JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.5b04406 • Publication Date (Web): 12 Oct 2015

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Matching *in vitro* bioaccessibility of polyphenols and antioxidant capacity of soluble coffee by Boosted Regression Trees.

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ABSTRACT

The aim of this study was to evaluate changes in polyphenol profile and antioxidant capacity of five soluble coffees throughout a simulated gastro-intestinal digestion, including absorption through a dialysis membrane. Our results demonstrate that both polyphenol content and antioxidant capacity were characteristic for each type of studied coffee, showing a drop after dialysis. Twenty-seven compounds were identified in coffee by HPLC-MS, while only 14 of them were found after dialysis. Green+roasted coffee blend and chicory+coffee blend showed the highest and lowest content of polyphenols and antioxidant capacity before *in vitro* digestion and after dialysis, respectively. Canonical correlation analysis showed significant correlation between the antioxidant capacity and the polyphenol profile before digestion and after dialysis. Furthermore, boosted regression trees analysis (BRT) showed that only 4 polyphenol compounds (5-*p*-coumaroylquinic acid, quinic acid, coumaroyl tryptophan conjugated and 5-O-caffeoylquinic acid) appear to be the most relevant to explain the antioxidant capacity after dialysis, being these compounds the most bio-accessible after dialysis. To our knowledge, this is the first report matching the antioxidant capacity of foods with the polyphenol profile by BRT, which opens an interesting method of analysis for future reports on the antioxidant capacity of foods.

KEYWORDS

polyphenol profile, *in vitro* gastro-intestinal digestion, bioaccessibility, chemometrics, boosted regression trees.

INTRODUCTION

Coffee is one of the most popular beverages consumed in the world, which has been consumed for its pleasant flavor and aroma as well as its stimulatory properties due to its caffeine content. In recent years, there has been an increasing interest in the possible positive implications of coffee consumption for human health[1].

Coffee has been proposed as an important source of antioxidants in the human diet. Epidemiological studies show that moderate coffee consumption may help to prevent chronic diseases such as Type 2 Diabetes Mellitus[2], Parkinson's[3] and liver[4] diseases. Species (*Coffea arabica*, Arábica or *Coffea canephora*, Robusta), cultivars[5], origin[6], process and degree of roasting[7] and different extraction processes[8] can influence the chemical composition and biological activity of coffee infusions. Among the compounds having antioxidant activity in coffee, we can find phenolic compounds[1], melanoidins and other products of Maillard reaction[9].

Mullen *et al.*[10] found a statistically significant correlation between the content of caffeoylquinic acids in coffee berries and its ability to scavenge free radicals. On the other hand, Somoza *et al.*[11] demonstrated that chlorogenic acid was the compound with the highest influence on the antioxidant activity, evaluated *in vitro* via the inhibition of the peroxidation of linoleic acid. However, some phenolic compounds can be lost in the roasting process[12], forming new compounds derived from Maillard reaction, with antioxidant properties[8]. Nicoli *et al.*[13] found that grains with intermediate roasting conditions had high antioxidant capacity. Daglia *et al.*[14] showed that melanoidins resulting from Maillard reaction would be responsible for the antioxidant capacity found in high molecular weight fractions in roasted coffee.

These studies demonstrate that *in vitro* antioxidant capacity of coffee would be influenced by its composition and by the roasting process, but little is known about the

bioavailability that these antioxidants have in the human digestive system, as a previous step to their absorption and distribution to exert effects at the cellular level. Thus, only the compounds that are able to tolerate the conditions found throughout the gastro-intestinal tract, crossing the intestinal membrane, will be able to produce physiological changes in the human body. So far, models simulating *in vitro* digestion have been developed to investigate the digestibility and bioaccessibility of polyphenols[15]. These models simulate the movement of food through the digestive tract, exposing the food to the conditions encountered in the gastric and intestinal canals. This involves the addition of digestive enzymes (pepsin, pancreatin) and bile salts, with pH and temperatures similar to the conditions found *in vivo*. Then dialysis may be performed, where the substances that could potentially pass through intestinal wall, can be measured by one or more chemical-biochemical assays. This methodology has been proposed as an estimation of bioaccessibility of food components in different food matrices[15–17]. However, to our knowledge, it has been used in only one research work to evaluate the bioaccessibility of polyphenols in coffee, but without using the dialysis step[18].

The main goal of this study was to assess the bioaccessibility of polyphenols arising from soluble coffees by *in vitro* digestion, including a final dialysis to simulate intestinal absorption, looking to understand how antioxidants present in coffee and other foods may exert their effect in the human body.

MATERIAL AND METHODS

Coffee samples. Soluble coffee samples (regular, decaffeinated, Arabic, green + roasted blend and chicory + coffee blend) were commercially obtained in supermarkets from

100 Tres Cantos, Madrid, Spain. Three random samples from each variety, corresponding to
101 different commercial brands, were obtained (n= 15).

102 **Chemicals and Materials.** Ultra-pure water (<5 μg L^{-1} TOC) was obtained from a
103 purification system Arium 61316-RO plus Arium 611 UV (Sartorius, Germany).
104 Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were
105 provided by J. T. Baker (Edo. de México, México) and Fluka (Steinheim, Germany),
106 respectively. Commercial standards of ferulic acid and caffeic acid were obtained from
107 Extrasynthèse (Genay, France), 5-O-caffeoylquinic acid and quinic acid were purchased
108 from Sigma-Aldrich (Steinheim, Germany) and *p*-coumaric acid was provided by Fluka
109 (Dorset, U.K.). Filters (0.45 μm, HVLP04700) were obtained from Millipore (São
110 Paulo, Brazil). ABTS (2,2'-azino-bis-(3-thylbenzothiazolne-6-sulfonic acid)
111 diammonium salt), TPTZ (2,4,6-tripyridyl-S-triazine), Trolox (6-hydroxy-2,5,7,8-
112 tetramethyl-chroman-2-carboxylic acid), 1,10-phenanthroline, pepsin (P-7000, from
113 porcine stomach mucosa), pancreatin (P-1750, from porcine pancreas) and bile extract
114 (B-6831, porcine) were provided by Sigma-Aldrich (Switzerland). Folin-Ciocalteu
115 reagent was obtained from Panreac (Barcelona, Spain). Dialysis bag was Medicell
116 7000/2, width 34 mm, 7000 MW cut off. All other reagents were of analytical grade.

117 **Sample preparation.** Coffee brews were prepared according to manufacturer's
118 instructions: 3 g of regular (RC), decaffeinated (DC), Arabic (AC), and green + roasted
119 (GRC) coffee samples, and 6 g of chicory + coffee blend (CC) samples, were dissolved
120 with 200 mL of hot ultra-pure water (70-75°C). Coffee brews obtained were then
121 filtered, fractionated and stored at - 20°C until analysis.

122 ***In vitro* digestion.** The *in vitro* digestion procedure was performed according to the
123 method described by Ramírez-Moreno *et al.*[17] with minor modifications. Briefly, 25 mL
124 of sample were adjusted to pH 2.0 with 6 M HCl and successively incubated in a

shaking water bath for 2 h at 37°C with 120 μL pepsin solution (40 mg mL^{-1} in 0.1 M HCl) to simulate gastric digestion. After incubation 1.5 mL pancreatin-bile solution (5 mg pancreatin plus 25 mg porcine bile mL^{-1} in 0.1 M $NaHCO_3$), was added to simulate intestinal digestion. The digestion products were placed in a dialysis bag and dialyzed in 250 mL of sodium bicarbonate solution (pH 7.5) for 3 h. Dialyzed samples were then filtered, fractionated and stored at - 20°C until analysis.

Total polyphenol content (TP), polyphenolic profile (PP) and antioxidant capacity of different coffee samples were studied before and after undergoing *in vitro* gastro-intestinal digestion and subsequent dialysis.

Total Polyphenols Assay. TP of coffee was measured by the Folin-Ciocalteu (FC) method, in accordance to the technique reported by Vignoli *et al.*[8] and slightly modified according to Parry *et al.*[19]. Coffee brews (100 μL), 10-fold diluted with ultrapure water, and 500 μL of dialyzed sample were added to 3.3 and 2.9 mL of ultrapure water, respectively. Folin-Ciocalteu reagent (200 μL) was added to each sample and stirred (vortex). After 1 min, 600 μL of sodium carbonate solution (20% v/v) were added. The samples were stirred and incubated for 2 h at room temperature in the dark. The absorbance was then read at 750 nm. Gallic acid was used as standard; results are expressed as g gallic acid equivalents (GAE) per 100 g fresh weight. All samples were analyzed in triplicate.

LC-MS. Polyphenols were analyzed in coffee and dialyzed samples by HPLC-MS/MS method, using an Agilent Technologies 1200 Series UPLC equipped with a gradient pump (Agilent G1312B SL Binary), solvent degasser (Agilent G1379 B) and autosampler (Agilent G1367 D SL+WP). The chromatographic separation was achieved

150 on a LUNA (Phenomenex, Torrance, CA, USA) C18 column (5 µm, 250 mm × 4.60 mm
151 i.d.) at 35°C using a column heater module (Agilent G1316 B). The mobile phase
152 consisted of 0.5% formic acid (v/v, solvent A) and 0.5% acid formic in methanol (v/v,
153 solvent B). The solvent gradient started with 20% B and changed to 50% B along 3 min,
154 kept 5 min, followed by a second ramp to 70% B along 7 min, maintained 5 min, a third
155 ramp to 80% B along 1 min, remaining at this last condition for 9 min before the next
156 run. The flow rate was set at 0.4 mL min^{-1} and the injection volume was 40 µL. The
157 HPLC system was connected to a photodiode array detector (Agilent G1315 C Starlight
158 DAD) and subsequently to a QTOF mass spectrometer (micrOTOF-QII Series, Bruker),
159 equipped with electro spray ionization (ESI) source. UV-Vis spectra were registered
160 from 200 to 600 nm. Mass spectra were recorded in negative ion mode between *m/z* 50
161 and 1000. The working conditions for the ionization source were as follows: capillary
162 voltage, 4500 V; nebulizer gas pressure, 4.0 bar; drying gas flow, 8.0 L min^{-1} and 180°C
163 for the drying gas. Nitrogen and argon were used as nebulizer/dryer and collision gases,
164 respectively. The MS detector was programmed to perform MS and alternative MS/MS
165 from the three most abundant ions obtained in MS. MS/MS was performed using
166 collision energy of 13.0 eV. Exact mass was verified by introducing sodium formiate at
167 the beginning and at the end of each chromatographic run through the multipath valve
168 of the MicroQTOF II, located between the DAD and the ESI source. Data acquisition
169 and processing were performed using Compass Version 3.1 software and DataAnalysis
170 Version 4.0 software, respectively (Bruker Daltonics, MA-USA).
171 Polyphenols present in samples were characterized according to their retention times,
172 exact mass, UV/Vis spectra, MS and MS/MS spectra in addition to comparison with
173 authentic standards when available. When authentic standards were not available, a
174 tentative identification was performed using UV-VIS, exact MS and MS/MS,

considering reports from tentative compounds in the literature. Quantification of polyphenols was based on external calibration curves from available phenolic standards, using the mass peak areas obtained from the extracted ion chromatograms, at concentrations between 1 and 100 mg L^{-1}. When the corresponding standards were not available, the quantification was performed using an external standard with a similar structure of the tentative compound in question. Samples and standards solutions were filtered (0.45 μm) and injected in HPLC-MS/MS system. All samples were analyzed in duplicate.

***In vitro* antioxidant analysis.**

In vitro antioxidant activity was measured using trolox equivalent antioxidant capacity (TEAC) assay, ferric reducing ability of plasma (FRAP) assay and 1, 10-phenanthroline (PHEN) assay.

TEAC assay. TEAC assay was performed using adaptations of the methodology described by Re *et al.*[20] The ABTS radical was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in 10 mL of water), keeping the mixture in the dark at room temperature for 16 h before use. The aqueous $ABTS^{\bullet+}$ solution was diluted with PBS (pH = 7.4) to an absorbance of 0.80 ± 0.02 at 734 nm. Hundred fifty microliters of coffee brews (diluted 1:37.5 with ultra pure water) or dialyzed samples were added to 4 mL of the TEAC solution plus 100 μL of methanol, incubated for 30 min in the dark, and measured at 734 nm. The standard curve used was linear between 0 and 0.02 mM trolox. Results are expressed in mmol trolox equivalents (TE) per 100 g fresh weight. All samples were analyzed in triplicate.

FRAP assay. FRAP assay was performed according to Benzie and Strain[21] with some modifications. The fresh working solution was prepared by mixing 25 mL acetate buffer pH 3.6 (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), 2.5 mL of a 10 mM TPTZ solution

200 in 40 mM HCl, and 2.5 mL of a 20 mM $FeCl_3.6H_2O$ solution. Three hundred microliters
201 of coffee brews (diluted 1:60 with ultra pure water) or dialyzed samples were added to 4
202 mL of the FRAP solution plus 200 μL of methanol, incubated for 30 min in the dark and
203 measured at 593 nm. A linear dynamic range between 0 and 0.02 mM trolox was
204 observed. Results are expressed in mmol TE per 100 g fresh weight. All samples were
205 analyzed in triplicate.

206 **PHEN assay.** PHEN reagent and assay was performed according to Berker *et al.*[22]. Five
207 hundred microliters of coffee brews (diluted 1:10 with ultra pure water) or 1 mL of
208 dialyzed samples were added to 1 mL of PHEN reagent adding 4.5 mL of ethanol (96%)
209 to coffee samples and 4 mL to dialyzed samples, and diluting to 25 mL with ultra pure
210 water. Samples were incubated for 30 min at 50°C and measured at 510 nm. The
211 method was linear between 0 and 0.04 mM trolox. Results are expressed in mmol TE
212 per 100 g fresh weight. All samples were analyzed in triplicate.

213

214 **Statistical Analysis.**

215 Results were analyzed using the statistical package Statistica 8.0 from StatSoft Inc.
216 (2007) and the Infostat software package[23].

217 *Analysis of Variance*. ANOVA was performed using mixed models[24]; in the case of
218 significance ($P < 0.05$), a DGC[25] comparison test was performed to reveal paired
219 differences between means.

220 *Canonical Correlation Analysis* (CCA). CCA was used to study the correlation between
221 antioxidant capacity (FRAP, TEAC and PHEN) and polyphenol profile of coffee
222 samples, before and after *in vitro* digestion and the subsequent dialysis.

223 *Boosted Regression Trees Analysis* (BRT). Development of predictive models to get
224 evidences on the relationship between the polyphenol profile and the antioxidant

activity was carried out using a statistical technique called boosted regression trees[26]. BRT is a powerful modeling method that combines regression trees and boosting algorithm. This method can handle predictor variables with different types and distributional characteristics. Variable selection with this model is robust to co-linearity amongst predictors, outliers and lack of data and, therefore, does not require prior variable selection or data reduction. Models were constructed with the "gbm" library[27] using the R software (version 3.0.3). Model over fitting was avoided by cross validation (CV). In CV, the function selects a fraction of the data provided, according to the parameters set, to build a model. The latter is validated with the fraction of remaining data, allowing the evaluation and confirmation of the predictive quality of the model built[26]. Three parameters were adjusted to maximize model performance: the proportion of data randomly selected at each iteration of the CV procedure (the "bag fraction"), the contribution of each tree to the growing model (the "learning rate") and the number of nodes (interactions) in each tree ("tree complexity"). Model performance was evaluated using the CV correlation (the correlation between predicted and raw data withheld from the model). CV correlations close to 1 indicates good model predictions. The importance of predictor variables in BRT models was evaluated using the function previously described, which calculates the contribution to the model fit attributable to each predictor, averaged across all trees[26].

RESULTS AND DISCUSSION

Polyphenol analysis.

Table 1 shows mean values and standard deviations of total polyphenol content (TP) of 5 types of instant coffees. Green + roasted coffee blend (GRC), Arabic coffee (AC) and

regular coffee (RC) samples showed similar TP values (average 15.8 g GAE/100g), higher than decaffeinated coffee (DC) samples (14.8 g GAE/100g), and much higher than chicory + coffee blend (CC) samples, which showed the lowest TP values (8.99 g GAE/100g), attributable to the substitution of coffee by chicory (60% of chicory and 38% of coffee). These results are in agreement with other authors. Vignoli *et al.*[8] showed TP values in instant coffee samples between 14.6 and 15.1 g GAE/100g. Del Pino-García *et al.*[28] showed TP values between 13.2 and 22.2 g GAE/100g in instant regular coffee samples. On the other hand, Alves *et al.*[29] suggested that decaffeination process has influence on TP levels. They detected greater amounts of TP in regular coffee than in decaffeinated ones. Additionally, chicory is a plant used in Europe and USA as a coffee substitute because it does not have caffeine. Normally it is used in chicory + coffee blends to reduce dietary caffeine intake[30], however this plant has lower amount of bioactive constituents (flavonoids, caffeic acid derivatives and other polyphenols) than coffee[31,32].

With respect to individual polyphenol constituents, 27 compounds were identified in coffee samples, which can be divided into 5 groups: 1 quinic acid, 20 free chlorogenic acids, 3 chlorogenic lactones and 3 hydroxycinnamoyl-amino acid conjugates. **Table 2** shows the parameters used for its identification.

Compounds identified in this study agree with those informed by others authors. Mullen *et al.* (2011)[10] found 16 compounds derived to chlorogenic acids in coffee fruit sample, whereas Rodrigues *et al.* (2013)[33] found 26 phenolic compounds in coffee brews.

The most abundant compounds in coffee samples were QA, 5-CQA and 5-FQA (**Table 3**). Our current results showed that the content of QA ranged between 576 and 1700 mg/100g, 5-CQA ranged between 353 and 1549 mg/100g, while 5-FQA ranged between 429 and 1327 mg/100g. All the compounds presented significant differences among

studied coffee samples and its values are in agreement with those reported in the literature[33,34]. GRC, RC and AC showed the highest values in practically all the compounds while CC displayed the lowest ones.

In vitro digestion model gives an indication as to the availability of coffee antioxidants in a biological system, because this model simulates *in vivo* digestion. It is assumed that the amount of dialyzable polyphenol compounds could be bio-accessible in the intestine.

After *in vitro* digestion, a decrease of TP was observed during dialysis through the semi permeable cellulose membrane (**Table 1**). The dialyzed samples showed TP values 5-fold lower than the coffee samples. GRC dialysates showed the highest TP values (3.82 gGAE/100g), followed by AC (3.63 gGAE/100g), and while CC dialysates showed the lowest TP values (1.78 g GAE/100 g), following the same trend that was observed in non-digested samples.

Individual results for each of the polyphenol compounds investigated and their recovery percents (R%) after dialysis are presented in **Table 3**. We observed substantial losses in some of the polyphenol compounds after dialysis in relation to their initial content in coffee samples. Thus, only 14 out of 27 compounds identified in the coffee samples were quantified in dialyzed samples. Nine of them were quantified in all dialysates. Conversely, CA and 5-diMCiQA were only quantified in AC and GRC dialysates; while 3,4-diCQA and 4,5-diCQA were quantified in GRC, and 3-diMCiQA was only quantified in AC.

QA (431.6 - 810 mg/100g) was the most abundant compound found in dialyzed samples, with R% ranging from 47 to 75%, followed by CoT (22 – 40 mg/100g; R% 19-42%). It is worthy to remark that 3-diMCiQA presented the highest R% (84.6) in

dialysates of Arabic coffee (AC). Conversely, the greatest drop was observed with 5-FQA, showing an overall R% of 2.4, followed by 5-CQA (R% = 2.62).

Several studies have shown that the bioaccessibility of different families of polyphenols in different matrices was lower than 40% when a dialysis bag was used. Gil-Izquierdo *et al.*[15] observed bioaccessibilities between 11% and 36% in flavanones from orange juice. Vallejo *et al.*[35] obtained a total flavonoid bioaccessibility of 6% in broccoli. Akillioglu and Karakaya[16] reported that the bioaccessibility of TP verified by Folin´s method ranged 19% to 39%, in bean varieties. However, to our knowledge, there is only one report on the *in vitro* bioaccessibility of polyphenols in instant coffee, using an *in vitro* digestion model and ultrafiltration step[18]. Our current work uses dialysis bags, which is more close to physiological conditions, avoiding the use of high pressure (ultrafiltration), so our results could be better extrapolated with results from *in vivo* models, in which the bioavailability of polyphenols is studied[36–38].

Then, this methodology constitutes a feasible approach to determine the potential availability of polyphenols. The polyphenols released from the food matrix during the digestive process (named bio-accessible polyphenols) are potentially bio-available to absorption through the gut barrier, and these may be useful for the interpretation of the effects of food polyphenols on health.

Antioxidant capacity.

Table 4 shows antioxidant capacity of different coffee samples before *in vitro* digestion and after dialysis, using TEAC, FRAP and PHEN *in vitro* assays.

A similar trend was observed from three assays: RC and AC presented the highest antioxidant capacity, while the lowest values were observed in CC samples. Significant correlation was observed between different antioxidant tests. This was confirmed by a

study of the correlations between them: FRAP/ PHEN (r = 0.911; P < 0.01), FRAP/ TEAC (r = 0.745; P < 0.01), and PHEN /TEAC (r = 0.682; P < 0.01). These findings are consistent with those reported in other studies involving TEAC and FRAP assays. Vignoli *et al.*[8] reported similar values of FRAP (76.99-139.99 mmol ET/100g) and TEAC (74.99-144.03 mmol ET/100g) in Arabic and Robust coffee with different levels of roasted, while Rufián-Henares *et al.*[39] showed similar ABTS and FRAP values (127 and 48 mmol ET/100g, respectively) in soluble coffee. On the other hand, Del Pino-García *et al.*[28] found elevated correlation between ABTS and FRAP values in instant coffee samples (r = 0.9311).

Differences between caffeinated and decaffeinated samples were also reported by others authors. Pellegrini *et al.*[40] showed differences between caffeinated and decaffeinated espresso coffee samples when they studied antioxidant capacity by TEAC and FRAP assays, while Niseteo *et al*.[41] found the same differences in instant coffee brews.

Chicory + coffee blend (CC) samples showed an antioxidant capacity nearly half less than in coffee samples (RC, AR and GRC) (P < 0.01). These results are consistent with those found by Rautenbach *et al.*[42] They found that blended coffee-chicory had 2.5-fold less antioxidant capacity than pure coffee (ORAC assay).[42] To our knowledge, there are not reports showing values of antioxidant capacity using TEAC, FRAP and PHEN assays in chicory + coffee blended.

After dialysis a similar trend was also observed. The antioxidant capacity of GRC dialysates was the highest followed by RC and AC, while CC dialysates remained with the lower antioxidant capacity. The correlation between antioxidant capacity assays was also confirmed with dialysates: FRAP/ PHEN (r = 0.537; P < 0.01), FRAP/ TEAC (r = 0.824; P < 0.01), and PHEN/TEAC (r = 0.663; P < 0.01). FRAP, TEAC and PHEN

showed a drop in all dialyzed samples, regarding the antioxidant capacity in coffee samples. Recovery percents range from 12.7 to 35.7 %, being higher in the mixture with green coffee (GRC), which means a higher antioxidant capacity in the material potentially absorbed from this type of coffee, in agreement with the higher R% obtained in this sample for TP and the content of caffeic acid (**Tables 1** and **3**).

Several studies showed that the antioxidant capacity decrease after dialysis in different matrices. Bouayed *et al.*[43] reported a decrease in the total antioxidant capacity of dialyzable compounds, compared to those observed in fresh apples (57% and 46% for FRAP and ABTS test, respectively). Rodríguez-Roque *et al.*[44] showed that the antioxidant capacity after dialysis decreased by 73% in soymilk. Akillioglu and Karakaya[16] observed a reduction ranging 1.6 to 2.1-fold in the DPPH test of pinto beans after *in vitro* gastrointestinal digestion. However, to our knowledge, there are not reports that show values of antioxidant capacity after dialysis step in coffee samples.

Matching between *in vitro* antioxidant capacity and polyphenol profile.

The antioxidant potential of coffee is attributed to the presence of polyphenols and melanoidins, but their contribution to the antioxidant capacity varies with the intensity of the roasting process, showing discrepancies in the results obtained by different authors[7,8,45]. The contribution of high molecular weight melanoidins to the antioxidant capacity of coffee brews was estimated in the range of 26-38 %.[39] However Delgado-Andrade *et al.*[45] found that the antioxidant capacity of melanoidins depends on the presence of low molecular weight compounds (polyphenols), linked non-covalently to the melanoidin skeleton. Other authors[46] found that only a small proportion of melanoidins could by absorbed through the intestinal wall.

374 To evaluate if the antioxidant capacity found in this work is dependent on the
375 polyphenol content (before *in vitro* digestion and after dialysis), we applied simple
376 correlation analysis. We observed statistically significant correlation between TP and
377 the antioxidant capacity determined by FRAP, TEAC and PHEN assays before *in vitro*
378 digestion (r = 0.753; 0.871 and 0.693, respectively) and after dialysis (r = 0.771; 0.851
379 and 0.717, respectively). These results are in agreement with others authors, who
380 evaluated the antioxidant capacity in coffee samples.[41,47] So, the antioxidant capacity
381 found in this work, evaluating instant coffee samples (before *in vitro* digestion and after
382 dialysis) could be attributed to their polyphenol profile. To confirm this hypothesis, we
383 evaluated the correlation between antioxidant capacity (FRAP, TEAC and PHEN) and
384 the entire polyphenol profile of coffee and dialyzed samples using canonical correlation
385 analysis (CCA). Before *in vitro* digestion CCA showed significant correlation between
386 antioxidant capacity and polyphenol profile of coffee samples (r^2 = 0.99; P < 0.001).
387 Additionally, CCA showed significant correlation between FRAP, TEAC and PHEN
388 with the polyphenol profile after dialysis (r^2 = 0.93; P < 0.001, using 9 polyphenols
389 quantified in all dialyzed samples). So far, CCA evidenced that the antioxidant capacity
390 of coffee samples can be reasonably linked to the polyphenol profiles in both pre-
391 digested and dialyzed samples.
392 We were also interested in evaluating the contribution of individual polyphenols to the
393 antioxidant capacity, looking for evidences on different contribution of individual
394 compounds to the antioxidant capacity. To solve this question, we applied Boosted
395 Regression Trees (BRT). Although BRT methods is applied in various fields including
396 ecology[48], epidemiology[49], agriculture[50] and highway safety[51]; to our knowledge, this
397 methodology has never been applied in food science. BRT identifies important predictor
398 variables, enabling complex functions to be modeled (antioxidant capacity), without

399 making assumptions about the type of data. BRT have some advantages over other
400 multivariate statistical techniques, such as multiple regression, because it is robust to
401 missing data, variable outliers, variable co-linearity, focusing on predictive accuracy
402 rather than *P*-values to indicate the significance of model coefficients[48].
403 The adjusted parameters (bag fraction, learning rate and tree complexity), performance
404 (CV correlation and number of trees) and relative influence of polyphenols for each
405 model (to TEAC, FRAP and PHEN) in coffee and dialyzed samples are presented in
406 **Table 5**. Before *in vitro* digestion and after dialysis BRT models showed good
407 performance (CV correlation) in TEAC as well as FRAP and PHEN analysis (**Table 5**).
408 BRT models showed that 90 % of the variability found in TEAC, FRAP and PHEN
409 analyses before *in vitro* digestion could be explained using 14 or 16 (relative influence
410 in bold, **Table 5**) out of 27 quantified compounds. On the other hand, after dialysis only
411 5 (relative influence in bold, **Table 5**) out of 9 quantified polyphenols are necessary to
412 explain 95 % of the variability observed in TEAC, FRAP and PHEN assays.
413 4-FQA was the most influential variable with a relative contribution of 15.9% to TEAC
414 BRT model in coffee samples, while CQL was the most significant variable for FRAP
415 and PHEN BRT models (27.3 % and 30.1 %, respectively). Additionally, other
416 predictor variables (CoT, FT, 3-FQL, 4-FQA, QA, 5-CQA, FA and 3-CQA) were also
417 important in TEAC, FRAP and PHEN models. Although these techniques explain
418 different mechanisms of action of the polyphenols, these compounds appear to be the
419 most relevant to explain the total antioxidant capacity before *in vitro* digestion.
420 On the other hand, QA was the most significant predictor for TEAC BRT model (51.8
421 %) in dialyzed samples, whereas 5-CoQA showed the highest contribution to FRAP and
422 PHEN BRT models (45.3 % and 37.6 %, respectively). Moreover, 5-CoQA, QA, CoT
423 and 5-CQA appeared in all models; so these variables appear to be the most relevant to

424 explain the antioxidant capacity after dialysis. In addition, QA, CoT and 5-CoQA were
425 the most bio-accessible compounds after dialysis (**Table 3**), with R% of 56, 27 and 27,
426 respectively, and QA, CoT and 5-CQA were also important in TEAC, FRAP and PHEN
427 BRT models before *in vitro* digestion, while 5-CoQA was important in both FRAP and
428 PHEN BRT models.
429 The marginal effect of these polyphenols (QA, CoT, 5-CoQA and 5-CQA) on
430 antioxidant capacity could be demonstrated using partial dependence plots. The plots
431 show the association of each compound with the antioxidant capacity (fitted function)
432 while all other compounds have an average effect in the model[26]. **Figure 1** provides the
433 partial dependence plots of QA, CoT, 5-CQA and 5-CoQA on TEAC (**A**), FRAP (**B**)
434 and PHEN (**C**) BRT models of dialyzed samples. Plots demonstrate a complex pattern
435 of variation between polyphenols (QA, CoT, 5-CQA and 5-CoQA) and antioxidant
436 capacity (TEAC, FRAP and PHEN). In general, it can be seen that antioxidant capacity
437 (TEAC, FRAP and PHEN) is high when the content of QA is greater than 0.7 g/100g,
438 values of 5-CoQA is equal to 0.0195 g/100g, CoT is approximately 0.03 g/100g and 5-
439 CQA is greater than 0.0235 g/100g (**Figure 1 A, B** and **C**). This condition is satisfied
440 mostly by GRC samples (**Table 3**), which would explain the greater antioxidant
441 capacity found in these samples after dialysis.
442
443 These results demonstrate that the antioxidant capacity of coffee samples before and
444 after *in vitro* digestion and subsequent dialysis can be explained by the polyphenol
445 profile. It is worthy to remark that four compounds (5-CoQA, QA, CoT and 5-CQA)
446 appear to be the most relevant to explain the antioxidant capacity found after dialysis,
447 regardless of the different method used to determine the action of polyphenols (TEAC,
448 FRAP or PHEN). Additionally, three out of these four compounds (5-CoQA, QA and

CoT) were the most bio-accessible after dialysis. Assuming that dialysis simulates the absorption of compound in the small intestine, we could affirm that these compounds could be available for absorption *in vivo*, influencing cellular activities that moderate the risk of several diseases and could be potentially beneficial for human health.

Our study has also shown that BRT method is a useful analytical tool to study the contribution of polyphenols to the antioxidant capacity. To our knowledge, this is the first report using this approach in food science.

ABBREVIATIONS

Regular (RC), decaffeinated (DC), Arabic (AC), green + roasted coffee blend (GRC) chicory + coffee blend (CC). Total polyphenols content (TP). Quinic acid (QA), 3-O-caffeoylquinic acid (3-CQA); 4-O-caffeoylquinic acid (4-CQA); 1-O-feruloylquinic acid (1-FQA); *cis*-5-O-caffeoylquinic acid (*cis*5-CQA); 5-O-caffeoylquinic acid (5-CQA); 3-O-feruloylquinic acid (3-FQA); 4-O-feruloylquinic acid (4-FQA); caffeoylquinic lactone (CQL); 5-*p*-coumaroylquinic acid (5-CoQA); 5_O-feruloylquinic acid (5-FQA); caffeic acid (CA); 3-O-dimetoxicinamoylquinic acid (3-diMCiQA); 3,4-O-dicaffeoylquinic acid (3,4-diCQA); 3-O-feruloylquinic lactone (3-FQL); 4-O-dimetoxicinamoylquinic acid (4-diMCiQA); 5-O-dimetoxicinamoylquinic acid (5-diMCiQA); coumaroyl,caffeoylquinic acid (CoCQA); ferulic acid (FA); 3-O-caffeoyl,4-O-feruloylquinic acid (3,4-CFQA); 4,5-O-caffeoylquinic acid (4,5-diCQA); diferuloylquinic acid (diFQA); 4-O-caffeoyl,5-O-feruloylquinic acid (4,5-CFQA); caffeoyl tryptophan conjugated (CT); dicaffeoylquinic lactone (diCQL); coumaroyl tryptophan conjugated (CoT) and feruloyl tryptophan conjugated (FT). Boosted Regression Trees (BRT).

ACKNOWLEDGEMENTS

N. S. Podio would like to acknowledge to the Complutense University of Madrid (UCM), to the Universidad Nacional de Córdoba (UNC) for granting a scholarship for a stay at the UCM, and to CONICET for a fellowship. Authors acknowledge grants from ANPCyT (PICT 2008-0554), SECyT-UNC (Res. 162/12 and 203/14) and ALIMNOVA research group (UCM-GR35/10A).

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FIGURE CAPTIONS

Figure 1. Functions fitted for boosted regression trees (BRT) model, showing the influence of QA, 5-CQA, CoT and 5-CoQA (g/100g) and their contribution (between square brackets) to fit the TEAC (**A**), FRAP (**B**) and PHEN (**C**) BRT models after dialysis.

TABLES

Table 1. Total Polyphenol content expressed in g gallic acid equivalents (GAE)/100g sample, before *in vitro* digestion and after dialysis. Values are reported as means ± SD.

	Coffee	Dialysates	R%
Regular Coffee (RC)	15.6 ± 0.4 **a**	3.33 ± 0.20 **C**	21.3
Arabic Coffee (AC)	15.8 ± 0.6 **a**	3.63 ± 0.09 **B**	23.0
Green + Roasted Coffee blend (GRC)	16.0 ± 0.5 **a**	3.82 ± 0.09 **A**	23.9
Chicory + Coffee blend (CC)	9.0 ± 0.2 **c**	1.78 ± 0.07 **D**	19.8
Decaffeinated Coffee (DC)	14.8 ± 0.4 **b**	3.26 ± 0.22 **C**	22.0

R%: percent recovery of dialysate with respect to coffee. Different letters (a > b > c > d > e to coffee, or A > B > C > D to dialysates) in the same column indicate significant differences ($P < 0.05$).

Table 2. Polyphenols identified in coffee samples.

N°	RT (min)	Abreviature	Compound	Molecular formula	$[M-H]^-$ (m/z) experimental	$[M-H]^-$ (m/z) calculated	error (ppm)	MS^2 (m/z)	λmáx (nm)
1	7,2	**QA***	**Quinic acid**	C7H11O6	191.0565	191.0561	-1.9	-	223
2	11,2	**3-CQA**	**3-O-caffeoylquinic acid**	C16H17O9	353.0883	353.0878	-1.5	179, 191, 173	227, 288sh, 321
3	11,6	**4-CQA**	**4-O-caffeoylquinic acid**	C16H17O9	353.0887	353.0878	-2.5	191, 179	228, 292sh, 326
4	12,2	**1-FQA**	**1-O-feruloylquinic acid**	C17H19O9	367.1039	367.1035	-1.2	193, 173	-
5	12,2	**cis5-CQA**	**putative cis-5-O-caffeoylquinic acid**	C16H17O9	353.0893	353.0878	-4.1	191, 179, 173	227, 289sh, 325
6	12,6	**5-CQA***	**5-O-caffeoylquinic acid**	C16H17O9	353.0887	353.0878	-2.6	191, 179, 173	230, 301sh, 326
7	12,7	**3-FQA**	**3-O-feruloylquinic acid**	C17H19O9	367.1045	367.1035	-2.9	193	-
8	13,8	**4-FQA**	**4-O-feruloylquinic acid**	C17H19O9	367.1043	367.1035	-2.3	173, 191, 193, 335	229, 325
9	13,8	**CQL**	**Caffeoylquinic lactone**	C16H15O8	335.0798	335.0772	-7.7	161, 173, 179	229, 325
10	13,9	**5-CoQA**	**5-*p*-coumaroylquinic acid**	C16H17O8	337.0905	337.0929	7.1	191, 173	-
11	14,1	**5-FQA**	**5-O-feruloylquinic acid**	C17H19O9	367.1064	367.1035	-7.9	191, 173	228, 326
12	14,2	**CA***	**Caffeic acid**	C9H7O4	179.0353	179.0350	-1.8	-	225, 300sh, 325
13	14,6	**3-diMCiQA**	**3-O-dimetoxicinamoylquinic acid**	C18H21O9	381.1187	381.1191	1.2	207, 337	-
14	15,2	**3,4-diCQA**	**3,4-O-dicaffeoylquinic acid**	C25H23O12	515.1207	515.1195	-2.2	353, 179, 173	226, 292sh, 323
15	16,0	**3-FQL**	**3-O-feruloylquinic lactone**	C17H17O8	349.0933	349.0929	-1.3	175, 193	227, 287sh, 317
16	17,1	**4-diMCiQA**	**4-O-dimetoxicinamoylquinic acid**	C18H21O9	381.1192	381.1191	-0.1	173, 207, 337	226, 285, 320
17	17,5	**5-diMCiQA**	**5-O-dimetoxicinamoylquinic acid**	C18H21O9	381.1192	381.1191	-0.3	173, 207, 193	226, 288, 317
18	17,8	**CoCQA**	**Coumaroyl,caffeoylquinic acid**	C25H23O11	499.1280	499.1246	-6.9	191, 173, 353, 311	226, 287sh, 322
19	17,8	**FA***	**Ferulic acid**	C10H9O4	193.0501	193.0506	2.8	-	226, 291sh, 322
20	18,1	**3,4-CFQA**	**3-O-caffeoyl, 4-O-feruloylquinic acid**	C26H25O12	529.1360	529.1351	-1.6	367, 173, 335, 179, 193	226, 300sh, 327
21	18,2	**4,5-diCQA**	**4,5-O-caffeoylquinic acid**	C25H23O12	515.1201	515.1195	-1.1	353, 173, 179	226, 294sh, 324
22	20,3	**diFQA**	**diferuloylquinic acid**	C27H27O12	543.1523	543.1508	-2.9	349, 193, 367, 173	-
23	20,7	**4,5-CFQA**	**4-O-caffeoyl, 5-O-feruloylquinic acid**	C26H25O12	529.1364	529.1351	-2.4	353, 367, 173, 179, 191	227, 287sh, 320
24	21,3	**CT**	**Caffeoyl tryptophan conjugated**	C20H17N2O5	365.1144	365.1143	-0.4	229, 186, 203, 161	226, 286, 317
25	22,2	**diCQL**	**putative dicaffeoylquinic lactone**	C25H21O11	497.1128	497.1089	-7.7	335	226, 283sh, 319
26	22,9	**CoT**	**Coumaroyl tryptophan conjugated**	C20H17N2O4	349.1184	349.1194	2.7	229, 186, 203	226, 282, 316
27	23,1	**FT**	**Feruloyl tryptophan conjugated**	C21H19N2O5	379.1286	379.1299	3.7	203, 229	227, 281, 318

RT, retention time; $[M-H]^-$ (m/z), negatively charged molecular ion; M^2 (m/z), daughter ions produced from $[M-H]^-$ fragmentation; λ_{max}, maximum absorbance for compound identification by UV-VIS spectra; sh, shoulder. Compounds marked with * were identified and quantified using authentic standards. Other compounds are tentatively proposed based on RT, accurate MS and MS/MS in according to data from the literature.

Table 3. Polyphenolic compounds identified in coffee samples before *in vitro* digestion and after dialysis. Values (mg standard/100g sample) are reported as means ± SD.

	Regular coffee			Arabic coffee			Green and roasted coffee blend			Chicory and coffee blend			Decaffeinated coffee		
	Coffee	Dialysate	R%	Coffee	Dialysate	R%	Coffee	Dialysate	R%	Coffee	Dialysate	R%	Coffee	Dialysate	R%
QA[1]	1700 ± 230**a**	810 ± 80**A**	48	1440 ± 230**b**	780 ± 100**A**	54	1314 ± 24**b**	708 ± 18**B**	54	576 ± 4**c**	431.6 ± 1.7**C**	75	1700 ± 300**a**	800 ± 80**A**	47
3-CQA	92 ± 12**b**	< LOD	0	104 ± 14**a**	< LOQ	0	98.7 ± 0.5**a**	< LOQ	0	26.6 ± 0.3**d**	< LOD	0	77 ± 3**c**	< LOD	0
4-CQA	550 ± 120**b**	16.9 ± 1.1**C**	3,1	720 ± 140**a**	18.1 ± 0.6**B**	2,5	787 ± 16**a**	20.60 ± 0.11**A**	2,6	136.0 ± 1.9**d**	16.7 ± 0.4**C**	12	460 ± 40**c**	16.7 ± 0.9**C**	3,6
1-FQA	28 ± 4**a**	< LOD	0	25 ± 4**b**	< LOD	0	21.0 ± 0.3**c**	< LOD	0	13.55 ± 0.09**d**	< LOD	0	25 ± 3**b**	< LOD	0
cis5-CQA	134 ± 10**b**	8 ± 8**C**	6,0	134 ± 13**b**	15.43 ± 0.23**B**	12	145 ± 4**a**	15.63 ± 0.25**B**	11	38.4 ± 1.1**d**	16.06 ± 0.21**A**	42	111 ± 9**c**	10 ± 8**C**	9
5-CQA	1050 ± 200**c**	19.7 ± 1.9**C**	1,9	1340 ± 240**b**	23.1 ± 1.7**B**	1,7	1549 ± 19**a**	32.40 ± 0.25**A**	2,1	352.9 ± 0.3**e**	18.7 ± 0.3**C**	5,3	940 ± 100**d**	19.4 ± 2.1**C**	2,1
3-FQA	280 ± 80**a**	16.0 ± 0.5**A**	5,7	250 ± 90**b**	16.0 ± 0.5**A**	6,4	195.2 ± 1.7**b**	16.09 ± 0.17**A**	8,2	104.1 ± 1.9**c**	16.1 ± 0.5**A**	16	220 ± 60**b**	15.67 ± 0.21**B**	7,1
4-FQA	110 ± 30**a**	15.85 ± 0.22**A**	14	90 ± 40**b**	15.8 ± 0.4**A**	18	49.5 ± 0.5**c**	15.61 ± 0.24**B**	32	46.5 ± 1.2**d**	16.1 ± 0.5**A**	35	69 ± 16**b**	15.90 ± 0.12**A**	23
CQL	430 ± 210**b**	< LOQ	0	740 ± 140**a**	< LOQ	0	674 ± 16**a**	< LOQ	0	233.8 ± 0.5**c**	< LOQ	0	220 ± 90**c**	< LOD	0
5-CoQA	90 ± 22**c**	18.9 ± 1.2**B**	21	120 ± 23**b**	20.7 ± 0.6**A**	17	136.7 ± 1.9**a**	20.6 ± 0.5**A**	15	33.9 ± 0.8**e**	16.7 ± 0.3**C**	49	51 ± 12**d**	17.2 ± 1.0**C**	34
5-FQA	1150 ± 250**b**	19.0 ± 1.1**B**	1,6	1000 ± 300**b**	19.9 ± 2.5**B**	2	1327 ± 6**a**	20.89 ± 0.20**A**	1,6	429 ± 7**d**	18.23 ± 0.18**C**	4,2	660 ± 90**c**	17.3 ± 0.4**D**	2,6
CA[2]	19 ± 7**a**	< LOQ	0	17.2 ± 1.8**a**	6.4 ± 0.4**B**	37	14.77 ± 0.12**b**	6.97 ± 0.12**A**	47	4.131 ± 0.014**d**	< LOD	0	13.7 ± 1.8**c**	< LOQ	0
3-diMCiQA	24 ± 15**a**	< LOQ	0	18.2 ± 1.2**a**	15.39 ± 0.23	85	15.54 ± 0.08**a**	< LOQ	0	< LOD **b**	< LOD	0	19 ± 7**a**	< LOQ	0
3,4-diCQA	120 ± 40**c**	< LOD	0	168 ± 23**b**	< LOD	0	466 ± 6**a**	16.04 ± 0.25	3,4	28.4392 ± 0.0006**e**	< LOD	0	89 ± 15**d**	< LOD	0
3-FQL	79 ± 23**b**	< LOD	0	108 ± 19**a**	< LOD	0	65.5 ± 0.3**c**	< LOD	0	65.90 ± 0.13**c**	< LOD	0	70 ± 30**c**	< LOD	0
4-diMCiQA	24 ± 16**a**	< LOQ	0	16.9 ± 1.0**a**	< LOQ	0	14.69 ± 0.12**b**	< LOQ	0	7.15 ± 0.05**c**	< LOD	0	20 ± 8**a**	< LOQ	0
5-diMCiQA	40 ± 40**a**	< LOQ	0	26 ± 4**a**	17.0 ± 0.6**A**	65	19.53 ± 0.11**b**	15.8 ± 0.4**B**	81	8.49 ± 0.4**c**	< LOD	0	29 ± 16**a**	< LOQ	0
CoCQA	19.7 ± 2.3**c**	< LOD	0	22.4 ± 1.8**b**	< LOD	0	28.2 ± 0.3**a**	< LOD	0	8.25 ± 0.10**e**	< LOD	0	17.8 ± 2.0**d**	< LOD	0
FA[3]	90 ± 30**b**	< LOD	0	130 ± 60**a**	< LOD	0	146.0 ± 1.4**a**	< LOD	0	68 ± 4**c**	< LOD	0	90 ± 40**b**	< LOD	0
3,4-CFQA	67 ± 22**b**	< LOD	0	58 ± 25**b**	< LOD	0	111.3 ± 1.5**a**	< LOD	0	18.7 ± 0.3**d**	< LOD	0	39.8 ± 1.3**c**	< LOD	0
4,5-diCQA	140 ± 50**c**	< LOD	0	180 ± 60**b**	< LOD	0	675.4 ± 0.6**a**	15.68 ± 0.25	2,3	30.441 ± 0.013**e**	< LOD	0	89 ± 18**d**	< LOD	0
diFQA	32 ± 16**a**	< LOD	0	29 ± 6**a**	< LOD	0	23.4 ± 0.6**b**	< LOD	0	10.7 ± 0.3**c**	< LOD	0	21 ± 5**b**	< LOD	0
4,5-CFQA	43 ± 14**b**	< LOD	0	39 ± 12**b**	< LOD	0	105.6 ± 0.3**a**	< LOD	0	12.55 ± 0.11**d**	< LOD	0	27.6 ± 0.9**c**	< LOD	0
CT[2]	160 ± 40**b**	< LOD	0	120 ± 70**b**	< LOD	0	287 ± 4**a**	< LOQ	0	52.2 ± 0.4**d**	< LOD	0	59 ± 11**c**	< LOD	0
diCQL	19 ± 3**b**	< LOD	0	23 ± 4**a**	< LOD	0	21.25 ± 0.03**a**	< LOD	0	8.16 ± 0.11**d**	< LOD	0	16.2 ± 1.6**c**	< LOD	0

CoT[4]	200 ± 60**a**	40 ± 8**A**	20	160 ± 110**b**	30 ± 30**B**	19	123 ± 8**b**	30.9 ± 1.4**B**	25	113.6 ± 0.6**b**	33.4 ± 0.3**B**	29	52 ± 16**c**	22 ± 9**C**	42
FT[3]	53 ± 8**b**	< LOD	0	40 ± 30**c**	< LOD	0	61.0 ± 0.6**a**	< LOD	0	25.47 ± 0.12**c**	< LOD	0	25 ± 4**c**	< LOD	0

R%: percent recovery of dialysate with respect to coffee. Different letters (a > b > c > d > e to coffee, or A > B > C > D to dialysate) in the same row indicate significant differences ($P < 0.05$). All compound were quantified using 5-O-caffeoylquinic acid as reference compound, except: [1]quantified with quinic acid; [2]quantified with caffeic acid; [3]quantified with ferulic acid; [4]quantified with *p*-coumaric acid. < LOD, below limit of detection. < LOQ, below limit of quantification. IDL = 4 mg/100g to 3-CQA, CQL, 3-diMCiQA, 3,4-diCQA, 4-diMCiQA, 5-diMCiQA, CoCQA, 3,4-CFQA, 4,5-diCQA, 4,5-CFQA and diCQL. IDL = 0.6 mg/100g to 1-FQA, 3-FQL, FA, diFQA and FT. IDL = 2 mg/100g to CA and CT. IQL = 15 mg/100g to 3-CQA, CQL, 3-diMCiQA, 4-diMCiQA and 5-diMCiQA. . IQL = 6 mg/100g to CA and CT.

Table 4. Antioxidant capacity (mmoles eq TROLOX/100g sample), for coffee samples before *in vitro* digestion and after dialysis.

	FRAP			TEAC			PHEN		
	coffee	**dialysates**	**R (%)**	**coffee**	**dialysates**	**R (%)**	**coffee**	**dialysates**	**R (%)**
RC	71 ± 7 **a**	14.7 ± 0.9 **B**	20.7	133 ± 11 **a**	27 ± 4 **B**	20.3	113 ± 18 **a**	22 ± 9 **B**	19.5
AC	76 ± 12 **a**	13.8 ± 1.3 **B**	18.2	132 ± 18 **a**	23.4 ± 0.7 **C**	17.7	124 ± 15 **a**	28 ± 4 **B**	22.6
GRC	65.5 ± 1.7 **b**	17.20 ± 0.08 **A**	26.2	121 ± 4 **b**	33.3 ± 1.1 **A**	27.5	112 ± 6 **a**	40 ± 3 **A**	35.7
CC	42.1 ± 1.0 **d**	6.88 ± 0.19 **D**	16.3	72.3 ± 2.4 **c**	11.5 ± 0.9 **E**	15.9	66 ± 3 **c**	8.4 ± 0.4 **C**	12.7
DC	61 ± 8 **c**	10.5 ± 1.3 **C**	17.2	128 ± 9 **a**	22.7 ± 0.7 **D**	17.7	102 ± 14 **b**	24 ± 10 **B**	23.5

R%: percent recovery of dialysate with respect to coffee. Different letters (a > b > c to coffee, or A > B > C to dialysates) in the same column indicate significant differences ($P < 0.05$).

Table 5. Adjusted parameters, performance, and relative influence (%) of Boosted Regression Trees models for antioxidant capacity (FRAP, TEAC and PHEN) of coffee samples before *in vitro* digestion and after dialysis.

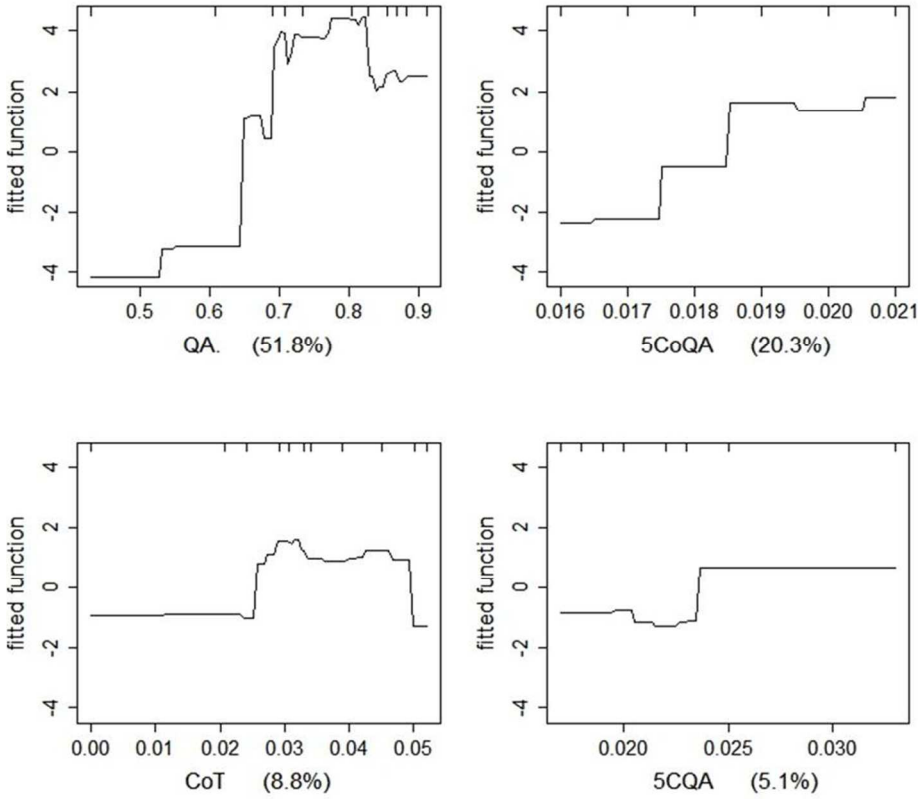
	Coffee			Dialysates		
	TEAC	**FRAP**	**PHEN**	**TEAC**	**FRAP**	**PHEN**
Adjusted parameters						
bag fraction	**0.55**	**0.55**	**0.50**	**0.55**	**0.60**	**0.60**
learning rate	**0.0006**	**0.0008**	**0.0006**	**0.001**	**0.0006**	**0.0005**
tree complexity	**2**	**2**	**2**	**2**	**2**	**2**
Model performance						
CV correlation	**0.969**	**0.979**	**0.926**	**0.985**	**0.970**	**0.982**
optimal number of trees	**48,250**	**39,500**	**29,750**	**38,250**	**32,250**	**60,500**
Relative influence of polyphenols (%)						
QA	**9.5**	**3.4**	**5.6**	**51.8**	**20.9**	**10.0**
3-CQA	**2.0**	**2.9**	**2.0**	-	-	-
4-CQA	**4.5**	1.0	**2.0**	**8.9**	0.3	0.7
1-FQA	**7.3**	**3.8**	**2.4**	-	-	-
cis5-CQA	0.8	0.4	0.8	2.7	1.5	**12.8**
5-CQA	**9.2**	**3.0**	**12.3**	**5.1**	**2.3**	**14.3**
3-FQA	**13**	2.5	**1.9**	1.1	2.1	2.2
4-FQA	**15.9**	**2.7**	**2.3**	0.3	1.9	0.6
CQL	5.6	**27.3**	**30.1**	-	-	-
5-CoQA	1.1	**5.3**	**2.8**	**20.3**	**45.3**	**37.6**
5-FQA	1.0	0.8	1.1	1.0	**15.5**	1.4
CA	0.8	0.5	0.7	-	-	-
3-diMCiQA	0.4	0.4	0.6	-	-	-
3,4-diCQA	1.4	**3.1**	**5.1**	-	-	-
3-FQL	**1.8**	**5.2**	**5.3**	-	-	-
4-diMCiQA	0.3	0.3	0.3	-	-	-
5-diMCiQA	0.2	0.3	0.6	-	-	-
CoCQA	0.1	0.1	1.1	-	-	-
FA	2.5	**2.9**	**2.1**	-	-	-
3,4-CFQA	1.6	**3.0**	**5.3**	-	-	-
4,5-diCQA	0.4	0.2	1.1	-	-	-
diFQA	**4.4**	0.8	0.7	-	-	-
4,5-CFQA	0.4	0.5	1.6	-	-	-
CT	**11.6**	1.7	1.5	-	-	-
diCQL	0.2	**3.8**	**4.7**	-	-	-
CoT	**2.3**	**13.4**	**3.6**	**8.8**	**10.3**	**20.3**

FT	**1.7**	**10.7**	**2.1**	**-**	**-**	**-**
Cumulative influence (%)	**100**	**100**	**100**	**100**	**100**	**100**

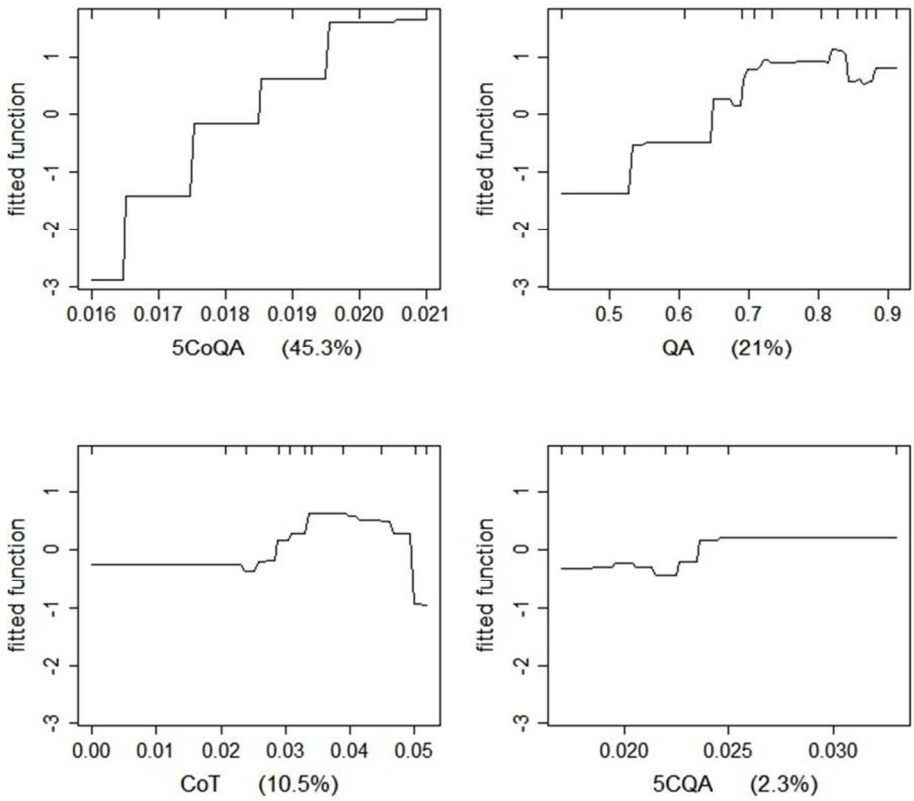
FIGURE GRAPHICS

Figure 1.

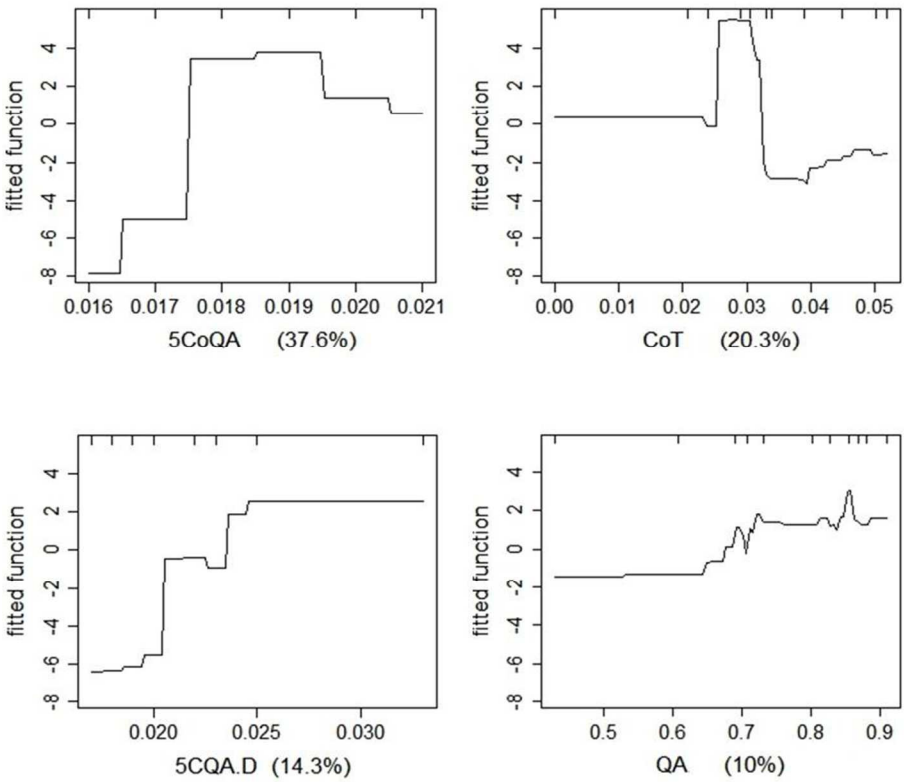
A



B


fitted function
-3
-2
-1
0
1
0.016 0.017 0.018 0.019 0.020 0.021
5CoQA (45.3%)
0.5 0.6 0.7 0.8 0.9
QA (21%)
0.00 0.01 0.02 0.03 0.04 0.05
CoT (10.5%)
0.020 0.025 0.030
5CQA (2.3%)

C


fitted function
-8 -6 -4 -2 0 2 4
0.016 0.017 0.018 0.019 0.020 0.021
5CoQA (37.6%)
fitted function
-8 -6 -4 -2 0 2 4
0.00 0.01 0.02 0.03 0.04 0.05
CoT (20.3%)
fitted function
-8 -6 -4 -2 0 2 4
0.020 0.025 0.030
5CQA.D (14.3%)
fitted function
-8 -6 -4 -2 0 2 4
0.5 0.6 0.7 0.8 0.9
QA (10%)

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