Development of an instant coffee enriched with chlorogenic acids

Marinês Paula Corso^{1,2} · Josiane Alessandra Vignoli³ · Marta de Toledo Benassi²

Revised: 17 November 2015 / Accepted: 29 December 2015 / Published online: 13 January 2016
© Association of Food Scientists & Technologists (India) 2016

Abstract The objective of this study was to present possible formulations for an instant coffee product enriched with chlorogenic acids for the Brazilian market. Formulations were prepared with different concentrations of freeze dried extracts of green *Coffea canephora* beans (G) added to freeze dried extracts of roasted *Coffea arabica* (A) and *Coffea canephora* (C). Medium (M) and dark (D) roasting degrees instant coffee were produced (AM, AD, CM and CD) to obtain four formulations with green extract addition (AMG, ADG, CMG and CDG). Chlorogenic acids were determined by HPLC, with average contents of 7.2 %. Roasted extracts and formulations were evaluated for 5-CQA and caffeine contents (by HPLC), browned compounds (absorbance 420 nm), and antioxidant activity (ABTS and Folin). Coffee brews of the four formulations were also assessed in a lab-scale test by 42 consumers for acceptance of the color, aroma, flavor and body, overall

acceptance and purchase intent, using a 10 cm hybrid scale. The formulations obtained acceptance scores of 6.6 and 7.7 for all attributes, thus they were equally acceptable. Greater purchase intent was observed for ADG, CDG and CMG (6.9) in comparison to AMG (6.1). The formulations had, on average, 2.5 times more 5-CQA than the average obtained from conventional commercial instant coffees. In addition to being more economically viable, the formulations developed with *C. canephora* (CDG and CMG) showed greater antioxidant potential (32.5 g of Trolox/100 g and 13.8 g of gallic acid equivalent/100 g) due to a balance in the amount of bioactive compounds.

Keywords Antioxidant activity · Acceptance · Roasting degree · Polyphenols · Green coffee · *Coffea*

Research highlights

The instant coffees developed had high ACG content and antioxidant activity. Formulations developed with *C. canephora* presented higher antioxidant potential. Instant coffees with the addition of green coffee extract had good acceptance.

✉ Marinês Paula Corso
corso@utfpr.edu.br

¹ Núcleo de Ciência dos Alimentos, Universidade Tecnológica Federal do Paraná, Avenida Brasil, n. 4232, Medianeira, Paraná 85884-000, Brazil

² Departamento de Ciência e Tecnologia de Alimentos, Centro de Ciências Agrárias, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Pr 445 Km380, Campus Universitário, Cx. Postal 6001, Londrina, Paraná 86051-980, Brazil

³ Departamento de Bioquímica e Biotecnologia, Centro de Ciências Exatas, UEL, Londrina, PR 86051-990, Brazil

Introduction

Coffee is an important product for the global economy, with a production of 143,253 million bags of 60 Kg in 2014, distributed from more than 40 exporting countries. Brazil, the second largest consumer of coffee (20,771 million bags), and the largest producer (45,342 million bags) and exporter (average of 2937 million bags for month at Apr-2015 to Sept-2015), is responsible for one-third of the production and more than one-fourth of the bean exports globally (Ico 2015). For instant coffee, world consumption increased from 22.8 million bags (equivalent in green grain) in 2000 to 31.1 million bags in 2010. In Brazil, the largest exporter of instant coffee, the general consumption represents approximately 5 % (Itc 2011), reaching 17 % of the Brazilian consumers (Abic 2010).

The production of soluble or instant coffee starts with coffee bean selection, followed by the processes of roasting, granulation, extraction, concentration and drying by

atomization or freeze drying (Nogueira and Trugo 2003; Itc 2011; Vignoli et al. 2011). Its composition depends on the coffee species and varieties used in the blends and on processing conditions (Nogueira and Trugo 2003; Vignoli et al. 2011; Marcucci et al. 2013). Between the most cultivated species, *Coffea arabica* and *Coffea canephora*, the latter is the most used in the production of instant coffee. Green (raw) *C. canephora* coffee beans have higher levels of caffeine and chlorogenic acids (CGA) and a lower trigonelline content than arabica (Nogueira and Trugo 2003; Hatzold 2012). Degradation of some bioactive compounds may occur during the extraction and drying processes, and in contrast, other compounds may have their concentrations increased proportionally, due to the removal of non-soluble components. However, it is during the roasting process that the most relevant changes take place (Farah and Donangelo 2006; Budryn et al. 2009; Vignoli et al. 2011). Recently, several studies have emphasized the effect of the roasting process on the composition, biologic activity and sensory characteristics of coffee (Bekedam et al. 2008; Hoelzl et al. 2010; Perrone et al. 2010; Bakuradze et al. 2011; Liu and Kitts 2011; Kotyczka et al. 2011; Vignoli et al. 2011; Perrone et al. 2012; Pino-García et al. 2012; Tfouni et al. 2012; Vignoli et al. 2014).

Coffee aroma and flavor are developed during the roasting process. With an increase in roasting intensity, the color becomes darker and there is a greater loss in coffee bean weight, the coffee brew loses acidity, gains body and a stronger flavor (Itc 2011). These physical and sensory changes are followed by alterations in the composition, especially due to the degradation of phenolic compounds and the development of Maillard reaction products (Farah and Donangelo 2006; Budryn et al. 2009; Hatzold 2012; Pino-García et al. 2012).

According to Farah and Donangelo (2006), CGAs and related compounds are the main components of the green coffee phenolic fraction. They are beneficial to our health not only for their powerful antioxidant activity (AA) but also as hepatoprotective, hypoglycemic and antiviral agents. Therefore, during processing, the CGA can be partially isomerized, hydrolyzed or degraded to low molecular mass compounds. High temperatures can also produce lactones and the polymerization of the CGA with the development of melanoidins (Perrone et al. 2012). Among the isomers, the 5-caffeoylquinic (5-CQA) is the most abundant form (Nogueira and Trugo 2003; Perrone et al. 2012; Tfouni et al. 2012).

There is a lack of data related to the absorption and metabolism of melanoidins in humans and, due to the great variety of products developed in different matrices, few structures have been described. Some positive effects of melanoidins on human health have been highlighted such as their AA, chemopreventive, antimicrobial and prebiotics action (Wang et al. 2011; Morales et al. 2012; Perrone et al. 2012). In contrast, there is also evidence that these substances may take part

in some pathological processes (Bastos et al. 2012). Liu and Kitts (2011) showed that Maillard reaction products, especially those of low molecular mass, are the predominant antioxidants in roast coffee brews.

The most highlighted bioactive compound in instant coffee, caffeine, is less affected by the roasting process and is more dependent on the raw material (Budryn et al. 2009; Hatzold 2012; Vignoli et al. 2011; Esquivel and Jiménez 2012). In addition to AA (Vignoli et al. 2011; Viana et al. 2012), caffeine has been connected to weight loss and the consequent reduction of overall risks for the development of the metabolic syndromes (Heckman et al. 2010).

Considering that both green and roasted coffees have positive effects on human health, instant coffees have been developed with the addition of green coffee beans in order to enrich products with CGA. Some in vivo studies with products in this category have reported on benefits related to the increased CGA content such as the reduction in glucose absorption (number of subjects (n) = 12) and body mass of obese people (n = 30) (Thom 2007); protection against oxidative damages to macromolecules (n = 36) (Hoelzl et al. 2010); reduction in DNA damages, with significant increase in glutathione and glutathione reductase, reduction of weight and body fat (n = 33) (Bakuradze et al. 2011); positive effects on humor and cognition, increased attention span and reduction in headaches and mental fatigue (n = 39) (Cropley et al. 2012). These products are being produced and commercialized in Europe, Asia and North America. In Brazil, consumers prefer coffee products with medium to dark roasts degree (Vignoli et al. 2011; Perrone et al. 2012; Marcucci et al. 2013), and products with green coffee are not available in the market yet.

In this study, possible formulations were proposed for an instant coffee product enriched with antioxidants produced by mixing different concentrations of dry extract from green *Coffea canephora* and dry extracts from roasted coffee of the *Coffea arabica* and *Coffea canephora* species with two different degrees of roasting (medium and dark). Products developed were evaluated regarding bioactive compounds, AA and sensory acceptance.

Materials and methods

Reagents and standards

Standards of 5 caffeoylquinic acid and gallic acid were acquired from Sigma (St. Louis, MO, USA). Caffeine standard and potassium persulfate were obtained from Acros Organics (NJ, USA). Acetonitrile (HPLC grade) was acquired from Fisher Scientific (NJ, USA). Acetic acid and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)

was obtained from Aldrich (Steinheim, NRW, Germany). Ethanol was obtained from Anidrol (Diadema, SP, Brazil). The Folin Ciocalteu reagent was purchased from Merk (Darmstadt, HE, Germany) and the sodium carbonate was obtained from Nuclear (Diadema, SP, Brazil). The water used in the preparation of solvents was obtained through the Milli-Q purification system (Millipore) (Molsheim, France).

Formulations

Freeze dried green coffee extract (G) from *Coffea canephora* and freeze dried extracts from processed coffee to obtain two degrees of roasting, medium roast (M) and dark roast (D), from two species *Coffea arabica* (A) and *Coffea canephora* (C) were produced (AM, AD, CM and CD). The extracts were processed in an industrial pilot plant following the internal processing standard for instant coffee. Samples (green and roasted coffees) were submitted to the conventional extraction in battery columns percolators. During this process, pressure water at 180 °C was introduced in the first percolation stage (column with the oldest coffee) and percolate through the next stages until, in the last stage, the extract reaches the recently loaded coffee. During the process, the instant solid extracts increase but the temperature decreases, and the final column (with fresh coffee) is extracted at a temperature close to 100 °C; therefore, under conditions that favor the preservation of aroma and flavor. The extracts were submitted to freeze drying. Freeze dried extracts, to be used in the formulations, were characterized according to color, moisture, contents of bioactive compounds (5-CQA, total CGA, caffeine and browned compounds) and AA, according to methodologies described in the following sections.

Four instant coffee formulations enriched with CGA were developed. The adopted method was to add the green bean dry extract to the four roasted bean dry extracts such that each product had around 7 % polyphenols, the average value obtained by commercial products (details in the Results section). The developed formulations were analyzed for moisture, coffee brew color, the contents of caffeine, 5-CQA, total CGA and browned compounds, AA and sensory acceptance.

Two commercial instant coffees from different origins (France and Spain), enriched with antioxidants, were used for comparative purposes (content of bioactive compounds and AA). Both had 35 % green coffee (reported on the label) but different processing, one product was granulated (freeze dried) and the other was agglomerated (spray dried).

Moisture and color characterization

The moisture of each instant coffee (3.000 g) was determined using an infrared equipment (OHAUS-MB200, EUA), at 105 °C for 7 min. Measurements were made in triplicate and results were used to calculate chemical constituents on dry basis.

Color was analyzed using a portable colorimeter (KONICA Minolta-CR400, Osaka, Japan) with a 45/0 geometry and a D65 illuminant. The colorimeter was coupled to a light projector tube (CR-A33). Instant coffees were packaged in a plastic recipient for granular product (CR-A50) with readings made directly on the surface. Coffee brews were prepared according to following Sensory analysis section and packaged in plastic cells coupled to a cells support. The analyses were made at room temperature and in genuine duplicate with measurements in triplicate. Values for L* (lightness), a* (red-green component) and b* (yellow-blue component) were obtained and used to determine hue as follows:

$$h = \tan^{-1} \left(\frac{b}{a} \right)$$

Bioactive compounds determination

A methodology adapted from Vignoli et al. (2011) and Marcucci et al. (2013) was used to determine caffeine and 5-CQA content and to estimate total CGA content. The instant coffees were dissolved in acetic acid solution 5 % to obtain extracts with concentration of 0.5 mg/mL. The extracts were filtered at 0.22 µm and directly injected. The chromatographic system (Shimadzu, Kyoto, Japan) consisted of a two-pump (LC-10 AD), Rheodyne injector valve with a 20 µL sampling handle, column furnace (CTO-20 A), UV/visible detector (SPD-10 A), CBM-101 interface, and the CLASS-CR10 program, version 1.2. A Spherisorb ODS1 column (250 × 4.6 mm, 5 µm) (Waters, Ireland) was used. The compounds were eluted with acetic acid solution 5 % (A) and acetonitrile (B) at a flow rate of 0.7 mL/min using the following gradient: 0–5 min: 8 %; 5–35 min: 15 %. Caffeine was detected at 272 nm, while 5-CQA and others CGAs were detected at 320 nm. The analyses were carried out in duplicate at 25 °C. Quantification was carried out by external standardization using a 6-point calibration curve with duplicate measurements in the concentration range of 1 to 31 µg/mL for 5-CQA and of 5 to 40 µg/mL for caffeine. In regards to polyphenols content, total CGA was estimated by the sum of the compounds detected at 320 nm based on Budryn et al. (2009), using the 5-CQA as a standard quantifier.

Browned compounds (melanoidins) were estimated according to the methodology described by Ludwig et al. (2012) and adapted by Marcucci et al. (2013). The instant coffees were diluted directly in water, at room temperature (25 °C) in the concentration of 0.57 mg/mL. Absorbance was measured at 420 nm by a UV-VIS Biochrom Libra S22 spectrophotometer (Cambridge, England). The analysis was carried out in genuine duplicate with triplicate measurements.

Antioxidant activity determination

Hydrogen ions donation activity to the ABTS radical (ABTS^{•+}) was assessed as described by Vignoli et al. (2012). The analyses were carried out in genuine duplicate of the samples, with measurements in triplicate. Ethanol solutions with six concentrations of Trolox in the range of 0.5 to 8 mM (in triplicate) were used for calibration. Results were expressed as antioxidant capacity equivalent to Trolox (TEAC) in g of Trolox/100 g of sample (dry basis).

The Folin-Ciocalteu method was carried out according to Vignoli et al. (2011). The analyses were carried out in genuine duplicate of samples with measurements in triplicate. Solutions with five known concentrations of gallic acid in the range of 0.5 mM to 7 mM (in triplicate) were used for calibration. The results were expressed in g of gallic acid equivalent (GAE)/100 g of sample (dry basis).

Sensory analysis

The formulated brews with green coffee were prepared following the proportion of 1.4 g of instant coffee for 50 mL of water. Coffees were dissolved in water at 95 °C and sweetened with 9.5 % of sugar, estimated as the ideal concentration for sweetening (Moraes and Bolini 2010). After the preparation, the samples were stored in thermal bottles and kept for at least 2 h, until being served at 70 °C.

A laboratory scale panel made up of 42 consumers was recruited at Universidade Estadual de Londrina, Paraná, Brazil. The participants, all regular consumers of coffee, were university students, teachers and employees, aged between 18 and 55 years old, with the prevalence of the female gender (69 %). This profile is adequate since Brazilian women are still the main responsible (77 %) for purchasing and preparing the product (Abic 2010). The panel presented diversified of family incomes despite the high level of education (93 % with higher education). All participants, at least occasionally, consumed functional foods and 88 % were instant coffee consumers. In general, the panel presented a high consumption rate, comparing to the ABIC research (2010) that states that, instant coffee is consumed only by 17 % of Brazilian consumers. This study was authorized by the Ethics Committee of Universidade Estadual de Londrina (certificate 0143.0.268.000–10).

Tests were conducted in a sensory analysis laboratory, under white light, in individual booths, in a single session. Coffee brews (30 mL) were served at 70 °C (Oliveira et al. 2009), in disposable styrofoam cups codified with three randomized digits. The samples were presented monadically according to an experimental design of complete balanced blocks, in a randomized order. A 10 cm hybrid scale (Villanueva et al. 2005) was used to assess acceptance (attributes color, flavor, body, and global acceptance) and purchasing intention.

Data analysis

The results were evaluated by one-way ANOVA, considering extracts and formulations as the source of variation, and Tukey's means test ($p \leq 0.05$). For the ABTS and Folin data, the difference between extracts and their respective formulations was tested by the t test for dependent sample ($p \leq 0.05$). Sensory analysis results were evaluated by two-way ANOVA, considering formulations and judges as the sources of variation, and Tukey's means test ($p \leq 0.05$). The analyses were carried out by Statistica 8.0 (Statsoft Inc., Tulsa, USA).

Results and discussion

Characterization of freeze dried extracts

Freeze dried extracts from green beans and roasted coffee (from both coffee species, *C. arabica* and *C. canephora*) were used for the development of instant coffee formulations enriched with CGA. The freeze drying process produces a better but more expensive product so it is generally used for *C. arabica* coffee or special products (Itc 2011). In Brazil, the majority of the commercial instant coffee is spray dried and produced with *C. canephora* or blends of the two species (Marcucci et al. 2013). In this research, the freeze drying process was chosen because does not use high temperatures, consequently preserving thermolabile compounds such as chlorogenic acids and improving sensory quality. It was also considered that all commercial enriched instant coffees available on the market, with the exception of a Spanish product, are freeze dried. In order to propose different formulations, the two species were tested to obtain the roasted coffee extracts. *C. canephora* was used to produce the green bean extract due to its greater viability for industry, lower price and higher chlorogenic and caffeine contents (Nogueira and Trugo 2003; Hatzold 2012; Perrone et al. 2012).

The roasting degrees used (medium and dark) were defined based on the characteristic L* values for industrial processes of freeze dried instant coffees. L* values from 36.2 to 43.9 were observed (Table 1), similar to what has been described for Brazilian commercial freeze dried instant coffees (L* from 34.6 to 43.7) (Marcucci et al. 2013). A lighter brown color was observed for the green bean extract compared to the roasted extracts (Table 1, Fig. 1).

The contents of bioactive compounds from each extract (Table 1) depended on the coffee species and on the roasting degree. *C. canephora* extracts (CD, CM and G) showed a greater caffeine content ($p < 0.05$) than *C. arabica* extracts; though the roasting degree had no significant effect on this

Table 1 Color parameters and content of bioactive compounds of dry extracts produced from roasted coffees and green coffee beans (dry basis)

Extract ¹	Lightness ²	Hue ²	Caffeine (g/100 g) ³	5-CQA (g/100 g) ³	Total CGA (g/100 g) ³	Browned compounds (Abs) ²
AD	37.75 ^c (2.97)	61.16 ^{bc} (0.46)	2.72 ^b (0.23)	1.21 ^c (0.99)	3.83 ^c (0.62)	0.281 ^b (3.14)
AM	43.93 ^b (1.34)	62.70 ^{bc} (1.23)	2.60 ^b (0.58)	2.06 ^b (0.79)	5.75 ^b (0.02)	0.227 ^d (2.41)
CD	36.22 ^d (0.45)	60.42 ^c (0.19)	3.98 ^a (1.25)	0.56 ^c (4.29)	2.39 ^d (1.37)	0.341 ^a (1.20)
CM	38.60 ^c (1.20)	63.27 ^b (4.75)	3.80 ^a (3.42)	1.04 ^d (3.83)	3.73 ^c (1.53)	0.259 ^c (2.73)
G	52.48 ^a (0.80)	67.09 ^a (0.37)	3.86 ^a (0.00)	7.52 ^a (0.96)	14.04 ^a (0.60)	0.126 ^e (5.00)

Different letters in the same column indicate a significant difference (Tukey, $p \leq 0.05$)

¹ *C. arabica* with dark (AD) and medium (AM) roasting degrees, *C. canephora* with dark (CD) and medium (CM) roasting degrees, *C. canephora* green (G).

² Values between parentheses represent coefficient of variation (CV%) of genuine duplicate with measurements in triplicate

³ or duplicate

compound. However, the increase in roasting degree reduced the contents of 5-CQA and, consequently CGA, in extracts of both species ($p < 0.05$). For each roasting degree, *C. arabica* extracts showed greater 5-CQA and CGA contents than *C. canephora* ($p < 0.05$). According to Vignoli et al. (2011), despite the higher content of CGA in green beans of *C. canephora*, these compounds are more sensitive to roasting processes in the *C. canephora* matrix than in *C. arabica*.

The green coffee extract had 14 % total CGA (Table 1). Farah and Donangelo (2006) reported CGA values from 7 to 14.4 % for green *C. canephora* coffee. It is possible to obtain extracts with a greater proportion of CGA using different extraction methods. For instance, Budryn et al. (2009) obtained extracts with up to 36 % CGA using water at 110 °C under high pressure (1.4×10^5 Pa). However, it should be noted that a high concentration of CGA may lead to lower sensory quality (Farah et al. 2006).

5-CQA was the most abundant isomer, representing 53 % of the total CGA in the green bean extract (Table 1), was

similar to the reported by Perrone et al. (2012) (51 to 63 %). With the more intense roasting degree (D), an 83 % loss was observed in total CGA content and 93 % in 5-CQA content; with the medium roasting degree (M) losses were 73 % and 86 %, respectively (Table 1). After roasting, the 5-CQA represented 23 to 35 % of the total CGA (Table 1), a portion of the 5-CQA was most likely isomerized to 3-ACQ and 4-ACQ (Budryn et al. 2009). However, it continued to be the main isomer (Fig. 2). Perrone et al. (2012) reported CGA content losses of 56 to 99 % depending on the roasting degree, with the 5-CQA proportion representing 32 to 36 % of the total CGA.

The presence of browned compounds increased with the increment in roasting degree for both coffee species ($p < 0.05$). Some browned compounds were also detected in green bean extract at 420 nm (absorbance), although in lower levels ($p < 0.05$). Budryn et al. (2009) also reported that browned compounds can be formed during the drying processes of the beans and/or during extraction and freeze drying of the extracts.

Fig. 1 Images of the dry extracts of *C. canephora* dark roast (CD), medium roast (CM), green (CG) and *C. arabica* dark roast (AD) and medium roast (AM)

Fig. 2 Chromatograms of dry extracts of *C. canephora*: dark roast (CD, L* 36) and green (G). Detection at 320 nm

Table 2 Characteristics (dry extract percentage of green coffee beans) and total CGA^a of developed formulations (dry basis)

Formulation	Roasted beans extracts		Green beans extract	Total CGA ^a
	Species	Degrees of roasting	(<i>C. canephora</i>) (%)	(g/100 g) ^b
ADG	<i>C. arabica</i>	Dark – L* 38	31	7.22 (2.32)
AMG	<i>C. arabica</i>	Medium – L* 44	15	6.88 (2.54)
CDG	<i>C. canephora</i>	Dark – L* 36	40	7.31 (0.27)
CMG	<i>C. canephora</i>	Medium – L* 39	32	7.54 (0.19)

^a Chlorogenic acids^b Values between parenthesis represent coefficients of variation (CV%) obtained in duplicate

Development of instant coffee formulations with green coffee

The four extracts of roasted coffee beans were enriched with CGA by the addition of different proportions (from 15 to 40 %) of green bean extract (Table 2). The formulations were developed to meet total polyphenolic content around 7 %, as described in the **Material** section. The total CGA contents varied from 6.9 to 7.5 %, a range close to the average content of two commercial products in this category used as references (7.02 %, with CV% = 17.66).

The formulations showed an average of 3.01 % (± 0.18) of 5-CQA (Table 3). This value is close to the content observed in enriched commercial products (Table 3) and is 2.5 times higher than the average content reported by Marcucci et al. (2013) for conventional instant coffees commercialized in Brazil. It is important to highlight that the Brazilian legislation recommends a minimum comparative difference of 25 % to define a product as “rich” in some component (Brasil 1998), although there are no specific references to antioxidants compounds. Hoelzl et al. (2010) assessed the composition of a

commercial instant coffee with 35 % green beans and reported contents of 8.9 % total CGA and 4.1 % 5-CQA.

The addition of green bean extract of *C. canephora* increased the caffeine content in the *C. arabica* formulations (ADG and AMG) (Tables 2 and 3), but they still had lower caffeine contents (~24 %) ($p < 0.05$) when compared to the *C. canephora* formulations (CDG and CMG). Browened compounds contents were higher ($p < 0.05$) in formulations developed with dark roast extracts, especially CDG. However, these were lower than the average content reported for conventional products obtained with only roasted bean extracts (Marcucci et al. 2013) and the enriched commercial products evaluated, in which a more intense roasting process was most likely used (Table 3).

Table 4 shows the AA of the original extracts and the formulations. An increase in the AA (evaluated by the ABTS and Folin methods) was observed with the addition of the green bean extract. There are no differences in AA ($p > 0.05$) when comparing extracts or formulations with different roasting degrees. Regarding of the species, *C. canephora* products showed a higher AA ($p < 0.05$) for both extracts and developed formulations.

Table 3 Bioactive compounds (on dry basis) of the formulations from *C. canephora* (C) and *C. arabica* (A) coffee dry extracts, with medium (M) and dark (D) roasting degrees, with green *C. canephora* coffee dry extract (G), and commercial products

Formulation ¹	5-CQA (g/100 g) ^{4*}	Caffeine (g/100 g) ^{4*}	Browened compounds (Abs) ^{5*}
ADG	3.03 ^b (1.21)	3.13 ^c (0.77)	0.248 ^b (3.66)
AMG	2.77 ^c (1.68)	3.02 ^d (0.44)	0.224 ^c (1.71)
CDG	3.22 ^a (0.10)	4.09 ^a (0.47)	0.273 ^a (0.98)
CMG	3.05 ^b (0.03)	3.99 ^b (0.15)	0.226 ^a (0.82)
Enriched commercial ²	3.18 (24.21)	3.62 (13.81)	0.339 (13.86)
Conventional commercial ³	1.20 (41.67)	3.51 (12.54)	0.361 (16.90)

¹ ADG (69 % AD and 31 % G), AMG (85 % AM and 15 % G), CDG (60 % CD and 40 % G) and CMG (68 % CM and 32 % G)² Mean values of two products enriched with antioxidants commercialized in European countries; values between parenthesis represent coefficients of variation (CV%)³ Mean values of 27 conventional products (17 brands) commercialized in Brazil (Adapted by Marcucci et al. 2013); values between parenthesis represent CV%⁴ Values between parentheses represent CV% of genuine duplicate with triplicate measurements⁵ Mean values of duplicate*Mean values followed by different low key letters in the same column indicate a significant difference (Tukey, $p \leq 0.05$)

Table 4 Antioxidant activity (dry basis) of *C. canephora* (C) and *C. arabica* (A) coffee spray dried extracts, with medium (M) and dark (D) roasting degrees, before and after the addition of green *C. canephora* coffee (G) (Formulations)

Roasted extracts/Formulation with Green coffee ¹	ABTS (g of Trolox/100 g)*		Folin (g of GAE/100 g)*	
	Extract	Formulation	Extract	Formulation
AD/ADG	25.62 ^{bb} (6.74)	28.13 ^{ba} (3.83)	11.55 ^{bb} (1.88)	12.27 ^{ba} (2.80)
AM/AMG	23.98 ^{bb} (6.06)	27.25 ^{ba} (1.63)	11.84 ^{bb} (2.03)	12.15 ^{ba} (2.69)
CD/CDG	30.94 ^{ab} (2.33)	32.63 ^{aa} (1.44)	13.25 ^{ab} (2.19)	13.78 ^{aa} (1.85)
CM/CMG	32.04 ^{aa} (8.48)	32.37 ^{aa} (3.93)	13.38 ^{ab} (3.29)	13.76 ^{aa} (1.67)
Enriched commercial ²	26.21(1.08)		13.26(2.93)	
Conventional commercial ³	30.41(14.27)		13.98(7.65)	

¹ ADG (69 % AD and 31 % G), AMG (85 % AM and 15 % G), CDG (60 % CD and 40 % G) and CMG (68 % CM and 32 % G)

² Mean values of two products enriched with antioxidants commercialized in European countries; values between parenthesis represent coefficients of variation (CV%)

³ Mean values of 27 conventional products (17 brands) commercialized in Brazil (Marcucci 2012); values between parenthesis represent CV%

* Mean values followed by different letters indicate a significant difference: lower case, on the same column (Tukey, $p \leq 0.05$); upper case, on the same line (t test, $p \leq 0.05$); values between parenthesis represent CV% obtained in genuine duplicate with assessments in triplicate

Similar results were reported by Vignoli et al. (2011) for the AA of instant coffees produced with different roasting degrees and coffee species. The authors reported that the AA was little affected by roasting processes since the reduction in chlorogenic acid content was balanced by the increase in melanoidin content. Thus, the higher content of caffeine in *C. canephora* was more relevant for the AA of the soluble coffees studied. This statement is corroborated by Marcucci (2012) who reported a high AA for commercial instant coffees largely produced with *C. canephora*.

In addition to the in vitro AA (Table 3), we should consider recent studies have reported that CGA has specific and effective health benefits (Thom 2007; Hoelzl et al. 2010; Bakuradze et al. 2011; Cropley et al. 2012). These studies suggest that coffee mixes/blends of roasted beans with high

contents of green beans (and consequently CGA) can contribute to an increase in antioxidant defense and other health benefits related to the prevention and delay of degenerative diseases, body weight control and positive effects on humor and cognition.

Acceptance of the formulations

A sensory analysis was carried out in a lab-scale test by a team of coffee consumers, to verify whether an increase in CGA content would affect the acceptance of the products. All formulations were accepted, obtaining average grades from 6.6 to 7.7, on a 10 scale. The addition of green bean extract, in the concentration range studied (15 to 40 %), had no effect on consumer acceptance of color, aroma, flavor, body and global acceptance ($p > 0.05$) (Table 5). A lower purchase intent was

Table 5 Sensory acceptance and purchase intent of instant coffee brews with different concentrations of green bean extract (G) added to dry extracts of *C. arabica* (A) and *C. canephora* (C) of medium (M) and dark (D) roasting degrees ($n = 42$)

Brew ¹	Attributes*				Global Acceptance*	Purchase intent*
	Color	Aroma	Flavor	Body		
ADG	7.67 ^a	6.82 ^a	7.23 ^a	7.56 ^a	7.28 ^a	6.99 ^a
AMG	7.54 ^a	7.05 ^a	6.64 ^a	7.01 ^a	6.81 ^a	6.05 ^b
CDG	7.70 ^a	7.27 ^a	6.86 ^a	7.28 ^a	7.18 ^a	6.62 ^{ab}
CMG	7.72 ^a	6.90 ^a	7.28 ^a	7.47 ^a	7.25 ^a	6.96 ^{ab}

¹ ADG (69 % AD and 31 % G), AMG (85 % AM and 15 % G), CDG (60 % CD and 40 % G) and CMG (68 % CM and 32 % G)

*Mean values followed by different letters on the same line indicate a significant difference (Tukey, $p \leq 0.05$), in reference to a 10 cm hedonic scale (0-disliked very much, 10-liked very much)

Table 6 Color parameters of the instant coffee brews with different concentrations of green bean dry extract (G) added to dry extracts of *C. arabica* (A) and *C. canephora* (C) of medium (M) and dark (D) roasting degrees

Brew ¹	Lightness*	Hue*
ADG	19.95 ^a (2.10)	16.06 ^{ab} (12.10)
AMG	20.22 ^a (1.43)	16.69 ^a (6.20)
CDG	20.36 ^a (0.83)	12.17 ^c (8.46)
CMG	20.23 ^a (0.87)	14.07 ^{bc} (12.86)

¹ ADG (69 % AD and 31 % G), AMG (85 % AM and 15 % G), CDG (60 % CD and 40 % G) and CMG (68 % CM and 32 % G)

*Means followed by different letters in the same column indicate a significant difference (Tukey, $p \leq 0.05$); values between parenthesis represent variation coefficients (CV%) of genuine duplicate with assessments in triplicate

observed for the formulation AMG compared to the formulation ADG ($p < 0.05$), but it was not different from the others (CDG and CMG). The AMG was prepared with the smallest percentage of green beans (15 %) and an extract of *C. arabica* after medium roasting presented the lowest contents of 5-CQA, caffeine, browned compounds ($p < 0.05$) (Table 3) and had a yellowish intensive brown color ($p < 0.05$) (Table 6). It should also be noted that *C. canephora* have a greater soluble solids extraction yield, which will most likely allow a commercial product of lower cost.

Conclusion

Instant coffee formulations enriched with CGA by mixing green beans extract in the range of 15 to 40 % and an average content of around 7 % CGA were proposed.

Based on the acceptance and purchase intent, dark and medium roasting degrees could be recommended for the roasted beans extracts of *C. canephora* and a dark roasting degree, for *C. arabica*.

Enriched formulations based only in *C. canephora* (green and roasted bean extracts) showed higher antioxidant potential.

Therefore, for the conditions studied, formulations combining extracts of *C. canephora* of medium ($L^* 39$) and dark ($L^* 36$) roasting degrees added of 32 and 40 % of green beans extract, were the best options for commercialization of an instant coffee enriched with CGA.

Acknowledgments The authors gratefully acknowledge the financial support of Conselho Nacional de Pesquisa (CNPq), Fundação Araucária, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundo de Apoio ao Ensino, à Pesquisa e à Extensão (FAEP/UDEL), and Companhia Iguatçu de Café Solúvel® (Cornélio Procópio, Brazil) that kindly produced the instant coffee extracts.

References

- Abic (2010) Associação brasileira da indústria de café. Tendências do consumo de café VII. http://www.abic.com.br/publique/media/EST_PESQTendenciasConsumo2010.pdf. Accessed 11 Nov 2015
- Bakuradze T, Boehm N, Janzowski C, Lang R, Hofmann T, Stockis JP, Albert FW, Stiebitz H, Bytof G, Lantz I, Baum M, Eisenbrand G (2011) Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: results from an intervention study. *Mol Nutr Food Res* 55:793–797. doi:10.1002/mnfr.201100093
- Bastos DHM, Ferreira EL, Siguemoto ES, Sefora MBS (2012) Maillard reaction products in processed foods: pros and cons. In: Valdez B (ed) Food industrial processes methods and equipment, 1st edn. In Tech, Rijeka, pp. 281–300
- Bekedam EK, Loots MJ, Schols HA, Boekel MAJSV, Smit G (2008) Roasting effects on formation mechanisms of coffee brew melanoidins. *J Agric Food Chem* 56:7138–7145. doi:10.1021/jf800999a
- Brasil (1998) Portaria n. 27. Regulamento Técnico referente à Informação Nutricional Complementar (declarações relacionadas ao conteúdo de nutrientes). http://portal.anvisa.gov.br/wps/wcm/connect/9180ca00474581008d31dd3fbc4c6735/PORTARIA_27_1998.pdf?MOD=AJPERES. Accessed 18 Nov 2014
- Budryn G, Nebesny E, Podsedek A, Zyzelewicz D, Materska M, Jankowski S, Janda B (2009) Effect of different extraction methods on the recovery of chlorogenic acids, caffeine and Maillard reaction products in coffee beans. *Eur Food Res Technol* 228:913–922. doi:10.1007/s00217-008-1004-x
- Cropley V, Croft R, Silber B, Neale C, Scholey A, Stough C, Schmitt J (2012) Does coffee enriched with chlorogenic acids improve mood and cognition after acute administration in healthy elderly? A pilot study. *Psychopharmacol* 219:737–749. doi:10.1007/s00213-011-2395-0
- Esquivel P, Jiménez VM (2012) Functional properties of coffee and coffee by products. *Food Res Int* 46:488–495. doi:10.1016/j.foodres.2011.05.028
- Farah A, Donangelo CM (2006) Phenolic compounds in coffee. *Braz J Plant Physiol* 18:23–36. doi:10.1590/S1677-04202006000100003
- Farah A, Monteiro MC, Calado V, Franca AS, Trugo LC (2006) Correlation between cup quality and chemical attributes of Brazilian coffee. *Food Chem* 98:373–380. doi:10.1016/j.foodchem.2005.07.032
- Hatzold T (2012) Introduction. In: Chu YF (ed) Coffee: emerging health effects and disease prevention, 1st edn. Blackwell, London, pp. 1–20
- Heckman MA, Weil J, Mejia EG (2010) Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. *J Food Sci* 75:R77–R87. doi:10.1111/j.1750-3841.2010.01561.x
- Hoelzl C, Knasmüller S, Wagner KH, Elbling L, Huber W, Kager N, Ferk F, Ehrlich V, Nersesyan A, Neubauer O, Desmarchelier A, Marin-Kuan M, Delatour T, Verguet C, Bezencon C, Besson A, Grathwol D, Simic T, Kundi M, Schilter C, Cavin C (2010) Instant coffee with high chlorogenic acid levels protects humans against oxidative damage of macromolecules. *Mol Nutr Food Res* 54:1722–1733. doi:10.1002/mnfr.201000048
- Ico (2015) Trade statistics http://www.ico.org/trade_statistics.asp. Accessed 12 Nov 2015
- Itc (2011) The coffee exporter's guide. 3rd edn. ITC, Geneva. <http://www.intracen.org/The-Coffee-Exporters-Guide—Third-Edition/>. Accessed 17 Mar 2013
- Kotyczka C, Boettler U, Lang R, Stiebitz H, Bytof G, Lantz I, Hofmann T, Marko D, Somoza V (2011) Dark roast coffee is more effective than light roast coffee in reducing body weight, and in restoring red blood cell vitamin E and glutathione concentrations in healthy volunteers. *Mol Nutr Food Res* 55:1582–1586. doi:10.1002/mnfr.201100248
- Liu Y, Kitts DD (2011) Confirmation that the Maillard reaction is the principle contributor to the antioxidant capacity of coffee brews. *Food Res Int* 44:2418–2424. doi:10.1016/j.foodres.2010.12.037
- Ludwig IA, Sanchez L, Caemmerer B, Kroh LW, De Peña MP, Cid C (2012) Extraction of coffee antioxidants: impact of brewing time and method. *Food Res Int* 48:57–64. doi:10.1016/j.foodres.2012.02.023
- Marcucci CT (2012). Atividade antioxidante e teores de compostos bioativos em cafés solúveis comerciais brasileiros <http://www.bibliotecadigital.uel.br/document/?code=vtls000176545>. Accessed 11 Nov 2015
- Marcucci CT, Benassi MT, Almeida MB, Nixdorf SL (2013) Teores de trigonelina, ácido 5-cafeoilquinico, cafeína e melanoidinas em cafés solúveis comerciais brasileiros. *Quim Nova*. 36:544–548. doi:10.1590/S0100-40422013000400011
- Moraes PCBT, Bolini HMA (2010) Different sweeteners in beverages prepared with instant and roasted ground coffee: Ideal and

- equivalent sweetness. *J Sens Stud* 25:215–225. doi:[10.1111/j.1745-459X.2010.00275.x](https://doi.org/10.1111/j.1745-459X.2010.00275.x)
- Morales FJ, Somoza V, Fogliano V (2012) Physiological relevance of dietary melanoidins. *Amino Acids* 42:1097–1109. doi:[10.1007/s00726-010-0774-1](https://doi.org/10.1007/s00726-010-0774-1)
- Nogueira M, Trugo LC (2003) Distribuição de isômeros de ácido clorogênico e teores de cafeína e trigonelina em cafês solúveis brasileiros. *Cienc Tecnol Aliment* 23:296–299. doi:[10.1590/S0101-20612003000200033](https://doi.org/10.1590/S0101-20612003000200033)
- Oliveira AL, Cabral FA, Eberlin MN, Cordello HMAB (2009) Sensory evaluation of black instant coffee beverage with some volatile compounds present in aromatic oil from roasted coffee. *Food Sci Technol* 29:76–80. doi:[10.1590/S0101-20612009000100012](https://doi.org/10.1590/S0101-20612009000100012)
- Perrone D, Donangelo R, Donangelo CM, Farah A (2010) Modeling weight loss and chlorogenic acids content in coffee during roasting. *J Agric Food Chem* 58:12238–12243. doi:[10.1021/jf102110u](https://doi.org/10.1021/jf102110u)
- Perrone D, Farah A, Donangelo CM (2012) Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *J Agric Food Chem* 60:4265–4275. doi:[10.1021/jf205388x](https://doi.org/10.1021/jf205388x)
- Pino-García RD, González-SanJosé ML, Rivero-Pérez MD, Muñiz P (2012) Influence of the degree of roasting on the antioxidant capacity and genoprotective effect of instant coffee: contribution of the melanoidin fraction. *J Agric Food Chem* 60:10530–10539. doi:[10.1021/jf302747v](https://doi.org/10.1021/jf302747v)
- Tfouni SAV, Serrate CS, Carreiro LB, Camargo MCR, Teles CRA, KMOVAB C, RPZ F (2012) Effect of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two *Coffea* cultivars: *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258. *Int J Food Sci Technol* 47:406–415. doi:[10.1111/j.1365-2621.2011.02854.x](https://doi.org/10.1111/j.1365-2621.2011.02854.x)
- Thom E (2007) The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used lon-term in owerweight and obese people. *J Int Med Res* 35:900–908
- Viana ALM, Fonseca MDM, Meireles ELJ, Duarte SMS, Rodrigues MR, Paula FBA (2012) Effects of the consumption of caffeinated and decaffeinated instant coffee beverages on oxidative stress induced by strenuous exercise in rats. *Plant Foods Hum Nut* 67:82–87. doi:[10.1007/s11130-011-0267-8](https://doi.org/10.1007/s11130-011-0267-8)
- Vignoli JA, Bassoli DG, Benassi MT (2011) Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: the influence of processing conditions and raw material. *Food Chem* 124:863–868. doi:[10.1016/j.foodchem.2010.07.008](https://doi.org/10.1016/j.foodchem.2010.07.008)
- Vignoli JA, Bassoli DG, Benassi MT (2012) Antioxidant activity of roasted and instant coffees: standardization and validation of methodologies. *Coffee Sci* 7:68–75
- Vignoli JA, Viegas MC, Bassoli DG, Benassi MT (2014) Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Res Int* 61:279–285. doi:[10.1016/j.foodres.2013.06.006](https://doi.org/10.1016/j.foodres.2013.06.006)
- Villanueva NDM, Petenate AJ, Silva MAAP (2005) Performance of the hybrid hedonic scale as compared to the traditional hedonic, self-adjusting and ranking scales. *Food Qual Prefer* 16:691–703. doi:[10.1016/j.foodqual.2005.03.013](https://doi.org/10.1016/j.foodqual.2005.03.013)
- Wang HY, Qian H, Yao WR (2011) Melanoidins produced by the Maillard reaction: structure and biological activity. *Food Chem* 128:573–584. doi:[10.1016/j.foodchem.2011.03.075](https://doi.org/10.1016/j.foodchem.2011.03.075)