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Spectrophotometric method for quantification of kahweol in coffee

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ABSTRACT

The diterpene kahweol, a component of the unsaponifiable matter of coffee oil, has anticarcinogenic and antioxidant properties. Kahweol is specific to *Coffea arabica*, so it can be used to discriminate between coffee species. Chromatographic or infrared techniques are usually required for the evaluation of kahweol. The objective of this study was to develop a methodology to quantify kahweol based in colorimetric reactions and spectrophotometric measurements. The best extraction conditions were achieved by direct saponification of roasted and ground coffees, extraction with MTBE, cleaning the extract with water, reaction of the extract with KI, dilution with HAc 50% and absorbance analysis at 620 nm for quantification. This method demonstrated good precision (RSD below 5%) considering different extractions and intraday repeatability. Linearity was also observed (R^2 = 0.996, $p \leq 0.05$), with low limits of detection (5.16 mg 100 g^{-1}) and quantification (17.2 mg 100 g^{-1}). Kahweol contents were assessed at similar levels to those obtained using a standard HPLC methodology, and a good recovery was observed (116%).

1. Introduction

The production and marketing of coffee has become an important economic activity as the popularity of this aromatic beverage has spread around the world. More than 80 species of the genus *Coffea* L. (Rubiacea) are known. The most important species are *Coffea arabica* (arabica) and *Coffea canephora* (robusta), which correspond to 63% and 37% of global production, respectively (ICO, 2011).

Arabica coffee has greater acceptability, better cup quality and a higher price than the robusta species. The green beans of the two species are distinctive: arabica is light green and oval in shape, whereas robusta is brown and more rounded. However, it is impossible to visually distinguish the species after roasting and grinding. As they belong to the same genus, few physical and chemical differences enable the detection and/or quantification of the addition of robusta coffee to arabica coffee (González et al., 2001; Kemsley et al., 1995).

The unsaponifiable matter content is approximately 12 g 100 g^{-1} of coffee oil, with diterpene kahweol present as a primary component (Lago, 2001). The level of kahweol has been cited as a possible discriminating factor between coffee species in blends of *C. arabica* and *C. canephora*, as this compound is considered to be specific to arabica coffee and is relatively stable at processing temperatures (Dias et al., 2011; Campanha et al., 2010; Kemsley et al., 1995).

The contents of diterpenes in coffee products have been investigated because of the physiological action of these compounds in human health. Some diterpenes, including kahweol, may induce the degradation of toxic substances and provide protective action against aflatoxin B1 (González et al., 2001). In addition, anti-inflammatory, anticarcinogenic and antioxidant properties, as well as hepatoprotection, have been reported (Muriel and Arauz, 2010; Kim et al., 2006, 2009; Nkondjock, 2009; Lee et al., 2007; Lee and Jeong, 2007; Cavin et al., 2002). The evaluation of kahweol in the coffee plant may help to elucidate the metabolic pathways and enzymes involved in the biosynthesis of this diterpene in the plant and fruit tissues during their development (Dias et al., 2010; Vieira et al., 2006).

Abbreviations: HAc, glacial acetic acid; MTBE, methyl *tert*-butyl ether; DS, direct saponification; DAB, days after bloom; ACN, acetonitrile..

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There are two general ways of extracting the unsaponifiable matter: direct saponification (DS) or the extraction of lipids followed by saponification. DS has been reported as the most rapid and efficient alternative, as it avoids the formation of artifacts (Saldanha et al., 2006). DS is considerably faster and demands lower amounts of solvent in comparison with the method using the pre-extraction of lipids (Mariutti et al., 2008).

Wurziger (1985) and Wurziger et al. (1979) described a method for differentiating coffee species (green and roasted beans) by reacting the petroleum ether extract with potassium iodide and hydrochloric acid in glacial acetic acid. KI and HCl react with the double bond of the kahweol structure. In an acidic medium, the reaction products absorb at 620 nm. The difference determined from the absorbance measurements at 290 nm (maximum wavelength, λ_{max}, for kahweol) and 620 nm (λ_{max} for the reaction products) was due to the high level of kahweol that is in arabica coffee and nearly absent in robusta coffee. However, the method was empirical and only semi-quantitative, and there is no mention of its later use.

The literature describes a variety of techniques based, in general, on analyzing various compounds for differentiating coffee species. High performance liquid chromatography (HPLC) stands out (Scharnhop and Winterhalter, 2009; Araújo and Sandi, 2006; Kurzrock and Speer, 2001a; Castillo et al., 1999; Pettitt, 1987), but techniques based on Raman spectrometry (Wermelinger et al., 2011; Keidel et al., 2010; Ribeiro et al., 2010; Rubayiza and Meurens, 2005) and gas chromatography (GC) have also been reported (Guerrero et al., 2005; Castillo et al., 1999; Urgert et al., 1995).

The detection of the fraudulent or accidental addition of robusta to arabica coffees is of interest to the cooperatives of coffee producers, coffee industry and regulatory authorities. However for the coffee-producing countries, chromatographic or infrared techniques are relatively expensive and not always available for routine analyses, since depend on equipment and a trained technician. In this study, the principles of the Wurziger technique were used to develop a methodology for quantification of kahweol based on reactions involving the unsaponifiable matter. The extraction steps were adapted, and the reaction conditions were standardized. After the development of the methodology, a validation step was performed to ensure that the results are reliable and to enable the method to be used routinely for the quantitative analysis of kahweol in roasted and ground coffees.

2. Materials and methods

2.1. Reagents and equipment

The reagents used on the method development were potassium hydroxide, KOH (analytical grade, Synth, Diadema, Brazil), ethanol (purity ≥99.5%; Merck, Darmstadt, Germany); methyl *tert*-butyl ether, MTBE (analytical grade; Vetec, Duque de Caxias, Brazil), sodium thiosulfate, $Na_2S_2O_5$ (anhydrous, analytical grade; Merck, Darmstadt, Germany), purified water (Milli-Q® purification system, Millipore, Billencia, USA), acetic acid, HAc (glacial; Merck, Darmstadt, Germany), hydrochloric acid, HCl (36.5–38.0%; Merck, Darmstadt, Germany), potassium iodide, KI (≥99.5%; Icasa SA., Monterrey, Mexico), hexane (purity ≥99.0%; Merck, Darmstadt, Germany), diethyl ether (purity ≥99.5%; Merck, Darmstadt, Germany). A kahweol standard (Axxora, San Diego, USA) of 98% purity, certified by Alexis Biochemicals (Lausen, Switzerland), was used to develop and validate the method. Water purified and acetonitrile, ACN (HPLC grade; Carlo Erba, Duque de Caxias, Brazil), used as mobile phase of HPLC analysis were filtered in a Millipore vacuum filtration system through 0.45 μm membranes.

A UV–vis GBC Cintra 20 spectrophotometer with a detection range from 190 to 1000 nm and a slit aperture of 2 nm was used for the absorbance measurements. A colorimeter (Color-guide portable BYK-Gardner, USA) with 45/0 geometry and D65 light source was used in color characterization of samples.

For HPLC analysis a Shimadzu liquid chromatograph equipment (Kyoto, Japan) was used, with a quaternary system of solvent pumping (LC10 ATvp model), online degasser DGU-14 Avp, aCTO-10ASvp column oven model, and a Rheodyne injection valve with a 20 μL loop. The system was coupled to a Shimadzu model SPDM10 Avp, UV–vis spectrophotometry diode array detector (spectral scan from 190 to 800 nm; sensitivity of 0.8×10^{-8} UA), connected to a PC through an interface (SCL-10Avp).

2.2. Material

The samples of green coffees *C. arabica* cv. IAPAR 59 and *C. canephora* cv. robusta of known origin (Paraná/Brazil and Rondônia/Brazil respectively) were supplied by the Instituto Agronômico do Paraná (IAPAR, Londrina, Brazil). The cherry fruits were sundried and the green coffee beans (at least 300 g per each sample) were processed. Roasting was performed using a pilot-type Rod Bel roaster with a capacity of 3.6 kg h^{-1} at a maximum temperature of 230 °C. The samples were ground to a granulometry of around 0.5 mm, stored in plastic bags, conditioned in a cold chamber (10 °C), and analyzed in the sequence. The samples were characterized as a medium roast degree according to their color (in triplicate). For the arabica coffee, a lightness (L^*) of 22.0 was observed, and an L^* 30.3 was observed for the robusta. A blend of the species robusta and arabica (70/30 w/w) was used for the recovery test.

For the accuracy test, commercial coffees from the local market (supplied by Companhia Iguaçu de Café Solúvel®, Cornélio Procópio, Brazil) were also used. These products were labeled as "traditional", indicating a blend of arabica and robusta coffees. Their color (L^* of 18.2, 17.6 and 19.1 for samples 1, 2 and 3, respectively) indicated a medium to dark roast degree.

Fruit tissues and coffee plant leaves from *Coffea arabica* cv. IAPAR 59, supplied by the Instituto Agronômico do Paraná, were also studied. Each tissue was dissected from fruits at different ripening stages (days after bloom, DAB) to obtain enough sample weight for analysis. The perisperm was obtained from fruit collected 83 DAB, and the endosperm and pericarp were obtained from fruit collected 240 DAB (Geromel et al., 2006). Leaves of young and mature *C. arabica* coffee trees were also evaluated. The fruit and leaves were conditioned in closed plastic tubes, immediately frozen in liquid nitrogen to avoid oxidation, and stored at −80 °C until analysis. After the tissue separation, each sample was macerated with the aid of a mortar and pestle in the abundant presence of liquid nitrogen, and analyzed in the sequence.

2.3. Method development

The extraction of kahweol and the conditions for the colorimetric reactions were based on the Wurziger (1985) method. To obtain a quantitative extraction, the technique and possible extraction solvents were studied, and the conditions most specific for kahweol used by authors working with chromatographic techniques were tested (Urgert et al., 1995). As the original method lacks details, standardization tests were conducted to determine the quantities and form for the addition of the reagents.

A full spectrum UV–vis (200–800 nm) was ordered after direct saponification and the reaction between kahweol and a colorimetric reagent, HCl or KI. The absorbance measurements were normalized after the reaction as a function of the initial