

Kahweol and cafestol in coffee brews: comparison of preparation methods¹

Caveol e cafestol em bebidas de café: comparação de métodos de preparo

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ABSTRACT - The profile of bioactive compounds in coffee brews depends on the coffee bean species and varieties, harvesting and post-harvesting practices, roasting processes, and also on the brewing method. The present research aimed to study the contents of cafestol and kahweol - coffee diterpenes with a known impact on human health - comparing coffee beverages prepared using common brewing methods (filtered, espresso, and instant coffee brews). Filtered (cloth-filtered and paper-filtered), espresso and instant brews were obtained from a medium-roasted *Coffea arabica* coffee (NY 2). Five genuine replicates of each coffee brew were prepared, and the extracts were lyophilized. A validated UPLC-based method provided the content of diterpenes. The results were reported in mg of diterpene per g of solids or per a standard dose of 50 mL of coffee brew. Solids content of coffee brews ranged from 2.06 to 2.46 g 100 mL⁻¹. All coffee brews presented low diterpene contents: 0.05 to 0.16 mg of kahweol and 0.11 to 0.14 mg of cafestol 50 mL⁻¹. Instant coffee brew showed the lowest content of kahweol and absence of cafestol; this reduction was related to the production process of soluble coffee. Diterpenes content was similar in espresso and paper-filtered brews. The cloth-filtered coffee had lower solids content, but higher levels of diterpenes (in mg g⁻¹ of solids). Similar cafestol and kahweol contents (mg 50 mL⁻¹) were observed in filtered and espresso brews.

Key words: *Coffea arabica*. Bioactive compounds. Espresso. Filtered coffee brew. Instant coffee.

RESUMO - O perfil dos compostos bioativos em bebidas café depende das espécies e variedades de café, métodos de plantio e processamento pós-colheita, processo de torra e também do método de preparação da bebida. O trabalho teve como objetivo estudar os teores de caveol e cafestol - diterpenos do café com efeitos conhecidos a saúde - comparando bebidas de café preparadas usando métodos usuais de preparo (filtrada, espresso e café solúvel). As bebidas filtradas (pano ou papel), espresso e café solúvel foram obtidas de grãos com torra média da espécie *Coffea arabica* (tipo 2). Cinco repetições genuínas de cada bebida foram preparadas e os extratos foram liofilizados. Os diterpenos foram quantificados por CLUE. Os resultados foram relatados em mg de diterpeno por g de sólidos ou por dose padrão de 50 mL. O teor de sólidos das bebidas de café variou de 2,06 a 2,46 g 100 mL⁻¹. Todas as bebidas apresentaram baixo teor de diterpenos: 0,05 a 0,16 mg de kahweol e 0,11 a 0,14 mg de cafestol 50 mL⁻¹. A bebida instantânea mostrou o menor teor de kahweol e ausência de cafestol, redução relacionada ao processo de produção de café solúvel. O teor de diterpenos foi semelhante em cafés espresso e filtrados em papel. O café filtrado em pano apresentou menor teor de sólidos, mas níveis mais elevados de diterpenos (em mg g⁻¹ de sólidos). Teores similares de cafestol e kahweol (mg 50 mL⁻¹) foram observados nas bebidas filtradas e no espresso.

Palavras-chave: *Coffea arabica*. Compostos bioativos. Espresso. Café filtrado. Café solúvel.

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INTRODUCTION

The composition of coffee depends on the genetics (species and varieties), edaphoclimatic conditions of plant growth, and processing parameters (CAMPANHA; DIAS; BENASSI, 2010; KITZBERGER *et al.*, 2013; SOUZA *et al.*, 2010; SPEER; KÖLLING-SPEER, 2006). However, the balance of compounds in the coffee beverage is also dependent on the brewing conditions (MESTDAGH; GLABASNIA; GIULIANO, 2017; VITTORI *et al.*, 2015). The method of coffee brewing varies with cultural habits and geographic traditions as well as personal preferences. For instance, American coffee is watery and soft, Italian coffee is known for its high solids content, and Turkish brew is prepared by boiling coffee in water (MESTDAGH; GLABASNIA; GIULIANO, 2017).

Brazil is the largest green coffee producer and exporter, and the second largest consumer of coffee. In 2017/18, 161.3 million 60-kg bags were consumed, mostly as coffee brews (INTERNATIONAL COFFEE ORGANIZATION, 2019). In parallel to modern preparation methods, as single dose capsules, filtered brews - particularly in cloth filters - are traditionally consumed in Brazil.

Kahweol and cafestol are the main diterpenes of coffee, constituting about 20% of the lipid fraction (BENASSI; DIAS, 2015; SPEER; KÖLLING-SPEER, 2006). These diterpenoids bring notable benefits to human health for its antioxidant capacity, anti-inflammatory effect, and protection against cancer and toxic substances (CÁRDENAS; QUESADA; MEDINA, 2015; KIM; KIM; JHON, 2018; LEE; JEONG, 2007). However, kahweol and, notably cafestol, are also known to raise serum cholesterol levels (NAIDOO *et al.*, 2011; PHAM; PREEDY, 2015; URGERT *et al.*, 1995). The profile of diterpenes and the resulting health impact depend on the coffee preparation method. Literature reports that unfiltered coffee brews have high contents of diterpenes, whereas in filtered beverages these values are reduced (DEL CASTILLO; HERRAIZ; BLANCH, 1999; GROSS; JACCAUD; HUGGETT, 1997; NAIDOO *et al.*, 2011; SRIDEVI; GIRIDHAR; RAVISHANKAR, 2011; URGERT *et al.*, 1995; VITTORI *et al.*, 2015).

In the single report on the use of cloth filter, Naidoo *et al.* (2011) reported on the *Sock* preparation, a typical Asian method based on the percolation of roasted and ground (RG) coffee with boiling water, using a cloth filter sock attached to a wooden stand. They investigated the relationship between commercial coffee brew consumption and the increase of serum lipids in Indonesian volunteers.

A wide range of diterpene content in coffee brews obtained by different preparation methods is reported. However, make a data comparison is very difficult, since the solids content of the brews and other relevant raw material information (such as coffee species and degrees of roasting and grinding) are usually not reported (MOEENFARD; ERNY; ALVES, 2016; NAIDOO *et al.*, 2011; SILVA *et al.*, 2012; SRIDEVI; GIRIDHAR; RAVISHANKAR, 2011). Additionally, the comparison between beverages prepared with soluble coffee and RG coffee is particularly complex due to the variation in the coffee species used. Another problem is related to the size of coffee cups, considering that there is no standard definition for coffee cup units.

Therefore, the goal of the present study was to compare the contents of kahweol and cafestol among some common types of coffee brews: filtered in cloth or paper filters, espresso, and instant coffee.

MATERIALS AND METHODS

Material: preparation of RG and instant coffee and brewing methods

Good quality *Coffea arabica* (NY 2 type) was used for the preparation of all coffee brews. The use of a standardized base material to produce RG and soluble coffees was adopted to allow an accurate quantitative comparison between brewing methods. The raw material was supplied by Companhia Iguau de Café Solúvel® (Cornélio Procópio, Paraná, Brazil), where the products were produced in a pilot plant of the same company.

Coffee beans were roasted in a pilot roaster Lilla model OPUS 2 industrial roaster (Guarulhos, Brazil), for small capacities (up to 24 kg per batch) for about 12 min at 218 to 223 °C. A grinder (Krupps GVX 2®, Solingen, Germany) was used to obtain a fine ground coffee (0% retained in sieve size 1.18 mm; 70% retained in sieve size 0.60 mm, and 30% passing a sieve size 0.60 mm) to be used for filtered and espresso coffee brews. The color of the RG coffee was evaluated using a portable colorimeter Konica Minolta® CR400 (Tokyo, Japan) under CIE standard illuminant D65 and directional 45°/0° geometry, characterizing a medium roasted coffee ($L^* = 29.1 \pm 0.6$).

For instant coffee production, the roasted beans were processed in the industrial pilot plant, being carried out in a batch of 30 kg, following the standard protocol used in Companhia Iguau. The roasted beans were granulated and subjected to percolation extraction (six stages). Pressurized water (180 °C) was introduced in the first percolation stage (column with the oldest coffee) and percolated through the following stages until the extract reaches the freshly loaded coffee in the last stage. During

this process, the amount of soluble solids increased, and the temperature decreased; fresh coffee was extracted at around 100 °C in the last column, causing minimum thermal damage, carefully preserving aroma and flavor. Extracts drying was done by freeze-drying process, obtaining about 0.8 kg of instant coffee.

Mineral water (Ouro Fino®, Campo Largo, Brazil) was used, following the brewing procedures described in the following.

Filtered/Dripped coffee: 50.0 g of the RG coffee was transferred to a Melitta® 102 paper filter (Guaíba, Brazil) in a pour-over coffee maker, or to a cloth strainer. Five hundred mL of water at 90 °C were then added.

Espresso: 7.0 g of RG coffee was used in an espresso machine (Krupps® XP5240, China). The coffee brew (50 mL) was extracted under 15 bars for 32 s.

Instant coffee: 1.2 g of freeze-dried instant coffee was dissolved in 50 mL of water (90 °C) (VIGNOLI *et al.*, 2016).

Five genuine replicates of each coffee brew were prepared. All the extracts were frozen (−18 °C, by 24 h), and lyophilized (Christ Alpha® 2-4 LD plus freeze dryer, Osterode am Harz, Germany), operating for about 72 h at −32 °C to constant weight. Coffee brews were characterized by the extracted solids' amount, determined by the weight difference between the ready-to-drink brew and the respective freeze-dried sample. These results were used to report diterpenes contents in mg g⁻¹ of solids.

Kahweol and cafestol analysis

The contents of kahweol and cafestol were assessed in the material (RG and soluble coffees) and in the coffee brews.

Potassium hydroxide (KOH) analytical grade (Synth®, Sao Paulo, Brazil), ethanol 96% analytical grade (Éxodo Científica®, Hortolândia, Brazil), acetonitrile HPLC grade (Fischer Scientific®, Bridgewater, NJ), methyl *tert*-butyl ether (MTBE) HPLC grade (Acrós Organics®, Morris Plains, NJ), and kahweol and cafestol standards (Axxora®, San Diego, USA) of 98% purity certified by Alexis Biochemicals® (Lausen, Switzerland) were used. The water was obtained by Elga Purelab Option-Q system (Veolia Water Technologies®, Saint-Maurice, France). Mobile phase and samples were filtered (nylon membranes, 0.22 µm; Millipore®, Billerica, MA). A Kinetex 2.6 µm C18 column (150 x 4.6 mm) (Phenomenex®, Torrance, USA) was used. The UPLC analysis was performed in an Acquity System (Waters®, Milford, USA) equipped with a flow-through needle injector, a quaternary solvent pumping system, column heater/cooler module, coupled with a DAD UV-Vis detector, controlled by the Empower3® software.

The extraction of diterpenes was performed as previously described (DIAS *et al.*, 2010). Samples (0.200 g) were saponified (80 °C, 1 h) with 2.0 mL solution of potassium hydroxide (2.5 mol L⁻¹) in ethanol 96% v/v. This step was followed by extraction with MTBE and clean up with water; the procedure was repeated three times. After evaporation to dryness in a water bath (70 °C, 15 min), the ether extract was resuspended in the mobile phase, filtered, and injected. Duplicate independent extractions were performed.

The UPLC analysis was carried out according to Wuerges *et al.* (2016). Detection at 230 nm (cafestol) and 290 nm (kahweol), isocratic elution of water:acetonitrile solution (45:55, v/v) at 1.2 mL min⁻¹, and an injection volume of 1.4 µL were applied. Identification was based on the retention time and UV spectrum. Quantification was carried out by external standardization using 6-point analytical curves with duplicate measurements ($r^2 \geq 0,999$, $p < 0,001$) in the range of 2.00 to 160 mg mL⁻¹. A limit of detection (LOD) of 0.5 mg mL⁻¹ for both diterpenes, and a limit of quantification (LOQ) of 1.4 and 1.6 mg mL⁻¹ for kahweol and cafestol were observed, respectively.

The experiments were carried out using a completely randomized design. Data were analyzed by one-way ANOVA, considering the brewing method the source of variation, and Tukey test ($p \leq 0.05$) using STATISTICA 7.0 software (StatSoft, USA).

RESULTS AND DISCUSSION

Solids content of coffee brews ranged from 2.06 to 2.46 g 100 mL⁻¹. Low variation was observed when comparing five replicates of each brewing method (CV from 0.5 to 1.2%). Cloth-filtered coffee presented the lowest solids content, while espresso showed more solids than the filtered coffee (Table 1).

There is a lack of information in the literature on coffee brews solids content; data comparison is even difficult because there are no standardized protocols for each brewing method. The solids content of a coffee brew varies with brewing temperature, particle size, coffee species, coffee to water ratio, and coffee machine pressure (for espresso) (VITTORI *et al.*, 2015). In the following discussion, the comparison was made just with data on Arabica coffee brews, since soluble solids content is highly dependent on the species (e.g., Robusta coffee usually present higher solids contents than Arabica coffee). No data were found for cloth-filtered brews. A range from 2.04 to 2.90 g of solids 100 mL⁻¹ was reported for Arabica paper-filtered coffee brews, and a large range (3.41 to 5.20 g of solids 100 mL⁻¹) was described for Arabica espresso

Table 1 - Solids content of coffee brews

Material	RG coffee			Instant coffee
Brewing method	Filtered /cloth filter	Filtered /paper filter	Espresso	Water addition
Solids content (g 100 mL ⁻¹) ^a	2.06 ^c (1.1%)	2.39 ^b (1.1%)	2.46 ^a (0.5%)	2.42 ^{ab} (1.2%)

^aMean of 5 genuine replicates of the brew (coefficient of variation in brackets). Different letters indicate significant differences ($p \leq 0.05$)

brews (MARTINS *et al.*, 2005; VIGNOLI *et al.*, 2016). Caprioli *et al.* (2012) verified that even a wider variation (1.4 to 24.1 g of solids 100 mL⁻¹) could be observed, since the efficiency of the solids extraction (inversely) varies with coffee brewing extraction time. In espresso brews prepared with Arabica coffee capsules, Wuerges *et al.* (2016) observed a range between 1.42 and 2.42 g of solids 100 mL⁻¹.

Diterpenes content was estimated for RG and instant coffees used for brewing. For RG coffee, which it was used as raw material for filtered and espresso coffee brews, 4.04 mg of kahweol and 3.18 mg of cafestol g⁻¹ were obtained. Contents from 3.71 to 10.1 mg of kahweol g⁻¹ and 2.21 to 7.42 mg of cafestol g⁻¹ were reported for Brazilian RG Arabica coffees (KITZBERGER *et al.*, 2013; KITZBERGER; SCHOLZ; BENASSI, 2014). In this research, soluble coffee presented kahweol content (0.04 mg g⁻¹) about 100 times lower than that found in RG coffee; cafestol was absent for instant coffee, indicating a substantial reduction of diterpene levels during the industrial process.

Kahweol and cafestol contents in coffee brews varied from 0.04 to 0.15 mg g⁻¹ of solids, and from absence to 0.11 mg g⁻¹ of solids, respectively. Thus, considering doses of 50 mL, less than 0.20 mg of each diterpene was found in the different brews (Table 2).

Considering the four brewing methods on trial, all beverages prepared with RG coffee showed about 0.14 mg of kahweol and 0.13 mg of cafestol per dose (Table 2).

The soluble coffee brew had the lowest kahweol content (0.05 mg 50 mL⁻¹) and absence of cafestol (Table 2), since diterpenes, notable cafestol, were already extracted at negligible levels during the industrial process of soluble coffee production. Literature surveys report contents of kahweol from absence to 0.60 mg 50 mL⁻¹, and cafestol between 0.01 and 0.70 mg 50 mL⁻¹ for soluble coffee brews (GROSS; JACCAUD; HUGGETT, 1997; SILVA *et al.*, 2012; URGERT *et al.*, 1995). These higher values probably occurred due to the soluble coffee content used in the brews' preparation, 4.0 g 100 mL⁻¹, twice as much as those used in this research (Table 1). The low content of diterpenes in soluble coffee brews reported in the literature is usually due to the use of robusta coffee as raw material, a species with lower content of diterpenes, notable kahweol (BENASSI; DIAS, 2015). Our data showed that even when beverages brewed with standardized procedures (the same Arabica coffee raw material and with a similar solid content) were compared, instant coffee brews presented an amount of kahweol three times lower than filtered and espresso brews and absence of cafestol (Table 2). This reduction, therefore, would be associated mainly with the industrial manufacturing process of soluble coffee.

Table 2 - Contents* of kahweol and cafestol in coffee brews prepared by different methods

Material	RG coffee			Instant coffee
Coffee brew	Cloth-filtered	Paper-filtered	Espresso	Instant
Kahweol				
mg g ⁻¹ of solids	0.15 ^a (0.10-0.17)	0.10 ^b (0.09-0.14)	0.12 ^b (0.10-0.13)	0.04 ^c (0.04-0.04)
mg 50 mL ⁻¹	0.16 ^a (0.11-0.18)	0.12 ^a (0.10-0.17)	0.14 ^a (0.12-0.16)	0.05 ^b (0.05-0.05)
CV%	19.0	20.3	9.2	2.7
Cafestol				
mg g ⁻¹ of solids	0.13 ^a (0.09-0.14)	0.09 ^b (0.08-0.12)	0.11 ^{ab} (0.10-0.13)	ND**
mg 50 mL ⁻¹	0.13 ^a (0.09-0.14)	0.11 ^a (0.10-0.14)	0.14 ^a (0.12-0.15)	ND
CV%	17.2	15.5	9.8	-

CV%: Coefficient of variation, in percentage, for five preparations. *Mean of five genuine replicates of the brew; range of variation in brackets. **ND: contents below LOD (0.5 mg mL⁻¹). Different letters in the line indicate significant differences ($p \leq 0.05$)

In order to compare results, the following literature data were converted into a common base (50 mL dose). For paper-filtered coffee brews, without information on the studied coffee species, Sridevi, Giridhar and Ravishankar (2011) reported on 0.12 mg kahweol 50 mL⁻¹ and 0.44 mg cafestol 50 mL⁻¹, and Silva *et al.* (2012) found 0.06 mg cafestol 50 mL⁻¹ and absence of kahweol (no information on dose volume). A wide range was reported even for Arabica coffee brews (50 mL dose): from 0.007 mg to 1.25 mg of kahweol and from 0.007 to 0.55 mg of cafestol (DEL CASTILLO; HERRAIZ; BLANCH, 1999; GROSS; JACCAUD; HUGGETT, 1997; RENDÓN; SCHOLZ; BRAGAGNOLO, 2018).

For cloth filtered coffee brews (Sock method), Naidoo *et al.* (2011) reported contents from absence to 0.08 mg of kahweol 50 mL⁻¹ and 0.48 mg of cafestol 50 mL⁻¹. Coffee brews were purchased in commercial shops, therefore, there was neither material nor process standardization (with variations on the filtration stage and water temperature, and the addition of other ingredients to the RG coffee), justifying the wide range of reported values.

We highlight that, among filtered coffees, the cloth-filtered brew presented the highest content of kahweol and cafestol, in mg g⁻¹ solids (Table 2). The use of a cloth filter yielded beverages with lower solids proportion (Table 1), but higher diterpenes content (Table 2). As previously reported, kahweol and cafestol are retained in spent coffee grounds, and filters are also a barrier (URGERT *et al.*, 1995). However, there is apparently less retention when using the cloth filter.

For espresso beverages, the literature reported content values in a wide range: kahweol between 0.06 and 6.6 mg 50 mL⁻¹, and cafestol between 0.2 and 5.0 50 mL⁻¹ (GROSS; JACCAUD; HUGGETT, 1997; SILVA *et al.*, 2012; SRIDEVI; GIRIDHAR; RAVISHANKAR, 2011; URGERT *et al.*, 1995; ZHAM *et al.*, 2010). For espresso coffees purchased in bars and restaurants, Urgert *et al.* (1995) reported contents from absence to 1.3 mg of kahweol and 1.0 mg of cafestol in 50 mL. Wuerges *et al.* (2016) described a range of 0.20 to 0.38 mg for kahweol contents and of 0.16 to 0.34 mg for cafestol contents per 50 mL for espresso with domestic preparation using commercial capsules of Arabica coffee. In addition to the raw material variability, the wide range of diterpenoid compounds reported for espresso coffee - greater than 50 times - could be explained by variations on coffee grind size and process conditions (coffee/water ratio, time, temperature and pressure). A significant influence of water temperature and grind size on cafestol content in espresso coffees was reported: the finer the particle size and the higher

the temperature, the higher the cafestol extraction yield (ZHAM *et al.*, 2010).

Coffee brews presented low levels of kahweol and cafestol for all the brewing methods assayed in this study, even when using Arabica coffee (coffee species with a high level of diterpenes), and fine grinding, which allows greater yield extraction. Furthermore, similar contents of diterpenes were observed for espresso and paper-filtered coffee brews, indicating no effect of pressure on diterpenes extraction yield, considering the standardized conditions (roasting degree and grinding conditions) applied in this study.

Considering our data and the essays of Urgert *et al.* (1995), a daily intake of at least 30 doses of filtered or espresso coffee would be necessary for an increase by approximately 2.5 mg 100 mL⁻¹ in serum cholesterol; thus, moderate intakes of these coffee brews would have negligible effects on serum cholesterol. No impacts on cholesterol levels would be expected by the intake of soluble coffee.

CONCLUSION

Similar contents of cafestol and kahweol (in mg per dose of 50 mL) were observed when comparing filtered and espresso brews. Soluble coffee brews presented absence of cafestol and reduced levels of kahweol, when compared to other methods of preparation. Among filtered brews, the cloth-filtered coffee showed lower solids content but higher content of cafestol and kahweol (in mg g⁻¹ of solids) than paper-filtered coffee brews.

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