Analysis of Diterpens in Green and Roasted Coffee of *Coffea arabica* Cultivars Growing in the Same Edapho-Climatic Conditions

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SUMMARY

Lipids are important components of coffee beverage flavor and aroma. Coffee oil is rich in diterpens of the kaurane family, mainly cafestol ($C_{20}H_{28}O_3$) and kahweol ($C_{20}H_{26}O_3$), which have increasingly received attention in recent years due to their physiological effects in human health. However, few studies have been conducted on the effects of the genetic variability for those lipids in *Coffea arabica*. In this work we initiate the characterization of cafestol and kahweol in different cultivars of *Coffea arabica,* growing in the same edaphoclimatic conditions. Mature coffee fruits from cultivars Catuaí, Icatu and three Catucaí derived the cultivars IPR 100, IPR 102 and IPR 106. They were harvested at the Agricultural Field Station of the Coop COCARI, Mandaguari, Paraná, Brazil, from May to July 2009. Although the time of harvesting was according to the maturation of each cultivar, harvesting and post-harvesting conditions were the same for all cultivars. The five samples were subjected to medium roasting for 8 to 11 minutes at 200-210 °C, until the degree of roasting light/media (L* around 28). The extraction of diterpens was carried out in green or roasted coffee by direct saponification with KOH, extraction with terc-butyl methyl ether, and clean up with water. A reverse-phase HPLC column with isocratic elution with acetonitrile/water (55/45 v/v) was used for detection and quantification of kahweol at 290 nm and cafestol at 220 nm. In green beans, the level of kahweol was higher than cafestol, for all three IPR cultivars. Meanwhile, the inverse was observed for green beans cultivars Catuaí and Icatu, where cafestol levels were higher than kahweol. The higher levels of kahweol in relation to cafestol were again observed in roasted coffee of the three IPR cultivars. In cultivars Icatu the values for kahweol and cafestol were similar (635 and 683 mg/100 g, respectively). The highest levels of kahweol were observed in cultivar IPR 106 (1096 mg/100 g). The cultivar IPR 102 showed the highest level of cafestol (394 mg/100g). Association of this data with gene expression profile can be useful to find genes involved in cafestol and kahweol metabolism as well as to develop molecular markers for diterpens in coffee.

INTRODUCTION

Coffee is the most consumed beverage in the world, and its quality and functional properties are being widely exploited and associated with compounds of interest like caffeine, minerals, amino acids, lipids and sugars (Higdon and Frei, 2006; Rufián-Henares and Morales, 2007; Marinova et al., 2009).

The lipid composition of coffee has been described as a major influence on human health. Compounds of the unsaponifiable fraction such as cafestol and kahweol had desirable effects against cancer (Roos et al., 1997; Cavin et al., 2002), induced degradation of toxic substances and protection against aflatoxin B1 (Cavin et al., 2002), and presented antioxidant and antiinflammatory action (Kim et al., 2006) and hepatoprotective effect (Lee et al., 2007). However, undesirable effects of cafestol on human health has also been reported as cholesterol raising factor (Urgert and Katan, 1997).

Coffee oil is rich in diterpens from kauren family, specially cafestol ($C_{20}H_{28}O_3$) and kahweol ($C_{20}H_{26}O_3$). Diterpens are pentacyclic alcohols based on fusion of isoprene units (C5) to form the skeleton of 20 kauren carbons. Kahweol differs from cafestol by a double bond between carbons 1 and 2 leading to a spectrum with maximum peak absorption at a different wavelength (Figure 1). Analysis of cafestol in coffee demonstrated a concentration higher in *Coffea arabica* than in *Coffea canephora* (Speer and Kölling-Speer, 2006). Kahweol was reported to be specific to arabica coffee beans (Campanha et al., 2010, Dias et al., 2010) or detected only in traces in *C. canephora* (Speer and Kölling-Speer, 2006,

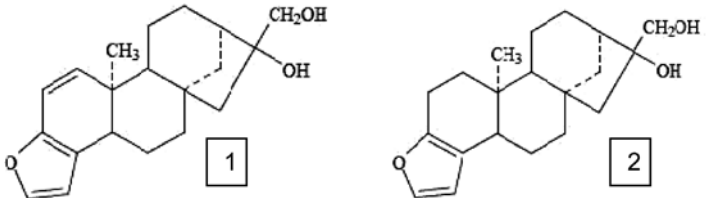

Figure 1. Structural formulas of kahweol (1) and cafestol (2).

From the factors affecting the composition of coffee, genetic variability has been highlighted for contributing directly to the diversity in terms of acidity, sugars, fat and caffeine (Sholz et al., 2000) and sensory quality (Medina Filho, 2007). It is also known that parameters such as altitude and temperature affect the composition in a different way (cell wall carbohydrates, chlorogenic acids, lipids and caffeine) depending on the variety (Jöet et al., 2010).

As for diterpens, there is little information about the influence of genetic variability in *C. arabica* cultivars, the aim of the study was to characterize the cafestol and kahweol contents in different coffee cultivars grown under the same edapho-climatic conditions.

MATERIALS AND METHODS

The samples of arabica coffee species were collected in Mandaguari – Paraná – Brazil at the Agriculture Technologic Park of Coop COCARI. Five cultivars were used: Catuaí vermelho, Icatu amarelo, IPR 100, IPR 102 and IPR 106 (Eira et al., 2007; Sera et al., 2005; Alteia et al., 2001; Ito et al., 2007). The samples were harvested from May to July 2009 at latitude 23°32'52" (South), altitude of 650 m and average annual temperatures of 22 to 23 °C. Cherry fruits were manually selected, washed and sun-dried in patio. The samples were processed, standardized in Grade 16 sieve size (6.5 mm) and characterized for their number of deffective beans (Brasil, 2003).

The samples of green coffee beans were frozen (-18 °C) and grounded (0.5 mm particles) in the disk mill (Perten 3600, Sweden) immediately prior to testing and using liquid nitrogen to prevent oxidation of compounds in the matrix (Dias et al., 2010). For roasted coffee, a roaster (Rod-Bel, São Paulo, Brazil) was used for 8-11 minutes at temperatures of 200 to 210 °C,

reaching light to medium roasting degree (L* about 28) as described by Scholz (2008). The samples were grounded in a manual disk grinder (FAMA, Indaiatuba, PR), stored in plastic bags and kept in a freezer (-18 °C).

For diterpens extraction, the samples were subjected to direct saponification with KOH and then extracting the unsaponifiable matter with t-BME, in duplicate. They were cleaned up with water after the extraction (Figure 2). The green coffee samples were weighed directly into the centrifuge container with 2 mL of KOH to prevent diterpens oxidation (Dias et al., 2010).

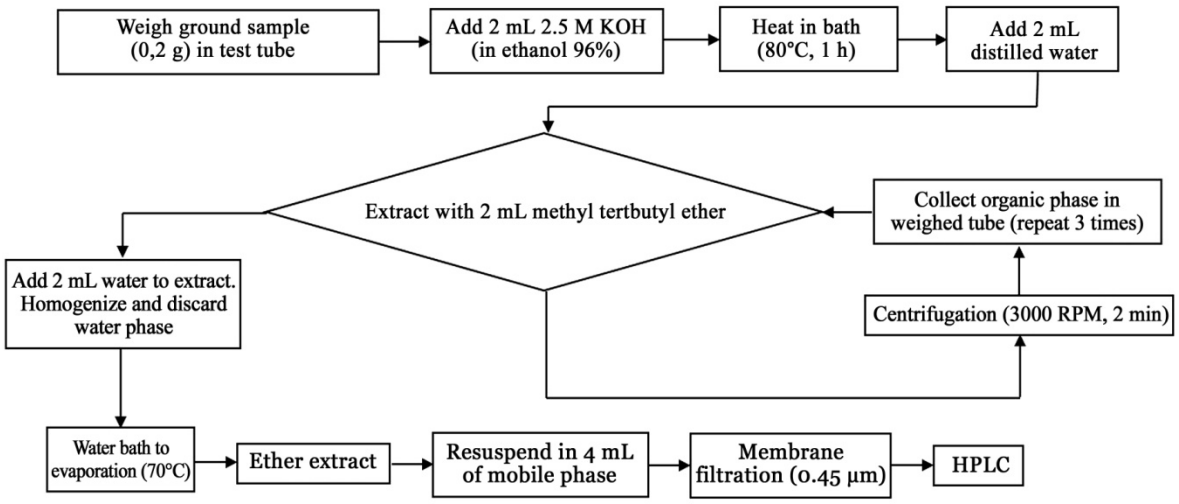

Figure 2. Flowchart of unsaponifiable coffee matter extraction (DIAS et al., 2010).

The analysis were performed in a High Pressure Liquid Chromatography Surveyor Plus (San Jose, USA) consisting of an autosampler with Peltier temperature control for rack of samples and oven integrated, quaternary gradient pump, diode-array detector, chromatography data system for ChromQuest 5.0 integrated via the network.

The analysis was conducted as described by Dias et al. (2010) using reversed-phase Spherisorb ODS 1 column (250 mm x 4.6 mm id 5 mm). It was used as mobile phase isocratic elution of acetonitrile/water (55/45 v/v) in 0.9mL/min flow rate. Detection was performed at 220 and 290 nm for cafestol and kahweol, respectively. It was applied oven temperature of 25 °C, and 20 minutes of running time. The identification was based on retention time comparison and coelution with the authentic standards. All samples were expressed on a dry basis (drying in the oven at 105 °C for 3 hours) and submitted to duplicate extraction and injection.

The quantification was carried out by external standardization, generating calibration curves for the compounds to be studied, with six different concentrations of standards (in triplicate) in the most appropriate concentration ranges. Calibration curves were constructed on concentrations of cafestol and kahweol (50-1000 mg/100 g of coffee). Data were analyzed by one-way ANOVA, considering the cultivar as the source of variation, and Tukey test ($p \leq 0.05$), using the Statistica 6.1 software.

RESULTS AND DISCUSSION

A great variability in the composition of diterpens was observed for both green and roasted coffee of different cultivars (Table 1). Roos et al. (1997) had already reported large differences in levels of cafestol and kahweol between coffees from different species grown in the same place. In this study, since the growing conditions, harvesting and processing were the same for all the plants and harvested coffee, the differences can be mainly attributed to the particular characteristics of each cultivar.

Table 1. Content of kahweol and cafestol (mg/100g) in green and roasted coffees of different cultivars on dry base*.

Cultivars	Green			Roasted		
	Cafestol	Kahweol	Total Diterpens	Cafestol	Kahweol	Total Diterpens
Catuaí	604±8a	371±6c	975±13b	668±52a	439±43c	1107±96b
Icatu	501±37a	433±18bc	934±56b	683±50a	635±51b	1318±99ab
IPR 100	328±32b	892±59a	1221±91ab	339±17b	939±25a	1278±42ab
IPR 102	356±34b	605±52b	960±86b	394±7b	691±4b	1086±3b
IPR 106	325±14b	986±46a	1312±60a	357±5b	1096±83a	1453±88a

**Mean of four values. Different letters in the column indicate significant differences ($p \leq 0.05$).*

For green coffee, there was a difference in the level of cafestol and kahweol between the new IPR cultivars and both Catuai and Icatu (Table 1). IPR 100 and IPR 106 also showed a high level of kahweol when compared with the other cultivars. The concentration of cafestol ranged from 325 to 604 mg/100 g of green coffee (1.8 times) with the highest value observed for the variety Catuai and the lower values for IPR 100 and 106. Kahweol showed even higher variation 371-986 mg/100 g of green coffee (2.6 times greater), with the highest content found in IPR 106. However, no difference was observed in total diterpens between the cultivars, with the exception of IPR 106. Kurzock and Speer (2001) reported values around 270 to 670 and 110 to 350 mg/100 g for cafestol and kahweol, respectively. Interestingly, Catuaí and Icatu have values near this range, but the IPRs have a different profile. Among IPRs, the IPR 102, derived from a cross of Icatu and Catuaí, showed behavior closer to traditional cultivars than to cultivars IPR 106 (only Icatu genetic background) and 100 (Catuaí genetic background). Total concentrations are found in the range of 934 to1312 mg/100 g and are among the levels reported in the literature (700 to 1300 mg/100 g) by Speer; Kölling-Speer (2006).

For roasted coffee, the cafestol content ranged from 339 to 683 mg/100g and the kahweol from 439 to 1096 mg/100 g (Table 1). Campanha et al. (2010), observed in Brazilian arabica coffees levels of 360 to 478 and 661 to 866 mg/100g for cafestol and kahweol respectively. Nicolau-Souza et al. (2010) analyzed five brands of gourmet coffee (100% Arabica), and observed cafestol concentration from 460 to 470 mg/100 g and kahweol from 570 to 800 mg/100 g. Our results were slightly above the range of those reports. However, Campanha (2008) reports lower values of cafestol (275 to 282 mg/100g) and higher values of kahweol (787 to 933 mg/100g) for *C. arabica* cv IAPAR 59, with different degrees of roasting, in a diterpens profile, similar to that observed for IPRs.

We note that the content of cafestol and kahweol increased when compared to green coffee in all varieties (increase of 3 to 36% for cafestol and 5 to 47% for kahweol). The relative increase in concentration can be attributed to degradation of thermolabile constituents from the roasting (Table 1). There is disagreement in literature over the stability of diterpens with the processing. Some authors report that diterpens could form dehidro derivatives (dehydro cafestol and kahweol) and other degradation products from roasting, reducing their levels (Kurzock and Speer, 2001, Speer and Kölling-Speer, 2006). Urgert et al. (1995) evaluated the behavior of kahweol and cafestol in *C. arabica* of intense roasts (26.5% weight loss), and concluded that roasting did not reduce the concentration of these compounds. Campanha et al. (2010), working with three degrees of roasting for different blends of coffee, reported that increasing in the degree of roasting did not lead to a reduction in diterpenes content. DIAS (2009) evaluated the degradation of cafestol and kahweol (time of 2-10 min, maximum temperature of 230 °C) and observed that the levels of cafestol and kahweol remained stable, even though dehidro derivatives occurred with more intense processes of roasting, due to increased concentration of lipids during the roasting process.

The ratio of kahweol/cafestol for the different cultivars was calculated (Figure 3). For the cultivars IPR the kahweol/cafestol ratio ranged approximately from 1.70 to 3.07 for both green and roasted coffee, since the amount of cafestol was always higher than kahweol on those cultivars. An opposite behaviour was observed for the cultivars Catuaí and Icatu which showed a kahweol/cafestol relation of approximately 0.61 to 0.93 for both green and roasted coffee.

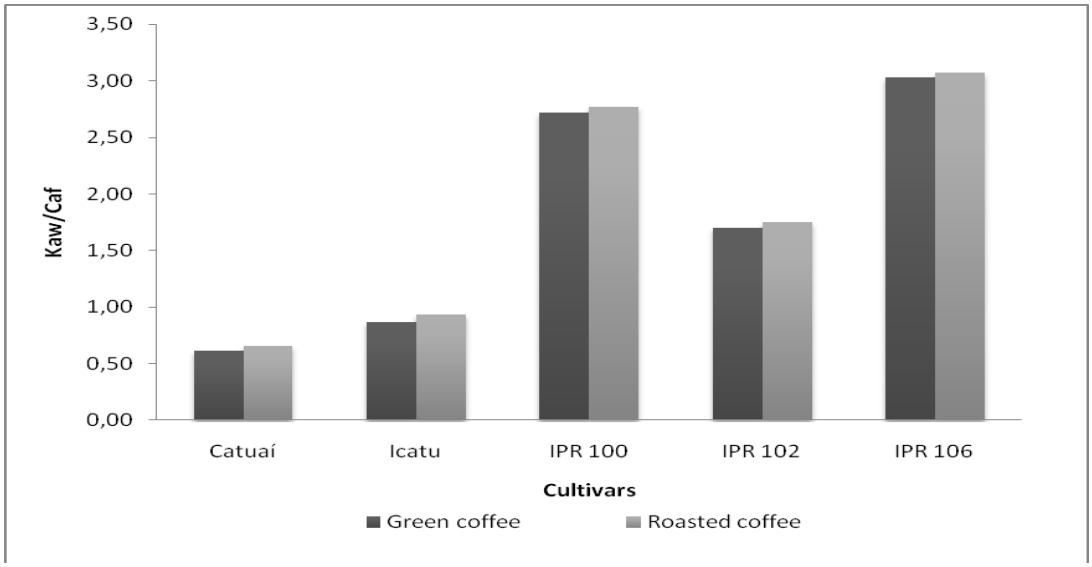

Figure 3. Relation kahweol/cafestol for green and roasted coffees.

This relation kahweol/cafestol was not affected by the roasting process, indicating that it could be used to characterize the cultivars regardless the degree of roasting (Figure 3).

It can be observed that the cultivars IPR 100 (Catuaí genetic background) and IPR 106 (Icatu genetic background) showed kahweol/cafestol relation around 3. Meanwhile, Catuaí and Icatu showed the lowest ratio. The cultivar IPR 102, which has both cultivars, Icatu and Catuaí in the genetic background showed a intermediated ratio.

Considering health issues, the crosses that generated the cultivars IPR were interesting as they showed higher levels of kahweol and, conversely, the levels of cafestol were reduced compared to traditional cultivars. Kahweol has been associated to beneficial health effects, but it has been proven that cafestol has a roll in raising cholesterol (Higdon and Frei, 2006), so the balance between cafestol and kahweol in cultivars IPRs, without altering the total amount of diterpens, can brings positive impact for consumers. Further work in the characterization of gene expression on those cultivars, associated to of diterpens profiles may be useful for finding genes involved in metabolism of cafestol and kahweol and to develop molecular markers for diterpens in coffees.

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