

## Article

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

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

3  
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26 **ABSTRACT**

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

44

45 **KEYWORDS**

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

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51 **INTRODUCTION**

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

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

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

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

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

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

96

## 97 MATERIAL AND METHODS

98 **Coffee samples.** Soluble coffee samples (regular, decaffeinated, Arabic, green + roasted  
99 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  $\mu\text{g L}^{-1}$  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  $\mu\text{m}$ , 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.*<sup>17</sup> with minor modifications. Briefly, 25 mL  
124 of sample were adjusted to pH 2.0 with 6 M HCl and successively incubated in a

125 shaking water bath for 2 h at 37°C with 120  $\mu\text{L}$  pepsin solution (40 mg  $\text{mL}^{-1}$  in 0.1 M  
126 HCl) to simulate gastric digestion. After incubation 1.5 mL pancreatin-bile solution (5  
127 mg pancreatin plus 25 mg porcine bile  $\text{mL}^{-1}$  in 0.1 M  $\text{NaHCO}_3$ ), was added to simulate  
128 intestinal digestion. The digestion products were placed in a dialysis bag and dialyzed in  
129 250 mL of sodium bicarbonate solution (pH 7.5) for 3 h. Dialyzed samples were then  
130 filtered, fractionated and stored at - 20°C until analysis.

131

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

135

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

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

150 on a LUNA (Phenomenex, Torrance, CA, USA) C18 column (5  $\mu\text{m}$ , 250 mm  $\times$  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  $\text{min}^{-1}$  and the injection volume was 40  $\mu\text{L}$ . 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  $\text{min}^{-1}$  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,



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

### 183 ***In vitro* antioxidant analysis.**

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

187 **TEAC assay.** TEAC assay was performed using adaptations of the methodology  
188 described by Re *et al.*<sup>20</sup> The ABTS radical was produced by reacting 7 mM ABTS and  
189 2.45 mM potassium persulfate (final concentration in 10 mL of water), keeping the  
190 mixture in the dark at room temperature for 16 h before use. The aqueous ABTS<sup>•+</sup>  
191 solution was diluted with PBS (pH = 7.4) to an absorbance of 0.80 ± 0.02 at 734 nm.  
192 Hundred fifty microliters of coffee brews (diluted 1:37.5 with ultra pure water) or  
193 dialyzed samples were added to 4 mL of the TEAC solution plus 100 µL of methanol,  
194 incubated for 30 min in the dark, and measured at 734 nm. The standard curve used was  
195 linear between 0 and 0.02 mM trolox. Results are expressed in mmol trolox equivalents  
196 (TE) per 100 g fresh weight. All samples were analyzed in triplicate.

197 **FRAP assay.** FRAP assay was performed according to Benzie and Strain<sup>21</sup> with some  
198 modifications. The fresh working solution was prepared by mixing 25 mL acetate buffer  
199 pH 3.6 (3.1 g C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>·3H<sub>2</sub>O and 16 mL C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>), 2.5 mL of a 10 mM TPTZ solution

200 in 40 mM HCl, and 2.5 mL of a 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O 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.*<sup>22</sup>. 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<sup>23</sup>.

217 *Analysis of Variance.* ANOVA was performed using mixed models<sup>24</sup>; in the case of  
218 significance ( $P < 0.05$ ), a DGC<sup>25</sup> 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

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

244

## 245 **RESULTS AND DISCUSSION**

246

### 247 **Polyphenol analysis.**

248 **Table 1** shows mean values and standard deviations of total polyphenol content (TP) of  
249 5 types of instant coffees. Green + roasted coffee blend (GRC), Arabic coffee (AC) and

250 regular coffee (RC) samples showed similar TP values (average 15.8 g GAE/100g),  
251 higher than decaffeinated coffee (DC) samples (14.8 g GAE/100g), and much higher  
252 than chicory + coffee blend (CC) samples, which showed the lowest TP values (8.99 g  
253 GAE/100g), attributable to the substitution of coffee by chicory (60% of chicory and  
254 38% of coffee). These results are in agreement with other authors. Vignoli *et al.*<sup>8</sup>  
255 showed TP values in instant coffee samples between 14.6 and 15.1 g GAE/100g. Del  
256 Pino-García *et al.*<sup>28</sup> showed TP values between 13.2 and 22.2 g GAE/100g in instant  
257 regular coffee samples. On the other hand, Alves *et al.*<sup>29</sup> suggested that decaffeination  
258 process has influence on TP levels. They detected greater amounts of TP in regular  
259 coffee than in decaffeinated ones. Additionally, chicory is a plant used in Europe and  
260 USA as a coffee substitute because it does not have caffeine. Normally it is used in  
261 chicory + coffee blends to reduce dietary caffeine intake<sup>30</sup>, however this plant has lower  
262 amount of bioactive constituents (flavonoids, caffeic acid derivatives and other  
263 polyphenols) than coffee<sup>31,32</sup>.

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

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

271 The most abundant compounds in coffee samples were QA, 5-CQA and 5-FQA (**Table**  
272 **3**). Our current results showed that the content of QA ranged between 576 and 1700  
273 mg/100g, 5-CQA ranged between 353 and 1549 mg/100g, while 5-FQA ranged between  
274 429 and 1327 mg/100g. All the compounds presented significant differences among

275 studied coffee samples and its values are in agreement with those reported in the  
276 literature<sup>33,34</sup>. GRC, RC and AC showed the highest values in practically all the  
277 compounds while CC displayed the lowest ones.

278

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

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

289 Individual results for each of the polyphenol compounds investigated and their recovery  
290 percents (R%) after dialysis are presented in **Table 3**. We observed substantial losses in  
291 some of the polyphenol compounds after dialysis in relation to their initial content in  
292 coffee samples. Thus, only 14 out of 27 compounds identified in the coffee samples  
293 were quantified in dialyzed samples. Nine of them were quantified in all dialysates.  
294 Conversely, CA and 5-diMCiQA were only quantified in AC and GRC dialysates; while  
295 3,4-diCQA and 4,5-diCQA were quantified in GRC, and 3-diMCiQA was only  
296 quantified in AC.

297 QA (431.6 - 810 mg/100g) was the most abundant compound found in dialyzed  
298 samples, with R% ranging from 47 to 75%, followed by CoT (22 – 40 mg/100g; R%  
299 19-42%). It is worthy to remark that 3-diMCiQA presented the highest R% (84.6) in

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

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

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

318

### 319 **Antioxidant capacity.**

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

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

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

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

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

344

345 After dialysis a similar trend was also observed. The antioxidant capacity of GRC  
346 dialysates was the highest followed by RC and AC, while CC dialysates remained with  
347 the lower antioxidant capacity. The correlation between antioxidant capacity assays was  
348 also confirmed with dialysates: FRAP/ PHEN ( $r = 0.537$ ;  $P < 0.01$ ), FRAP/ TEAC ( $r =$   
349  $0.824$ ;  $P < 0.01$ ), and PHEN/TEAC ( $r = 0.663$ ;  $P < 0.01$ ). FRAP, TEAC and PHEN

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

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

363

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

365 The antioxidant potential of coffee is attributed to the presence of polyphenols and  
366 melanoidins, but their contribution to the antioxidant capacity varies with the intensity  
367 of the roasting process, showing discrepancies in the results obtained by different  
368 authors<sup>7,8,45</sup>. The contribution of high molecular weight melanoidins to the antioxidant  
369 capacity of coffee brews was estimated in the range of 26-38 %.<sup>39</sup> However Delgado-  
370 Andrade *et al.*<sup>45</sup> found that the antioxidant capacity of melanoidins depends on the  
371 presence of low molecular weight compounds (polyphenols), linked non-covalently to  
372 the melanoidin skeleton. Other authors<sup>46</sup> found that only a small proportion of  
373 melanoidins could be 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.<sup>41,47</sup> 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<sup>48</sup>, epidemiology<sup>49</sup>, agriculture<sup>50</sup> and highway safety<sup>51</sup>; 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<sup>48</sup>.

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<sup>26</sup>. **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

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

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

456

#### 457 **ABBREVIATIONS**

458 Regular (RC), decaffeinated (DC), Arabic (AC), green + roasted coffee blend (GRC)  
459 chicory + coffee blend (CC). Total polyphenols content (TP). Quinic acid (QA), 3-O-  
460 caffeoylquinic acid (3-CQA); 4-O-caffeoylquinic acid (4-CQA); 1-O-feruloylquinic  
461 acid (1-FQA); *cis*-5-O-caffeoylquinic acid (*cis*5-CQA); 5-O-caffeoylquinic acid (5-  
462 CQA); 3-O-feruloylquinic acid (3-FQA); 4-O-feruloylquinic acid (4-FQA);  
463 caffeoylquinic lactone (CQL); 5-*p*-coumaroylquinic acid (5-CoQA); 5-O-feruloylquinic  
464 acid (5-FQA); caffeic acid (CA); 3-O-dimetoxycinamoylquinic acid (3-diMCiQA); 3,4-  
465 O-dicaffeoylquinic acid (3,4-diCQA); 3-O-feruloylquinic lactone (3-FQL); 4-O-  
466 dimetoxycinamoylquinic acid (4-diMCiQA); 5-O-dimetoxycinamoylquinic acid (5-  
467 diMCiQA); coumaroyl,caffeoylquinic acid (CoCQA); ferulic acid (FA); 3-O-caffeoyl,4-  
468 O-feruloylquinic acid (3,4-CFQA); 4,5-O-caffeoylquinic acid (4,5-diCQA);  
469 diferuloylquinic acid (diFQA); 4-O-caffeoyl,5-O-feruloylquinic acid (4,5-CFQA);  
470 caffeoyl tryptophan conjugated (CT); dicaffeoylquinic lactone (diCQL); coumaroyl  
471 tryptophan conjugated (CoT) and feruloyl tryptophan conjugated (FT). Boosted  
472 Regression Trees (BRT).

473

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480

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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  $\pm$  SD.

	Coffee	Dialysates	R%
<b>Regular Coffee (RC)</b>	15.6 $\pm$ 0.4 <b>a</b>	3.33 $\pm$ 0.20 <b>C</b>	21.3
<b>Arabic Coffee (AC)</b>	15.8 $\pm$ 0.6 <b>a</b>	3.63 $\pm$ 0.09 <b>B</b>	23.0
<b>Green + Roasted Coffee blend (GRC)</b>	16.0 $\pm$ 0.5 <b>a</b>	3.82 $\pm$ 0.09 <b>A</b>	23.9
<b>Chicory + Coffee blend (CC)</b>	9.0 $\pm$ 0.2 <b>c</b>	1.78 $\pm$ 0.07 <b>D</b>	19.8
<b>Decaffeinated Coffee (DC)</b>	14.8 $\pm$ 0.4 <b>b</b>	3.26 $\pm$ 0.22 <b>C</b>	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] <sup>-</sup> (m/z) experimental	[M-H] <sup>-</sup> (m/z) calculated	error (ppm)	MS <sup>2</sup> (m/z)	λ <sub>máx</sub> (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- <i>p</i> -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-dimetoxycinamoylquinic 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-dimetoxycinamoylquinic acid	C18H21O9	381.1192	381.1191	-0.1	173, 207, 337	226, 285, 320
17	17,5	5-diMCiQA	5-O-dimetoxycinamoylquinic 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]<sup>-</sup> (m/z), negatively charged molecular ion; M<sup>2</sup> (m/z), daughter ions produced from [M-H]<sup>-</sup> fragmentation; λ<sub>max</sub>, 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  $\pm$  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 <sup>1</sup>	1700 $\pm$ 230a	810 $\pm$ 80A	48	1440 $\pm$ 230b	780 $\pm$ 100A	54	1314 $\pm$ 24b	708 $\pm$ 18B	54	576 $\pm$ 4c	431.6 $\pm$ 1.7C	75	1700 $\pm$ 300a	800 $\pm$ 80A	47
3-CQA	92 $\pm$ 12b	< LOD	0	104 $\pm$ 14a	< LOQ	0	98.7 $\pm$ 0.5a	< LOQ	0	26.6 $\pm$ 0.3d	< LOD	0	77 $\pm$ 3c	< LOD	0
4-CQA	550 $\pm$ 120b	16.9 $\pm$ 1.1C	3,1	720 $\pm$ 140a	18.1 $\pm$ 0.6B	2,5	787 $\pm$ 16a	20.60 $\pm$ 0.11A	2,6	136.0 $\pm$ 1.9d	16.7 $\pm$ 0.4C	12	460 $\pm$ 40c	16.7 $\pm$ 0.9C	3,6
1-FQA	28 $\pm$ 4a	< LOD	0	25 $\pm$ 4b	< LOD	0	21.0 $\pm$ 0.3c	< LOD	0	13.55 $\pm$ 0.09d	< LOD	0	25 $\pm$ 3b	< LOD	0
cis5-CQA	134 $\pm$ 10b	8 $\pm$ 8C	6,0	134 $\pm$ 13b	15.43 $\pm$ 0.23B	12	145 $\pm$ 4a	15.63 $\pm$ 0.25B	11	38.4 $\pm$ 1.1d	16.06 $\pm$ 0.21A	42	111 $\pm$ 9c	10 $\pm$ 8C	9
5-CQA	1050 $\pm$ 200c	19.7 $\pm$ 1.9C	1,9	1340 $\pm$ 240b	23.1 $\pm$ 1.7B	1,7	1549 $\pm$ 19a	32.40 $\pm$ 0.25A	2,1	352.9 $\pm$ 0.3e	18.7 $\pm$ 0.3C	5,3	940 $\pm$ 100d	19.4 $\pm$ 2.1C	2,1
3-FQA	280 $\pm$ 80a	16.0 $\pm$ 0.5A	5,7	250 $\pm$ 90b	16.0 $\pm$ 0.5A	6,4	195.2 $\pm$ 1.7b	16.09 $\pm$ 0.17A	8,2	104.1 $\pm$ 1.9c	16.1 $\pm$ 0.5A	16	220 $\pm$ 60b	15.67 $\pm$ 0.21B	7,1
4-FQA	110 $\pm$ 30a	15.85 $\pm$ 0.22A	14	90 $\pm$ 40b	15.8 $\pm$ 0.4A	18	49.5 $\pm$ 0.5c	15.61 $\pm$ 0.24B	32	46.5 $\pm$ 1.2d	16.1 $\pm$ 0.5A	35	69 $\pm$ 16b	15.90 $\pm$ 0.12A	23
CQL	430 $\pm$ 210b	< LOQ	0	740 $\pm$ 140a	< LOQ	0	674 $\pm$ 16a	< LOQ	0	233.8 $\pm$ 0.5c	< LOQ	0	220 $\pm$ 90c	< LOD	0
5-CoQA	90 $\pm$ 22c	18.9 $\pm$ 1.2B	21	120 $\pm$ 23b	20.7 $\pm$ 0.6A	17	136.7 $\pm$ 1.9a	20.6 $\pm$ 0.5A	15	33.9 $\pm$ 0.8e	16.7 $\pm$ 0.3C	49	51 $\pm$ 12d	17.2 $\pm$ 1.0C	34
5-FQA	1150 $\pm$ 250b	19.0 $\pm$ 1.1B	1,6	1000 $\pm$ 300b	19.9 $\pm$ 2.5B	2	1327 $\pm$ 6a	20.89 $\pm$ 0.20A	1,6	429 $\pm$ 7d	18.23 $\pm$ 0.18C	4,2	660 $\pm$ 90c	17.3 $\pm$ 0.4D	2,6
CA <sup>2</sup>	19 $\pm$ 7a	< LOQ	0	17.2 $\pm$ 1.8a	6.4 $\pm$ 0.4B	37	14.77 $\pm$ 0.12b	6.97 $\pm$ 0.12A	47	4.131 $\pm$ 0.014d	< LOD	0	13.7 $\pm$ 1.8c	< LOQ	0
3-diMCiQA	24 $\pm$ 15a	< LOQ	0	18.2 $\pm$ 1.2a	15.39 $\pm$ 0.23	85	15.54 $\pm$ 0.08a	< LOQ	0	< LOD b	< LOD	0	19 $\pm$ 7a	< LOQ	0
3,4-diCQA	120 $\pm$ 40c	< LOD	0	168 $\pm$ 23b	< LOD	0	466 $\pm$ 6a	16.04 $\pm$ 0.25	3,4	28.4392 $\pm$ 0.0006e	< LOD	0	89 $\pm$ 15d	< LOD	0
3-FQL	79 $\pm$ 23b	< LOD	0	108 $\pm$ 19a	< LOD	0	65.5 $\pm$ 0.3c	< LOD	0	65.90 $\pm$ 0.13c	< LOD	0	70 $\pm$ 30c	< LOD	0
4-diMCiQA	24 $\pm$ 16a	< LOQ	0	16.9 $\pm$ 1.0a	< LOQ	0	14.69 $\pm$ 0.12b	< LOQ	0	7.15 $\pm$ 0.05c	< LOD	0	20 $\pm$ 8a	< LOQ	0
5-diMCiQA	40 $\pm$ 40a	< LOQ	0	26 $\pm$ 4a	17.0 $\pm$ 0.6A	65	19.53 $\pm$ 0.11b	15.8 $\pm$ 0.4B	81	8.49 $\pm$ 0.4c	< LOD	0	29 $\pm$ 16a	< LOQ	0
CoCQA	19.7 $\pm$ 2.3c	< LOD	0	22.4 $\pm$ 1.8b	< LOD	0	28.2 $\pm$ 0.3a	< LOD	0	8.25 $\pm$ 0.10e	< LOD	0	17.8 $\pm$ 2.0d	< LOD	0
FA <sup>3</sup>	90 $\pm$ 30b	< LOD	0	130 $\pm$ 60a	< LOD	0	146.0 $\pm$ 1.4a	< LOD	0	68 $\pm$ 4c	< LOD	0	90 $\pm$ 40b	< LOD	0
3,4-CFQA	67 $\pm$ 22b	< LOD	0	58 $\pm$ 25b	< LOD	0	111.3 $\pm$ 1.5a	< LOD	0	18.7 $\pm$ 0.3d	< LOD	0	39.8 $\pm$ 1.3c	< LOD	0
4,5-diCQA	140 $\pm$ 50c	< LOD	0	180 $\pm$ 60b	< LOD	0	675.4 $\pm$ 0.6a	15.68 $\pm$ 0.25	2,3	30.441 $\pm$ 0.013e	< LOD	0	89 $\pm$ 18d	< LOD	0
diFQA	32 $\pm$ 16a	< LOD	0	29 $\pm$ 6a	< LOD	0	23.4 $\pm$ 0.6b	< LOD	0	10.7 $\pm$ 0.3c	< LOD	0	21 $\pm$ 5b	< LOD	0
4,5-CFQA	43 $\pm$ 14b	< LOD	0	39 $\pm$ 12b	< LOD	0	105.6 $\pm$ 0.3a	< LOD	0	12.55 $\pm$ 0.11d	< LOD	0	27.6 $\pm$ 0.9c	< LOD	0
CT <sup>2</sup>	160 $\pm$ 40b	< LOD	0	120 $\pm$ 70b	< LOD	0	287 $\pm$ 4a	< LOQ	0	52.2 $\pm$ 0.4d	< LOD	0	59 $\pm$ 11c	< LOD	0
diCQL	19 $\pm$ 3b	< LOD	0	23 $\pm$ 4a	< LOD	0	21.25 $\pm$ 0.03a	< LOD	0	8.16 $\pm$ 0.11d	< LOD	0	16.2 $\pm$ 1.6c	< LOD	0

<b>CoT<sup>4</sup></b>	200 ± 60 <sup>a</sup>	40 ± 8 <sup>A</sup>	20	160 ± 110 <sup>b</sup>	30 ± 30 <sup>B</sup>	19	123 ± 8 <sup>b</sup>	30.9 ± 1.4 <sup>B</sup>	25	113.6 ± 0.6 <sup>b</sup>	33.4 ± 0.3 <sup>B</sup>	29	52 ± 16 <sup>c</sup>	22 ± 9 <sup>C</sup>	42
<b>FT<sup>3</sup></b>	53 ± 8 <sup>b</sup>	< LOD	0	40 ± 30 <sup>c</sup>	< LOD	0	61.0 ± 0.6 <sup>a</sup>	< LOD	0	25.47 ± 0.12 <sup>c</sup>	< LOD	0	25 ± 4 <sup>c</sup>	< 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: <sup>1</sup>quantified with quinic acid; <sup>2</sup>quantified with caffeic acid; <sup>3</sup>quantified with ferulic acid; <sup>4</sup>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 (%)
<b>RC</b>	71 ± 7 <b>a</b>	14.7 ± 0.9 <b>B</b>	20.7	133 ± 11 <b>a</b>	27 ± 4 <b>B</b>	20.3	113 ± 18 <b>a</b>	22 ± 9 <b>B</b>	19.5
<b>AC</b>	76 ± 12 <b>a</b>	13.8 ± 1.3 <b>B</b>	18.2	132 ± 18 <b>a</b>	23.4 ± 0.7 <b>C</b>	17.7	124 ± 15 <b>a</b>	28 ± 4 <b>B</b>	22.6
<b>GRC</b>	65.5 ± 1.7 <b>b</b>	17.20 ± 0.08 <b>A</b>	26.2	121 ± 4 <b>b</b>	33.3 ± 1.1 <b>A</b>	27.5	112 ± 6 <b>a</b>	40 ± 3 <b>A</b>	35.7
<b>CC</b>	42.1 ± 1.0 <b>d</b>	6.88 ± 0.19 <b>D</b>	16.3	72.3 ± 2.4 <b>c</b>	11.5 ± 0.9 <b>E</b>	15.9	66 ± 3 <b>c</b>	8.4 ± 0.4 <b>C</b>	12.7
<b>DC</b>	61 ± 8 <b>c</b>	10.5 ± 1.3 <b>C</b>	17.2	128 ± 9 <b>a</b>	22.7 ± 0.7 <b>D</b>	17.7	102 ± 14 <b>b</b>	24 ± 10 <b>B</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.

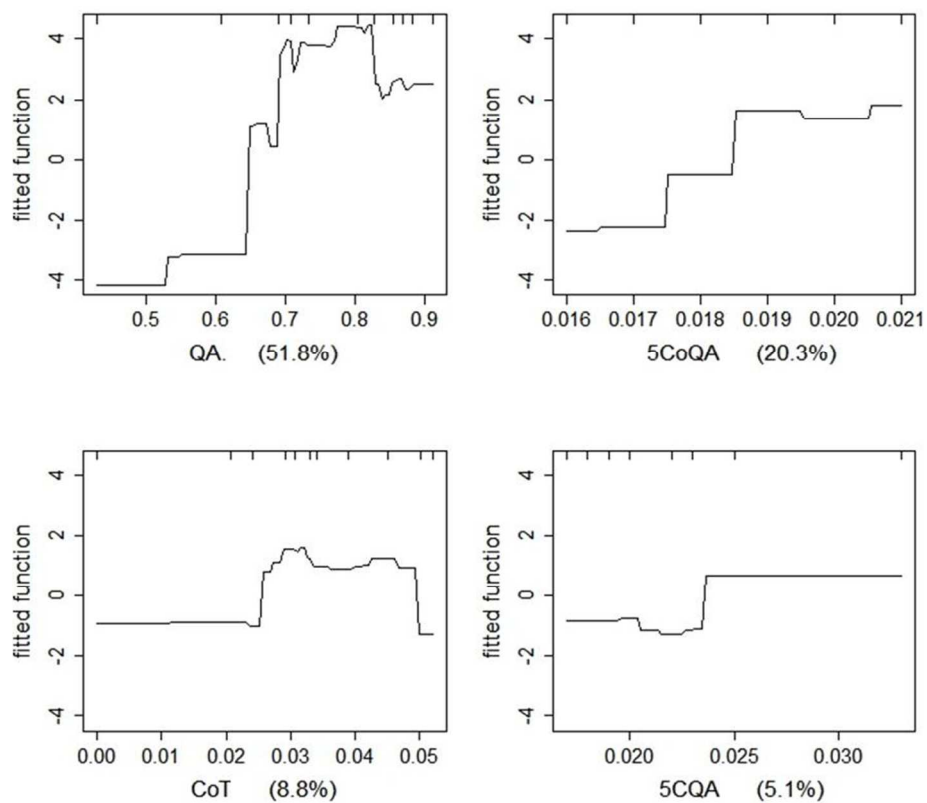
	Coffee			Dialysates		
	TEAC	FRAP	PHEN	TEAC	FRAP	PHEN
<i>Adjusted parameters</i>						
bag fraction	0.55	0.55	0.50	0.55	0.60	0.60
learning rate	0.0006	0.0008	0.0006	0.001	0.0006	0.0005
tree complexity	2	2	2	2	2	2
<i>Model performance</i>						
CV correlation	0.969	0.979	0.926	0.985	0.970	0.982
optimal number of trees	48,250	39,500	29,750	38,250	32,250	60,500
<i>Relative influence of polyphenols (%)</i>						
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

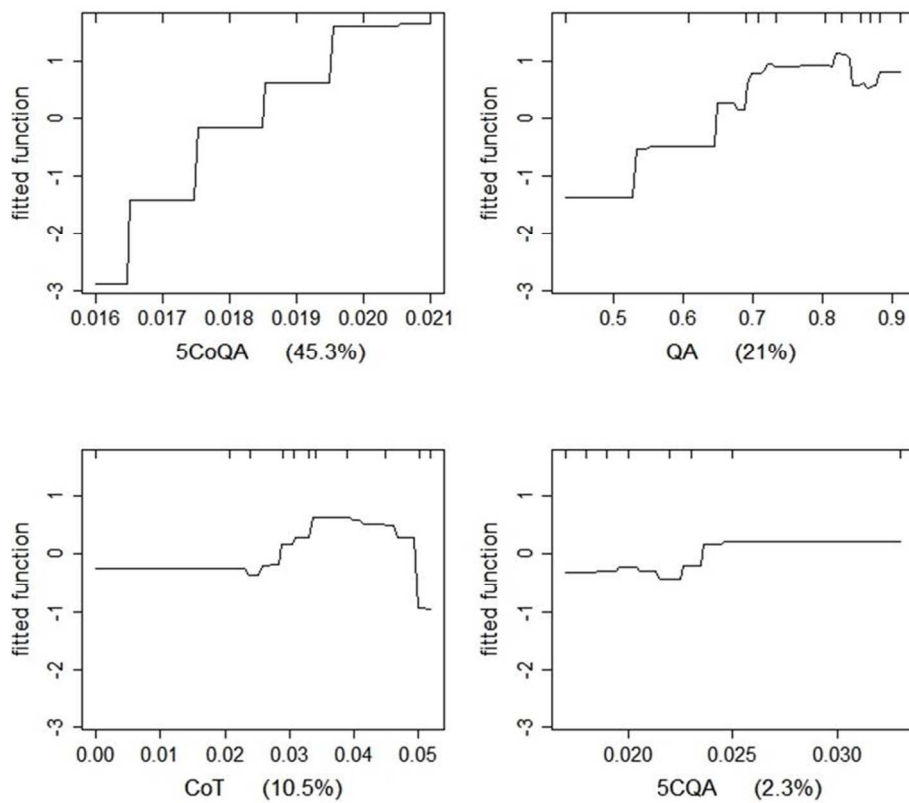
FT	1.7	10.7	2.1	-	-	-
<i>Cumulative influence (%)</i>	100	100	100	100	100	100

## FIGURE GRAPHICS

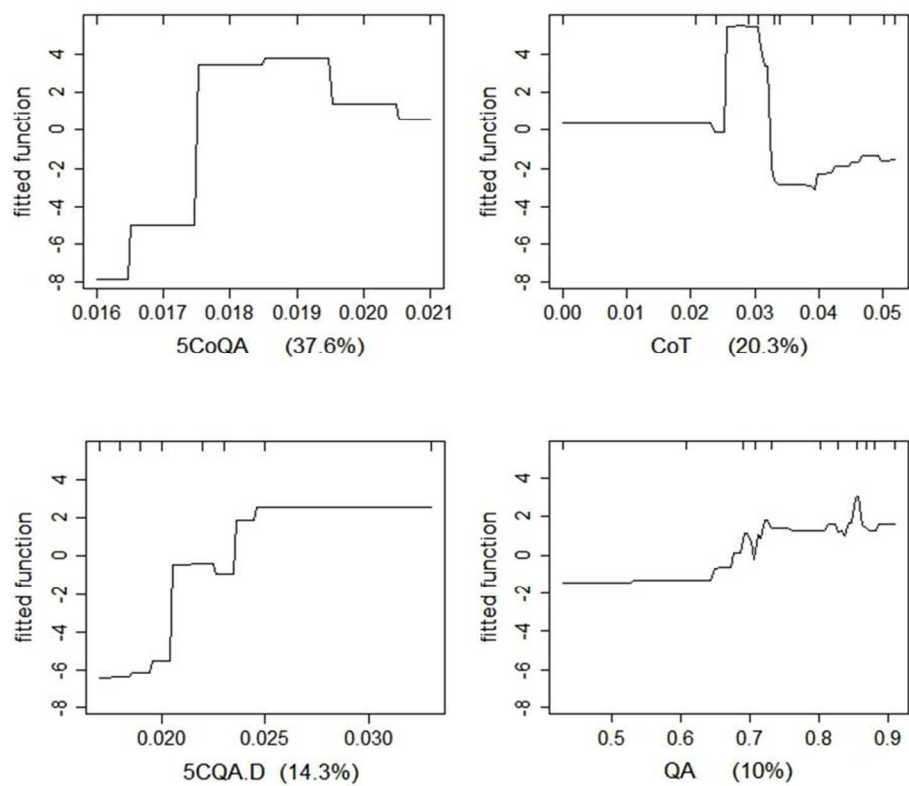
Figure 1.

A



**B**

C



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