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Antioxidant Activity of Commercial Soluble Coffees

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Abstract: A product of easy preparation and high added value, soluble/instant coffee is obtained by drying the aqueous extract of roasted coffee and presents a high amount of bioactive compounds. The aim of the study was to evaluate the antioxidant activity of 33 Brazilian commercial soluble coffees considering the radical scavenging activity (via the ABTS method) and the reducing capacity (via the Folin–Ciocalteu method). Soluble coffees of several brands and types (regular, gourmet, and decaffeinated), subjected to different drying processes (agglomeration, atomization, and freeze-drying) (n = 85), were evaluated. In general, regular and decaffeinated soluble coffees presented high antioxidant activity. The reducing capacity ranged from 9.9 to 15.4 g of gallic acid per 100 g, while the radical scavenging activity ranged from 20.4 to 37.0 g of Trolox per 100 g. Good repeatability—with coefficients of variation of 2.4% for Folin–Ciocalteu and of 5.2% for ABTS—and high correlations between the values of antioxidant activity obtained by both methods (r = 0.66) were observed. Gourmet coffees presented less antioxidant activity compared to the regular samples. No correlation was verified between drying processes and antioxidant activity.

Keywords: instant coffee; Folin-Ciocalteu; ABTS; reducing capacity; radical scavenging activity

1. Introduction

Coffee's health benefits come largely from its antioxidants. The concern with health is of general interest since coffee is one of the three most consumed beverages worldwide, including water and tea, coffee being one of the main commodities in the global economy. Brazil is the world's largest green coffee producer and exporter, and the second consumer [1].

Coffea arabica (arabica coffee) and *Coffea canephora* (robusta coffee) are the two most relevant commercial coffee species worldwide [1]. Roasted and ground coffee and instant/soluble coffee are commonly obtained from specific blends of coffee species and cultivars; robusta coffee is the most usual raw material for instant coffee production. The chemical composition of coffee products is influenced by genetic factors of the selected beans, and depends on post-harvest conditions, including the industrial process, particularly roasting, extraction, drying, and decaffeination steps [2–8]. Once the coffee beans have been selected, the soluble coffee manufacturing consists of producing a coffee extract by roasting, grinding, and extracting the roasted beans, removing the water from the brew using a spray-dryer (powder or granulated products) or a freeze-dryer process [9].

There are beneficial effects of the moderate consumption of coffee on health, with evidence of a reduction in the risk and severity of several chronic diseases, such as type 2 diabetes, neurodegenerative, cardiovascular, and liver diseases, and some cancers [10–14], and an association with a reduction in

mortality rate [15]. These positive health effects are mainly attributed to the significant antioxidant activity (AA) of coffee [16–18] associated with the presence of various bioactive compounds. The brews of coffee stand out as a significant dietary source of potential antioxidants as phenolic compounds, highlighting the chlorogenic acids, as well as trigonelline, caffeine, and melanoidins [10,19–21].

There is no general method for the AA assessment in food, and several techniques have been applied for coffee. DPPH, ABTS, FRAP, Folin-Ciocalteu (F-C), ORAC, the hydroxyl radical scavenging assay, and the O^{2-} scavenging capacity assay have been used to determine the antioxidant activity of coffee beans/brew [2,8,17,22]. Considering the previous experience of our research group (comparing several methods of AA assay for different coffee matrices) [23,24], evaluation of the reducing capacity via Folin-Ciocalteu analysis and the scavenging ability of the long-life radical cation $ABTS^{\cdot+}$ (the ABTS method) allowed for an accurate evaluation of AA in soluble coffee matrices, and the results presented a satisfactory correlation with other AA assays [24].

There is little research on the antioxidant activity of soluble coffee [23–27] and even less information regarding commercial instant coffees in the literature. Considering the current concern about the health benefits associated with the consumption of coffee products, this study presents data on the antioxidant activity (AA) of Brazilian commercial soluble coffees considering radical scavenging activity (via the ABTS method) and reducing capacity (via the Folin-Ciocalteu method). The results were also correlated with the main bioactive compounds in the samples, which were analyzed in a preliminary study [28].

2. Materials and Methods

2.1. Commercial Coffee Samples

Thirty-three regular or decaffeinated (decaf) commercial soluble coffees of 17 Brazilian brands were analyzed. A panel of 85 samples was evaluated. In most cases (25 products), three batches of each product were studied. Two batches were evaluated for two products, and one batch for six products. In order to have a wide range of commercial products, soluble coffees classified by industry as strong, extra strong, traditional (usually blends of arabica and robusta coffee), or gourmet (description for product obtained only from arabica coffee), and obtained by different dry processes, were evaluated. Gourmet coffees were freeze-dried, and the others were agglomerated or spray-dried. The moisture content was estimated using an infrared equipment (OHAUS MB45, Nänikon, Switzerland) at 105 °C for 7 min. In a previous report of this research group [28], differences in color and in the amount of bioactive compounds was observed for these soluble coffee products, indicating possible variations in the AA of samples (Table 1).

Table 1. Concentrations of bioactive compounds, and lightness (L*) of soluble coffees [28].

Parameter [1]	Regular		Decaffeinated
	Traditional	Gourmet	
L*	19.52–33.0	34.63–43.70	21.65–26.88
Trigonelline [1]	0.47–1.60	1.15–2.15	1.10–1.85
5-CQA [1]	0.38–2.66	1.15–2.37	1.18–2.43
Caffeine [1]	2.32–4.08	2.62–2.85	0.06–0.24
Melanoidins [2]	0.26–0.48	0.25–0.39	0.31–0.37

[1] In g/100 g (db); [2] AU at 420 nm.

2.2. Chemicals and Equipment

The following chemicals were used: Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and ABTS (2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt), from Sigma-Aldrich (Steinheim, Germany), sodium carbonate (Na_2CO_3), from Nuclear (Diadema, Brazil), potassium persulfate, from Across Organics (Morris Plains, NJ, USA), and Folin–Ciocalteu reagent,

from Laborclin (Pinhais, Brazil), gallic acid, from Vetec (Rio de Janeiro, Brazil). Purified Milli-Q® water (Millipore, Molsheim, France) and a spectrophotometer Biochrom Libra S22 (Cambridge, UK) were also used.

2.3. Antioxidant Activity Assessments

The soluble coffees were directly diluted in water (0.3000 g in 50.0 mL) at 25 °C just before analysis. All AA measurements were performed in triplicate (three genuine replicates).

The reducing capacity of the commercial coffee samples was evaluated by the Folin–Ciocalteu (F-C) method as previously described [24]. An aliquot of 100 µL of the sample was added to 7.5 mL of distilled water and 300 µL of 0.9 N F-C reagent. After stirring, 1 mL of a 20% of sodium carbonate solution and 1.1 mL of distilled water were added. The absorbance of solutions was obtained at 760 nm after 1 h at 25 °C. The analytical calibration curve was linear from 0.38 to 1.13 g/L (R^2 = 0.999) using gallic acid as standard. The results were expressed as g of gallic acid per 100 g (dry basis).

The ABTS·$^+$ radical scavenging capacity of the coffee samples was evaluated as previously described [24]. An aqueous solution of ABTS radical cation (ABTS·$^+$) was obtained by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate and by keeping the mixture in the dark at room temperature for 12 h. This solution was diluted with phosphate buffered saline (pH 7.4) to an absorbance (730 nm) of 0.70 ± 0.02. Ten microliters of sample or Trolox (used as standard) was added to 4.0 mL of a diluted ABTS·$^+$ solution, and absorbance measurements were taken after 6 min of reaction. The analytical calibration curve was linear in the range of 0.13 g/L to 2.00 g/L (R^2 = 0.999) using Trolox. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) in g of Trolox per 100 g of sample (dry basis).

2.4. Data Analysis

Data were evaluated by a one-way ANOVA, considering sample as the source of variation and an unequal N HSD Tukey test ($p \leq 0.05$). Principal component analysis (PCA) was also applied in order to correlate AA results with information on composition analyzed in a preliminary study [28]. The bioactive compound (trigonelline, 5-CQA, caffeine, and melanoidins) concentrations were used as active variables, and lightness (L*) and AA as supplementary variables (Statistica 7.1 software, Tulsa, OK, USA, 2006).

3. Results and Discussion

Table 2 presents the antioxidant activity of commercial soluble coffees.

The repeatability (coefficients of variation) between extractions ranged from 0.9% to 8.6% (average of 2.4%) in the F-C method, and from 0.5% to 8.9% (average of 5.2%) in the ABTS method, in accordance with previously reported data (3.7 to 4.9 for F-C, and 8% to 12% for ABTS) [24].

The reducing capacity values ranged from 9.91 to 15.4 g of gallic acid per 100 g, with minor differences verified among the samples (Table 2). In general, low variability among the batches (CVs up to 6%) was observed, except for regular coffee J1 (16%). Greater variation was noticed in the ABTS·$^+$ radical scavenging capacity values (from 20.39 to 37.02 g of Trolox per 100 g), as well as a larger variability between batches (CVs between 0.26% and 32.3%) (Table 2). Similar results were reported for freeze-dried soluble coffee of different species (arabica and robusta) in three roasting degrees (light, medium, and dark), also varying the extraction procedures: F-C values ranged from 12.08 to 18.54 g of gallic acid per 100 g, and ABTS from 18.77 to 36.05 g of Trolox per 100 g [23].

The gourmet products B2, C2, and K1 presented low AA: 22.5, 24.4, and 27.1 g of Trolox per 100 g; and 12.8, 12.5, and 12.8 g of gallic acid per 100 g, respectively (Table 2). The regular soluble coffee J1 was noted as presenting the lowest AA (9.91 g of gallic acid per 100 g and 20.4 g of Trolox per 100 g) (Table 2). The literature describes lower values of AA for *C. arabica* soluble coffees (from 12.1 to 15.1 g of gallic acid per 100 g, and from 18.8 to 36.1 g of Trolox per 100 g) comparing to *C. canephora* soluble coffees [23].

Table 2. Antioxidant activity of commercial soluble coffees: reducing capacity determined via the Folin–Ciocalteu (F-C) method (g of gallic acid per 100 g of sample), and free radical scavenging capacity via the ABTS method (g of Trolox per 100 g).

Sample [1]	Type [2]	Drying Process	F-C [3]	ABTS [3]
A1 *	R	Agglomerated	13.58 ^{c,d,e} (3.93)	28.42 ^{a,c,d,e,f,g,h,i} (18.10)
A2 *	R	Agglomerated	13.83 ^{b,c,d,e} (1.07)	26.20 ^{f,g,h,i,j} (17.61)
A3 *	R	Agglomerated	13.94 ^{b,c,d} (4.01)	28.41 ^{a,c,d,e,f,g,h,i} (2.67)
A4 *	R	Powder	13.93 ^{b,c,d} (1.88)	30.50 ^{a,b,c,d,e,f,g,h} (19.10)
A5 **	D	Agglomerated	14.19 ^{a,b,c,d,e,f} (1.40)	27.20 ^{c,d,e,f,g,h,i,j} (32.33)
B1 *	R	Agglomerated	14.04 ^{b,c} (1.83)	28.45 ^{a,c,d,e,f,g,h,i} (14.98)
B2 *	G	Freeze-dried	12.79 ^{e,f} (5.37)	22.48 ^{i,j} (7.79)
B3 *	R	Powder	14.40 ^{a,b,c} (4.20)	26.73 ^{e,f,g,h,i,j} (12.91)
C1 *	R	Agglomerated	14.41 ^{a,b,c} (2.57)	27.67 ^{d,e,f,g,h,i} (14.29)
C2 *	G	Freeze-dried	12.47 ^{f} (3.52)	24.34 ^{h,i,j} (10.01)
C3 *	R	Powder	14.23 ^{b,c} (5.11)	29.03 ^{a,c,d,e,f,g,h,i} (21.06)
C4 *	R	Agglomerated	13.55 ^{c,d,e,f} (3.41)	31.76 ^{a,b,c,d,e,f,g} (8.41)
C5 *	R	Agglomerated	14.16 ^{b,c} (3.98)	31.69 ^{a,b,c,d,e,f,g} (19.95)
C6 *	D	Agglomerated	14.06 ^{b,c} (1.81)	25.17 ^{g,h,i,j} (9.31)
D1 *	R	Agglomerated	14.77 ^{a,b} (0.93)	32.26 ^{a,b,c,d,e,f} (14.15)
D2 *	R	Powder	15.41 ^{a} (5.31)	32.14 ^{a,b,c,d,e,f} (9.23)
D3 *	D	Agglomerated	14.27 ^{b,c} (1.90)	29.30 ^{a,b,c,d,e,f,g,h,i} (23.39)
E1 *	R	Agglomerated	14.60 ^{a,b,c} (5.21)	29.14 ^{a,b,c,d,e,f,g,h,i} (1.87)
E2 ***	R	Powder	15.22 ^{a,b,c} (5.26)	36.65 ^{a,b,c,d,e,f,g} (10.49)
E3 *	D	Agglomerated	14.41 ^{a,b,c} (2.27)	34.92 ^{a,b,c} (1.01)
F1 *	R	Agglomerated	14.88 ^{a,b} (5.97)	33.51 ^{a,b,c,d,e} (7.42)
G1 *	R	Powder	14.39 ^{a,b,c} (2.81)	34.91 ^{a,b,c} (19.11)
G2 ***	D	Powder	13.64 ^{a,b,c,d,e,f} (0.78)	31.31 ^{a,b,c,d,e,f,g,h,i,j} (6.44)
H1 *	R	Powder	14.24 ^{b,c} (2.67)	33.16 ^{a,b,c,d,e} (0.26)
I1 *	R	Agglomerated	14.64 ^{a,b,c} (3.04)	34.32 ^{a,b,c,d} (12.10)
J1 *	R	Agglomerated	9.91 ^{g} (15.62)	20.39 ^{j} (31.88)
K1 *	G	Freeze-dried	12.83 ^{d,e,f} (4.92)	27.05 ^{e,f,g,h,i,j} (6.69)
L1 ***	R	Agglomerated	14.55 ^{a,b,c,d,e} (4.53)	32.95 ^{a,b,c,d,e,f,g,h,i} (2.98)
M1 **	R	Agglomerated	14.78 ^{a,b,c} (0.29)	36.69 ^{a,b} (3.05)
N1 ***	R	Agglomerated	13.87 ^{a,b,c,d,e,f} (4.37)	37.02 ^{a,b,c,d,e,f,g} (3.88)
O1 ***	D	Agglomerated	14.40 ^{a,b,c,d,e} (2.65)	25.72 ^{a,b,c,d,e,f,g,h,i,j} (3.68)
P1 ***	R	Agglomerated	13.72 ^{a,b,c,d,e,f} (2.49)	29.11 ^{a,b,c,d,e,f,g,h,i,j} (6.83)
Q1 *	R	Agglomerated	14.32 ^{a,b,c} (4.90)	36.06 ^{b} (2.60)
Average			14.01	30.14

[1] A–Q (brands); numbers refer to the product. [2] Type: gourmet (G), regular (R), and decaf (D). [3] Different letters in the column indicate significant differences (Tukey, $p \leq 0.05$). * Average of nine values (three batches and three genuine replicates). CV (%) among batches, in brackets. ** Average of six values (two batches and three genuine replicates). CV (%) among batches, in brackets. *** Average of three values (one batch and three genuine replicates). Analytical CV (%) in brackets.

The samples of decaffeinated soluble coffee A5, C6, D3, E3, G2, and O1 showed similarity in the reducing capacity (average of 14.2 g per 100 g of gallic acid), but a wide range in the ABTS·$^{+}$ radical scavenging results (from 25.2 to 34.9 g of Trolox per 100 g). Despite the relevant contribution of caffeine for AA [23,29], even the products with a reduced amount of this alkaloid showed significant AA.

Considering the drying process, no correlation was found in the AA values comparing the powder, agglomerated, and freeze-dried samples (Table 2).

The first two components in the PCA analysis explained 90% of the data variability. The first component (PC1) was positively correlated to levels of trigonelline and 5-CQA, and negatively to the amount of melanoidins, suggesting that PC1 was related to the roasting process (when the compounds were probably formed or decomposed). On the other hand, the second component (PC2) had higher

correlation (positively) with the level of caffeine, indicating that the effect was associated with the raw material (coffee species) or with the decaffeination conditions (Figure 1).

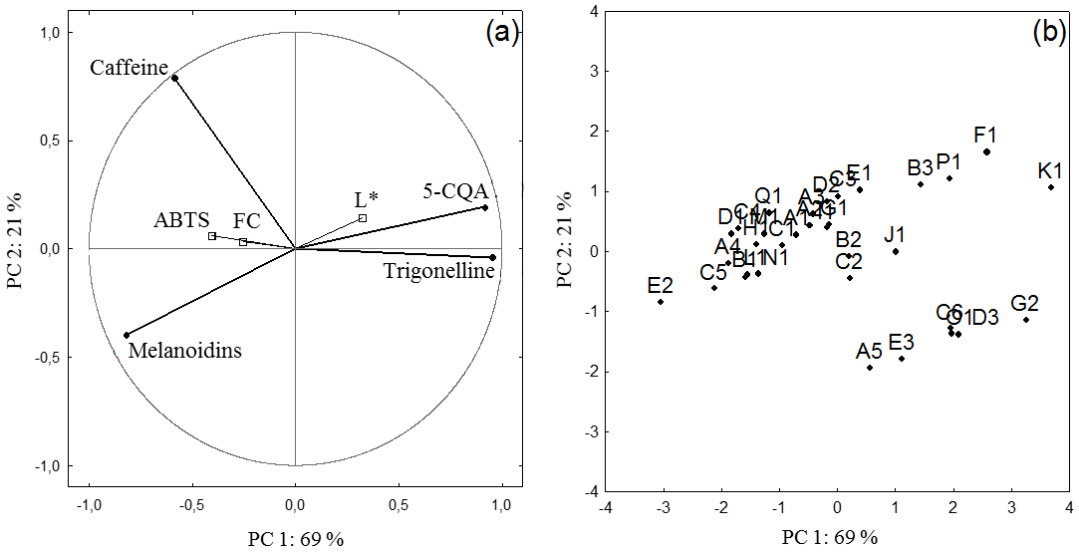

Figure 1. Principal component analysis: (**a**) projection of variables: active (●) and supplementary (□); (**b**) score plots of the samples.

A high correlation (r = 0.66) was observed among F-C and ABTS results (Figure 1), which agrees with the literature [23,25].

AA values were positively associated with caffeine and melanoidin content and negatively associated with L*, and 5-CQA and trigonelline content. Thus, soluble coffee with a higher caffeine concentration and/or a darker roasting also has greater AA (Figure 1, Table 2).

In general, decaf and gourmet coffees were on the right side of the plots (Figure 1b), indicating a relatively low AA. These samples have less caffeine and present a lighter roasting degree (a higher L* and a lower melanoidin concentration, Table 1). A similar behavior (low AA) was reported for a light-roasted arabica instant coffee [23]. A higher AA for dark-roasted robusta can be attributed to the presence of a greater amount of melanoidins, compared to arabica, as reported in [30].

Sample J1 was highlighted because of its low AA (Table 2), and it was plotted close to the gourmet and decaf samples (Figure 1), probably due to its low level of caffeine [28]. The remaining samples (B3, E1, F1, and P1) in this region (Figure 1) have high amounts of trigonelline and/or 5-CQA [28], indicating a low degree of roasting in these samples.

The results show that the AA of soluble coffees is a result of the balance of the bioactive compounds. Coffee brews have been highlighted by their antioxidant efficacy compared to other beverages (wine, tea, beer, and juices) and by their relevant contribution to dietary antioxidant intake [22]. From this perspective, considering the high AA values observed for commercial soluble coffee products regardless of the raw material and the drying process used to obtain the product, soluble coffee brews stand out due to their convenience of consumption and potential health benefits.

4. Conclusions

The antioxidant activity (AA) of commercial soluble coffees was assessed. Soluble coffee has high AA. No correlation was observed between the dry process (agglomeration, atomization, and freeze-drying) and the AA. Gourmet coffees, compared to regular ones, have a lower AA value. Despite the lower caffeine content of decaffeinated soluble coffees, high AA was still observed.

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Conflicts of Interest: The authors declare no conflict of interest.

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