# Cafestol and Kahweol Content in Espresso Coffees as Influenced by Preparation Parameters

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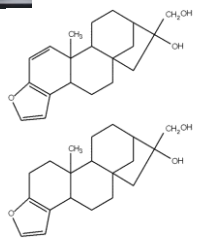

The effect of preparation parameters on the diterpenes profile of espresso coffee (EC) and the extraction efficiency of diterpenes from roast and ground (R&G) Arabica coffee were evaluated. The influence of water quantity, amount of ground coffee, grinding size, percolation time, water temperature and pressure on cafestol and kahweol content of ECs were investigated. Diterpenes were analysed by liquid-liquid extraction followed by HPLC-DAD analysis. The average cafestol and kahweol content of R&G Arabica coffee were 467.62 ± 20.02 and 638.04 ± 33.64 mg / 100 g. All preparation parameters influenced the diterpenes content of final EC, nevertheless grinding size and water quantity had greater effect on diterpenes concentration. This study clearly shows that parameters for coffee brew preparation may be changed for the intended purpose.

Espresso coffee (EC) is an enjoyable coffee brew that has a great popularity throughout the world. In espresso preparation, a limited amount of hot water (90±5 °C) under pressure (9±2 bar) passes through a compact roast and ground coffee (R&G, 6.5±1.5 g) in a short time (30±5 s), producing a brew (15-50 mL) with strong taste and flavour topped with crema (espresso coffee foam) [1]. Coffee is a complex beverage rich in large amount of chemical compounds that may contribute to biological activity [2]. Coffee oil is a source of unsaponifiable compounds from kauran family called diterpenes, especially cafestol and kahweol [3] which may exist in free form (0.7-3.5 %) [4] or esterified form (almost 98 %) [5].

Coffee diterpenes are responsible for elevation of liver enzymes [6]. Moreover, anticarcinogenic [7] anti-inflammatory and antiangiogenic properties [8] are some of the beneficial effect of these diterpenes. Besides that, cafestol and, to a lesser extent, kahweol are the main hyperlipidemic components in coffee [2] as ingestion of 10 mg cafestol per day can promote serum cholesterol increase by 5 mg/dl (0.13 mmol/L) [10]. Originally, EC is an Italian beverage but recently it is widely consumed in Latin European countries, USA and Japan [1] so in addition to study the body, taste and flavour, EC have caught recently more attention due to its high consumption and its subsequent impact on human health. The evaluation of the effects of changes in extraction parameters on diterpenes levels in EC is of interest due to the biological effects of these compounds and that EC is a popular brew among Portuguese consumers.

Few studies compare different preparation parameters in terms of diterpenes contents of this type of brew [10]. However, there is no comprehensive study regarding the effect of all preparation parameters on cafestol and kahweol content of EC. As far as the authors know, there are no legal limits for individual diterpenes content. Studying the various types of ECs prepared by different technical conditions is valuable for the modification of brewing procedures in order to adjust diterpenes concentration and cafestol / kahweol ratio in final EC. This may be useful when data about diterpenes effects on health is robust enough to allow the establishment of safe or beneficial levels of intake. Therefore this work aims to evaluate diterpenes level differences in EC arising from changes in preparation parameters including coffee/water ratio, volume of coffee, particle size, extraction time, extraction temperature and pressure. Cafestol and kahweol content of ECs prepared by coffee-shop espresso machine will be measured. Efficiency of extraction (%) of cafestol and kahweol was also evaluated in final brews.

The reagents used in this work were analytical or HPLC grade. Chemicals were acetonitrile, methanol (HPLC gradient grade, VWR, Belgium) and diethyl ether (VWR, Belgium). Other chemicals used were potassium hydroxide with purity of ~85 % (Merck, Germany) and sodium chloride (Panreac Quimica SA, Spain). Free cafestol and kahweol standards (purity of 98 %) were purchased from Chroma Dex (Irvine, CA, USA) and LKT Laboratories (MN, USA), respectively.

Roasted Arabica coffee (100 % Coffea arabica, 2.34 % water content) was supplied by a local company in Porto, Portugal. Roasted beans were ground by means of automatic grinder (La Cimbali®, grinder-doser 6/SA) just before the EC preparation. The particle size distribution for very fine, fine and coarse ground is presented in Figure 1.

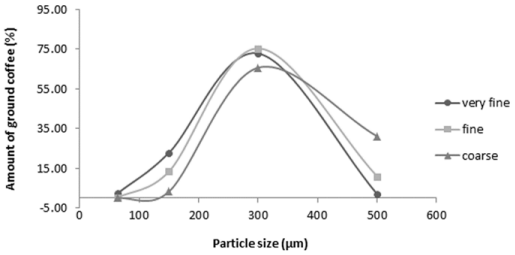


**Figure 1.** Particle size distribution of very fine, fine and coarse ground roasted Arabica coffee.

ECs were prepared using semiautomatic espresso machine (La Cimbali M31 Classic). Standard EC was prepared by 7.5 g of finely R&G Arabica coffee. Water temperature was fixed at 90±2 °C (temperature of water at the exit of the heating unit). For the preparation of EC in a volume of 40 mL the pressure of around 9±1 bar was needed. The percolation time was kept on 20±3 s. The variables were the R&G coffee weight (6.5, 7.5, 8.5 and 9.5 g), cup size equivalent volume (amount of water) (30, 40, 50 and 60 mL), particle size (very fine, fine and coarse grind size), percolation time (10, 21 and 30 s), water temperature (70, 80 and 90 °C) and water pressure (7, 9 and 11 and 14 bar).

For the diterpenes extraction from coffee brew, three cups of coffee brews (for each variable) were defrosted and mixed to reach a homogeneous mixture then 2.5 mL of heated brew (60 °C) and 2.5 mL of distilled water were pipetted into an amber glass flask and saponified with 3 g of potassium hydroxide powder in water bath (80 °C - 60 min). Diterpenes extractions were performed twice with 5 mL of diethyl ether and subsequently the combined ether phase was washed with 5 mL of 2 M NaCl solution. After a 10 min centrifugation at 4000 rpm, the clean organic phase was collected and brought to dryness under $N_2$ stream.

Regarding coffee beans, extraction of diterpenes was achieved by saponification of 200 mg R&G Arabica coffee (≤ 300 µm) in 5 mL of methanol and 2.5 mL of distilled water (2:1 v/v) with 3 g of potassium hydroxide powder (80 °C, 60 min). Saponified solution was subjected to liquid-liquid extraction using diethyl ether (repeated 4 times) and after centrifugation (4000 rpm, 10 min) the combined ether phase was washed with 5 mL of 2 M NaCl solution and dried under $N_2$ stream.

HPLC analyses of all samples were performed in duplicate on Merck Hitachi Elite LaChromatogragh with Purospher® STAR LiChroCART® RP-18 end-capped (250 x 4 mm, 5 µm) column attached to a guard column (4x4 mm, 5µm) of the same type with L-2455 UV/vis spectrophotometry diode array detector. Wavelength used were 225 nm for cafestol and 290 nm for kahweol. EZChrom Elite 3.1.6 software was used for data acquisition and peak integration.

The dried extracts were made up to 2.5 mL (brews) or 10 mL (R&G coffee) with acetonitrile and 20 µL was injected after filtration (0.45 µm PTFE membranes). The chromatographic conditions were: mobile phase of acetonitrile / water (55 %/ 45 %) with an isocratic flow rate of 0.8 mL/min during 15 min. By comparing the spectrum and retention time of analytes with standard solutions, target compounds were identified. Quantitative analysis was performed using external standard calibration curves.

Calibration curves for cafestol and kahweol were plotted by injection of 9 standards in the range of 2-200 mg/L. The coefficients of determinations ($R^2$) were 0.999, both for cafestol and kahweol. Good recovery was observed both for cafestol and kahweol regarding coffee brew (around 85 %) and coffee bean (around 95 %).

Differences were considered significant when $p \leq 0.05$. Differences between different levels of each variable in ECs preparation was evaluated by ANOVA-one way at four replications. Data are reported as mean ± standard deviation. All statistical analysis was carried out by Matlab 7.12.0 software. Figures were plotted by means of Matlab 7.12.0 and Excel (2010).

Obtained results indicated that water quantity significantly affected the level of diterpenes in resulting ECs ($p \leq 0.05$) and the highest cafestol and kahweol concentration (mg/L) was observed in samples prepared using 30 mL of water which contained 55.78 ± 0.83 mg/L (total cafestol and kahweol). A positive relation was also observed within different amount of R&G coffee. Mean total diterpenes (total cafestol and kahweol) at a constant water quantity (40 mL) were 31.92 ± 1.09, 40.39 ± 3.97, 40.74 ± 3.42 and 42.53 ± 4.49 mg/L for 6.5, 7.5, 8.5 and 9.5 g of R&G coffee, respectively. Variation of diterpenes levels from coffees at different particle size was expected because very fine grinds provided large surface area and more diterpenes could be extracted. With

regards to percolation time the increase was not significant between 10 and 21 sec ($p \geq 0.05$). This may be due to this fact that diterpenes were mainly extracted at the beginning of the brewing process although brewing coffee during 30 sec produced a beverage with higher diterpenes concentration ($p \leq 0.05$). Concerning the extraction temperature, total diterpenes content of 30.77 ± 0.80, 39.24 ± 3.41 and 40.39 ± 3.97 mg/L were found in ECs prepared at water temperature of 70, 80 and 90 °C, respectively. Differences among 80 and 90 °C were not remarkable ($p \geq 0.05$). With regards to the pressure, increasing the pressure from 7 to 11 bar led to extracts richer in diterpenes ($p \leq 0.05$) while the water pressure at 14 bar resulted in reduced amount of diterpenes in EC samples. Results revealed that the pressure of 11 bar, provided the highest diterpenes concentration followed by 9, 14 and 7 bar.

For majority of ECs, an extraction yield of 1.5 - 2.5 % was found both for total cafestol and kahweol. However, very fine particles can produce high diterpenes brew concentration with diterpenes extraction yield of about 2.8 %. In EC brewed with cup size of 60 mL, up to 2.6 % of diterpenes were transferred to the brew. EC prepared at cup size of 60 mL contained less diterpenes than 30 mL but since extraction yield is dose dependent so their diterpenes extraction yield would be different. Cafestol and kahweol extraction yield (%) were shown in Figure 2.

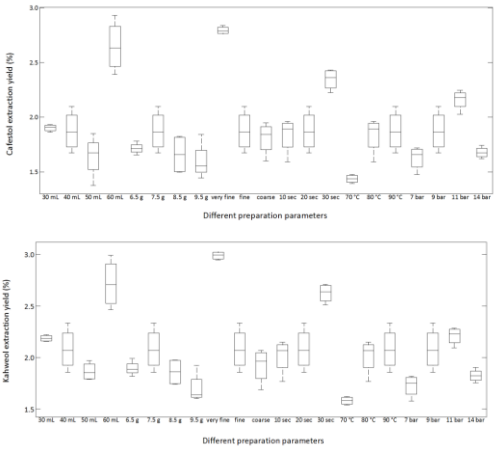


**Figure 2.** Cafestol and kahweol extraction yield (%) in espresso coffee as influence by preparation parameters. Yield= [brew diterpenes concentration (mg/L) × total brew volume (L)] / [R&G diterpenes concentration (mg/kg) × total R&G (kg)] × 100, where R&G is roasted and ground coffee.

The role of coffee diterpenes on human health is still far from established and therefore the doubt still remains about the health effects of increasing or reducing coffee diterpenes levels. However, this study clearly shows that changes in parameters of coffee brew preparation may be used to modulate EC diterpenes content. This data may also prove useful when assessing diterpenes exposure through coffee intake in a population.

***Acknowledgements***

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