amount of other bioactive compounds with positive impact on the consumers' health (ESQUIVEL; JIMÉNEZ, 2012; MURIEL; ARAUZ, 2010).

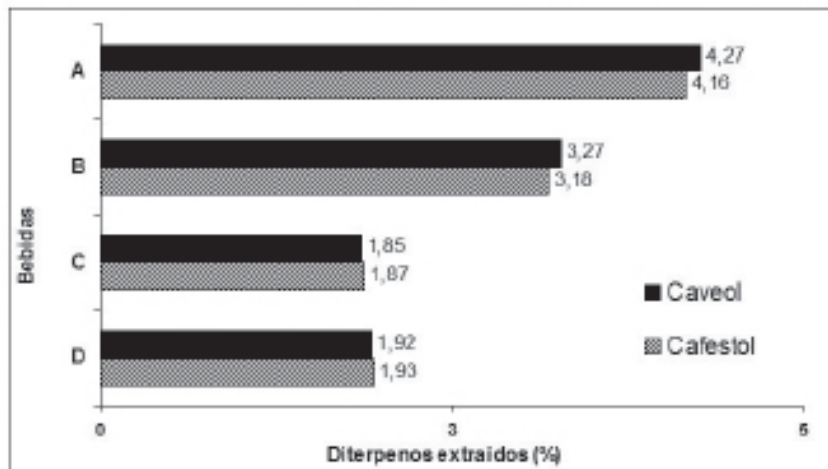


FIGURE 2 - Percentage of extracted diterpenes.

#### 4 CONCLUSIONS

Variation in the contents of diterpenes in the roasted and ground coffee used in commercial capsules as well as in coffee brews originated from the product are observed. Higher contents of kahweol and cafestol are obtained for brews prepared in smaller volume (smaller doses).

Considering the contents of cafestol observed in this study, there is no evidence of a hypercholesterolemic effect due to a moderate consumption of coffee brews prepared with these commercial capsules.

#### 5 ACKNOWLEDGMENTS

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