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Impact of *in-vitro* gastro-pancreatic digestion on polyphenols and cinnamaldehyde bioaccessibility and antioxidant activity in stirred cinnamon-fortified yogurt

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1 **Abstract**

2 In this study, cinnamon powder was supplemented into yogurt as a functional ingredient. The
3 total phenolic compounds, individual phytochemicals and radical scavenging activity of the
4 yogurts were measured and compared with a cinnamon water extract treated in the same way as
5 the fortified yogurt. Cinnamon-fortified yogurt displayed higher total phenolic content ($P<0.05$)
6 and higher radical scavenging activity ($P<0.05$) compared to plain yogurt. Phenolic acids,
7 flavonols and cinnamaldehyde were identified in the cinnamon-fortified yogurt. Results showed
8 that only the 34.7% of the total phenolic compounds present in the cinnamon water extract were
9 found in the cinnamon-fortified yogurt, the remaining being bound to milk proteins. A low
10 recovery was also found for the individual phytochemicals. However, *in-vitro* digestion of the
11 cinnamon-fortified yogurt resulted in the release of phenolic compounds from milk proteins so
12 that at the end of the digestion the amount of phenolic compounds recovered in the cinnamon-
13 fortified yogurt was higher than that found in the digested cinnamon water extract ($P<0.05$).
14 These results clearly showed that yogurt matrix enhance the gastro-intestinal stability and the
15 bioaccessibility of cinnamon polyphenols. Cinnamon-fortified yogurt can be considered an
16 important source of dietary bioaccessible polyphenols.

17 **Keywords:** functional yogurt, cinnamon, phenolic compounds, radical scavenging activity,
18 bioaccessibility

19 **1. Introduction**

20 Developing of functional foods with health promoting natural ingredients has increased in the
21 past decade (Granato, Nunes, & Barba, 2017). The development of new products with
22 potentially positive effect on health using traditional herbs and food, which are known to be safe
23 from the toxicological standpoint, is generally desirable since there is an increasing interest
24 among consumers to look for healthier and natural food (Granato et al., 2017). Traditional herbs
25 and food used to improve the functionality of food are normally chosen because rich in phenolic
26 compounds, which possess strong antioxidant activity and show protective effects against
27 chronic diseases including diabetes, cardiovascular diseases and cancer (Del Rio et al., 2013). In
28 the Middle East and Arab countries, cinnamon powder is a well-known and commonly used
29 food and traditional herbal medicine. Cinnamon showed several beneficial health properties
30 such as anti-tumoural, cardiovascular, cholesterol lowering, and antioxidant activities
31 (Gruenwald, Freder, & Armbruester 2010; Hlebowicz, Darwiche, Bjorgell, & Almer, 2007;
32 Hlebowicz et al., 2009). Cinnamon polyphenols mainly consist of condensed tannins
33 (oligomeric and polymeric procyanidins) and monomeric phenolic compounds such as flavonols
34 and phenolic acids (Gu et al., 2004; Helal, Tagliazucchi, Verzelloni & Conte, 2014).
35 Cinnamaldehyde is also a major component in cinnamon bark, which exhibits several biological
36 effects such as anti-tumoural, pro-apoptotic and anti-inflammatory activities (Chao, et al., 2008;
37 Roussel, Hininger, Benaraba, Ziegenfuss, & Anderson, 2009).
38 Yogurt is the most popular fermented dairy product and is highly appreciated for its nutritional
39 value and good digestibility (Saint-Eve, Levy, Martin, & Souchon, 2006). Recently, numerous
40 studies underlined the health benefits of yogurt consumption in terms of enhancement of the
41 immune system, improvement of bowel function, protection against colon cancer and
42 *Helicobacter pylori* infection (El-Abbadi, Dao, & Meydani, 2014). The health benefits of yogurt
43 have been ascribed to the presence of bioactive peptides and probiotics (Rutella, Tagliazucchi,

44 & Solieri, 2016). However, it is not considered a source of phenolic compounds and therefore
45 traditional herbs or food such as spices, fruit juices and grape seed or extract had been used to
46 enhance the phenolic content of yogurt (Karraslan, Ozden, Vardin, & Turkoglu, 2011;
47 Chouchouli et al., 2013; Illupapalayam, Smith, & Gamlath, 2014; Oliveira et al., 2015). Yogurt
48 matrix seems to be an excellent delivery vehicle for plant-derived phenolic compounds. The low
49 pH increase the stability of phenolic compounds during storage (Chouchouli et al., 2013),
50 whereas the presence of proteins or large peptides and fat should maintain the integrity of
51 phenolic compounds during digestion increasing their bioaccessibility (Tagliazucchi, Helal,
52 Verzelloni, & Conte, 2012; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014).
53 Bioaccessibility is defined as the amount of a specific compound solubilized in the small
54 intestine and available for the subsequent absorption. The bioaccessibility definition comprises
55 the release of compounds from food matrices and their stability under the gastro-intestinal
56 condition (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). This latter point is of paramount
57 importance since only the compounds released from the food matrix and stable in the gastro-
58 intestinal condition are potentially bioavailable and in condition to exert their beneficial effects
59 on the gastro-intestinal tract.

60 The main objective of the present study was to fortify the phenolic content of yogurt, using
61 cinnamon powder and to evaluate the bioaccessibility of phenolic compounds and
62 cinnamaldehyde and the antioxidant activity during simulated gastro-pancreatic digestion of the
63 cinnamon-fortified yogurt.

64 2. Materials and methods

65 2.1 Materials

66 Dano® full cream milk powder was obtained from Arla Foods Ingredients (Viby J, Denmark).
67 YOFLEX® commercial yogurt starter culture of *Streptococcus thermophilus* and *Lactobacillus*
68 *delbrueckii* ssp. *bulgaricus* were obtained from Chr. Hansen, (Hoersholm, Denmark). Cinnamon
69 bark powder (*Cinnamomum cassia*) was purchased from local market (Damanhour, Egypt).
70 Enzymes and reagents for the *in-vitro* digestion, radical scavenging activity analysis as well as
71 phenolic standards were supplied by Sigma (Milan, Italy).

72

73 2.2 Preparation of stirred yogurts and cinnamon water extract

74 Yogurt preparation and experimental strategy are summarized in **Figure 1**.
75 Stirred yogurt was manufactured according to the instructions of Illupapalayam et al. (2014)
76 with some modifications. Briefly, plain yogurt was prepared by heat-treating reconstituted full-
77 fat milk powder (12% w/v) at 95°C for 5 min followed by cooling to 45°C. For the preparation
78 of plain yogurt with sucrose, 7.5% (w/v) of sucrose was added to the milk powder and treated as
79 reported above. The cinnamon-fortified yogurt was prepared by adding 1.5% (w/v) of cinnamon
80 powder to the reconstituted milk powder following by the same heat-treatment as reported
81 above. In the cinnamon fortified yogurt with sucrose, an amount of 7.5% of sucrose was also
82 added before the heat-treatment. All the treatments were then filtered using stainless-steel mesh
83 to remove the insoluble materials, inoculated with starter culture and incubated at 45°C until the
84 pH reached 4.4 (~8 h). Cooling to 5°C was done to halt further acidification. The yogurt was
85 manually stirred during the cooling using stainless-steel kitchen whisker. The stirred samples
86 were transferred into yogurt cups aseptically and stored in refrigerator at 5°C for one day.

87 A control (named cinnamon water extract) with cinnamon powder (1.5% w/v) but without milk
88 powder was also prepared and heat-treated, inoculated, stirred and cooled as the cinnamon
89 fortified yogurt.

90 Samples were collected from each treatment at the end of the procedure.

91

92 **2.3 *In-vitro* digestion**

93 Yogurt preparations and cinnamon water extract were subjected to *in-vitro* simulated digestion
94 to determine the effect of digestion on phenolic content and radical scavenging activity. The
95 recent standardized digestion method by Minekus et al. (2014) was followed with some
96 modification as reported in Tagliazucchi, Helal, Verzelloni, Bellesia, & Conte (2016). For the
97 gastric step, samples were diluted with simulated gastric fluid stock electrolyte solution (1:1)
98 and homogenized for 2 min in a laboratory blender. The pH was then lowered to 2.5 with 6
99 mol/L HCl before the addition of 2000 U/mL of pepsin. Samples were incubated for 2 hours at
100 37°C. The chyme was then subjected to the pancreatic phase of digestion. Simulated intestinal
101 fluid was added and the pH was brought to 7.5 with 20% Na₂CO₃ before adding 0.8 g/L of
102 pancreatin and 10 mmol/L of bile salts. The digestive mixture was incubated in a shaking bath
103 for additional 2 hours at 37°C.

104 Aliquots of the samples were collected before and after peptic digestion and after pancreatic
105 digestion. The digestions were carried out in triplicate.

106

107 **2.4 Samples preparation for analysis**

108 Samples from yogurt preparations, cinnamon-water extract and *in-vitro* digestions were
109 centrifuged at 17500g for 10 min at 5°C to eliminate the insoluble material. The clear
110 supernatants were then analysed for the content in total free phenolic compounds, total free

111 tannins and individual free phytochemicals as well as for the radical scavenging activity
112 analysis.

113

114 **2.5 Determination of total phenolic content total tannins content**

115 Quantification of total phenolic compounds was carried out with the Folin-Ciocalteu assay as
116 reported by Singleton, Orthofer, & Lamuela-Raventós (1999). The clear supernatant, obtained as
117 described in section 2.4, was diluted at least three times to reduce the interferences due to the
118 digestive enzymes, bile salts and sucrose (Helal et al., 2014). In a 1.5 mL Eppendorf tube
119 790 μ L of distilled water, 10 μ L of diluted sample and 50 μ L of the Folin–Ciocalteu reagent
120 were added and mixed. After exactly 1 min, 150 μ L of 20% aqueous sodium carbonate was
121 added, the mixture was mixed and left to stand at room temperature in the dark for 120 min.
122 Detection was achieved at 760 nm.

123 Total tannins were determined according to Hagerman and Butler (1978) on the clear
124 supernatant of the sample containing cinnamon (cinnamon water extract, cinnamon-fortified
125 yogurt and the corresponding digested samples). Briefly, 1 mL of three times diluted sample
126 was added to 2 mL of standard protein solution (bovine serum albumin dissolved at a
127 concentration of 1 mg/mL in 0.2 mol/L acetate buffer, pH 5, containing 0.17 mol/L sodium
128 chloride). The solutions were mixed and allowed to stand at room temperature for 15 min and
129 were then centrifuged for 15 min at 5000 *g*. After centrifugation, the pellet was washed with
130 acetate buffer and then dissolved in 4 mL of sodium dodecyl sulfate (SDS)-triethanolamine
131 solution (1% SDS and 5% (v/v) triethanolamine). Tannins were determined by mixing 2 mL of
132 tannin fraction with 0.5 mL of ferric chloride reagent (0.01 mol/L ferric chloride in 0.01 mol/L
133 HCl). The absorbance value was read at 510 nm.

134 The results were reported as mg catechin/100 g of yogurt or cinnamon water extract for both the
135 assays.

136 For the analysis of milk proteins-tannin interaction (Helal et al., 2014), 15 mg of cinnamon
137 powder was added to 1 mL of reconstituted milk so that the final concentration of milk proteins
138 in the assay was 3.5% (w/v) and of cinnamon powder was 1.5% (w/v). Samples were
139 immediately centrifuged after mixing and the pellets analysed for tannins content as reported
140 above.

141

142 **2.6 Radical scavenging activity analysis**

143 Two different methods were used to determine the radical scavenging activity, namely ABTS
144 and DPPH assays. ABTS radical scavenging activity was carried out according to Re et al.
145 (1999) and results expressed as mg ascorbic acid/100 g of yogurt or cinnamon water extract.
146 Three times diluted sample (40 μ L) was added to 1960 μ L of the resulting blue-green ABTS
147 radical cation. The mixture was incubated at 37°C for 10 min and the decrease in absorbance
148 measured at 734 nm.

149 DPPH method was carried out according to Behrad, Yusof, Goh, & Baba (2009) with slight
150 modification as reported by Illupapalayam et al. (2014). To 3 ml of 60 μ mol/L DPPH in
151 methanol, 250 μ L of three times diluted sample was added. Samples were incubated in the dark
152 and after 20 min, the absorbance was measured at 517 nm. Results were expressed as mg of
153 trolox/100 g of yogurt or cinnamon water extract.

154

155 **2.7 Identification of low molecular weight phenolic compounds and cinnamaldehyde by** 156 **high performance liquid chromatography (HPLC)**

157 Low molecular weight phytochemicals were identified by using high performance liquid
158 chromatography (HPLC) as previously described by Helal et al. (2014) using a Beckman HPLC
159 (Beckman Coulter, USA), fitted with UV absorbance detector (Perkin Elmer, USA) and
160 equipped with a C18 column (Ascentis® C18 HPLC Column 5 μ m particle size, 250×4.6 mm,

161 Sigma-Aldrich Co. LLC). The two solvents were as follows: solvent A mixture of water-formic
162 acid (0.1%) and solvent B acetonitrile. The gradient started at 3% B for 0.5 min then linearly
163 ramped up to 10% B in 10 min. The mobile phase composition was raised up to 40% B in 34
164 min, then 100% B in 1 min and maintained for 5 min in order to wash the column. The flow rate
165 was 1 mL/min. Peaks for samples and standards were monitored at 360 nm for flavonols and at
166 270 nm for phenolic acids and cinnamaldehyde. Identification and quantification of
167 phytochemicals in samples were performed comparing to chromatographic retention times and
168 areas of external pure standards.

169

170 **2.8 Statistical analysis**

171 All data are presented as mean \pm standard deviation (SD) for three replicates. The Student's t-test
172 was performed using XLSTAT-Pro 2007 (trial version 7.5, Addinsoft, Paris, France). Univariate
173 analysis of variance (ANOVA) with Tukey's post-hoc test was applied using statgraphics
174 16.1.11 (Stat PointTechnologies, Inc, Virginia, USA), when multiple comparisons were
175 performed. Differences were considered significant at $P < 0.05$.

176 3. Results and Discussion

177

178 3.1 Quantification and identification of phenolic compounds in cinnamon bark water 179 extract

180 Water extract of cinnamon bark powder, prepared using the same protocol as for yogurt
181 production but without milk (**Figure 1**), was characterized for its content in total and individual
182 phenolic compounds, total tannins and antioxidant activity. The total amount of phenolic
183 compounds extracted from cinnamon bark was 76.6 ± 4.2 mg of catechin/100 g of water extract.
184 Tannins were 62.1 ± 1.8 mg catechin/100 g of water extract (representing about the 81% of total
185 polyphenols). These values would correspond to 51.1 mg of total phenolic compounds/g of
186 cinnamon powder and 41.4 mg of total phenolic compounds/g of cinnamon powder. Klejdus &
187 Kováčic (2016) found a total soluble phenolic amount of 164 mg/g of cinnamon powder
188 (*Cinnamon cassia*) after extraction with a 60% ethanol solution. This higher value can be due to
189 the different solvent used in the extraction procedure. On the other hand, Shan, Cai, Sun, &
190 Corke (2005) found a total phenolic value in *Cinnamon cassia* of 63.4 mg/g of powder after
191 extraction with 80% methanol solution. Extraction with water resulted in a value of 43.8 mg of
192 total phenolic/g of cinnamon powder and 33.6 mg of tannin/g of cinnamon powder (Helal et al.,
193 2014) which is in agreement with the data found in this study.

194 Three phenolic acids and three flavonols were identified and quantified in the cinnamon extract
195 by HPLC. Among the phenolic acids, coumaric acid was found at the highest concentration
196 (2493.0 ± 15.6 µg/100 g of water extract) followed by syringic (484.0 ± 8.5 µg/100 g of water
197 extract) and ferulic (151.3 ± 8.1 µg/100 g of water extract) acids. The total amount of individual
198 phenolic acids identified and quantified in the cinnamon extract was 3128.3 µg/100 g of water
199 extract, corresponding to the 4.1% of total phenolic compounds. Quercetin-3-rhamnoside and
200 quercetin were the most represented flavonols found in the extract at a concentration of $41.3 \pm$

201 1.8 µg/100 g of water extract and 29.8 ± 1.1 µg/100 g of water extract. Kaempferol was instead
202 found at lower concentration (20.0 ± 0.2 µg/100 g of water extract) respect to the other
203 individual phenolic compounds. The total amount of individual flavonols identified and
204 quantified in the cinnamon extract was 91.1 µg/100 g of water extract, corresponding to the
205 0.12% of total phenolic compounds. Cinnamaldehyde was also quantified resulting in a
206 concentration in the cinnamon extract of 53.3 ± 3.3 mg catechin/100 g of water extract.
207 Quantification of phenolic compounds in *Cinnamon cassia* has not yet been performed in detail.
208 Klejdus & Kovâcic (2016) identified 10 phenolic acids in *Cinnamon cassia* being
209 protocatechuic acid the most representative whereas Helal et al. (2014) identified two phenolic
210 acids with coumaric acid present at the highest concentration. The phenolic acids identified in
211 this study have been already described in *Cinnamon cassia* in amount lower than that found in
212 this study (Helal et al., 2014; Klejdus & Kovâcic, 2016). Wide variation of phytochemical
213 concentration were found in *Cinnamon cassia* bark between single bark sticks, even within the
214 sticks of a package and also within bark samples originating from the same tree (Woehrlin, Fry,
215 Abraham, & Preiss-Weigert, 2010). Quercetin-3-rhamnoside, kaempferol and quercetin have
216 been already reported in *Cinnamon cassia* at concentration similar or lower than that found in
217 this study (Prasad et al., 2009; Helal et al., 2014). Solvent used in the extraction procedure as
218 well as the provenience of the samples and other parameters (age, bark thickness, duration of
219 storage) certainly affect chemical composition of cinnamon bark. The amount of
220 cinnamaldehyde found in this study was in the range already reported (from about 9 to more
221 than 50 mg/g) for *Cinnamon cassia* (Shan et al., 2005; Woehrlin et al., 2010; Helal et al., 2014).
222 The total antioxidant activity of cinnamon extract was 129.1 ± 5.6 mg of ascorbic acid/100 g of
223 cinnamon water extract when the ABTS assay was applied. In the DPPH assay, the antioxidant
224 activity was 77.9 ± 4.7 mg of trolox/100 g of cinnamon water extract.

225

226 **3.2 Total phenolic compounds, individual phytochemicals and antioxidant activity in the** 227 **supernatant of cinnamon-fortified yogurt**

228 The addition of cinnamon powder determined a significant ($P<0.01$) increase in total phenolic
229 compounds in the supernatant of fortified yogurt in comparison with the plain yogurt
230 supernatant (**Figure 2**, before digestion). No significant differences ($P>0.05$) were found
231 between the plain yogurt formulated with sucrose and the plain yogurt without sucrose neither
232 between the cinnamon formulated yogurt and the cinnamon-fortified yogurt with sucrose
233 (**Figure 2**, before digestion). The amount of phenolic compounds in cinnamon-fortified yogurt
234 was 45.0 ± 1.8 mg of catechin/100 g of yogurt, which resulted in a value of 28.3 mg of
235 catechin/100 g of yogurt when corrected for the contribution of plain yogurt (16.7 ± 1.8 mg of
236 catechin/100 g of yogurt). The Folin–Ciocalteu reactivity of plain yogurt is due to the presence
237 of milk compounds different from polyphenols such as low molecular weight antioxidants, free
238 amino acids, peptides and proteins. A comparison with the total phenolic compounds extracted
239 from cinnamon with only water revealed that the amount of total phenolic found in the
240 supernatant of the fortified yogurt was 34.7% of the theoretically expected. It is important to
241 note that total phenolic compounds were quantified in the supernatant of yogurt samples and, in
242 these conditions, only free or unbounded polyphenols are determined. Similarly, Oliveira et al.
243 (2015) and Trigueros, Wojdylo, & Sendra (2014) found a decrease in total phenolic content in
244 yogurts added of strawberry and pomegranate juice respect to the control strawberry and
245 pomegranate juice preparations without yogurts. The low recovery of phenolic compounds in
246 the supernatant of cinnamon-fortified yogurt can be due to the presence of milk proteins that can
247 bind and precipitate cinnamon polyphenols. In a previous study, Helal et al. (2014) found that
248 the addition of 25% milk to a cinnamon beverage determined a decrease of about 28% in total
249 polyphenols content and this decrease is a result of the formation of insoluble complexes
250 between cinnamon tannins and milk proteins. Indeed, the acidic pH, as that found in yogurt

251 because of fermentation, may enhance the binding affinity between phenolic compounds and
252 milk proteins. Hala Mohamed et al. (2015) found that the optimum pH of the interactions
253 between tannins and milk caseins was at pH 5. In general, the formation of insoluble complexes
254 between proteins and tannins is maximum at pH values near the isoelectric point of the protein
255 (Hagerman & Butler, 1978). To gain more information, the interaction of milk proteins with
256 cinnamon tannins was investigated by precipitation assay. Milk proteins at concentration of
257 3.5% (w/v) were able to precipitate 27.4 ± 0.6 mg of catechin/100 g. This amount of precipitated
258 tannins explain more than 77% of polyphenols lost during yogurt preparation.

259 The most representative cinnamon monomeric phenolic compounds and cinnamaldehyde were
260 identified and quantified using HPLC in the supernatant of cinnamon-fortified yogurt (**Table 1**).

261 As found in the cinnamon water extract, phenolic acids were present in higher concentration
262 than flavonols, and coumaric acid was the individual phenolic compound found at the highest
263 concentration in cinnamon-fortified yogurt. As expected, no phenolic acids and flavonols were
264 found in the plain yogurt. A comparison with the amount of phenolic compounds reported in the
265 cinnamon water extract revealed that only a part of free phenolic compounds was recovered in
266 the supernatant of cinnamon-fortified yogurt (**Table 1**). The recovery yield was different among
267 the different monomeric compounds. In the case of syringic acid, ferulic acid, quercetin and
268 quercetin-3-rhamnoside the recovery was higher than 50%, whereas coumaric acid and
269 especially kaempferol showed the lowest recovery. The addition of 7.5% sucrose had no
270 significant effect on monomeric phenolic content in the prepared yogurt mixture (**Table 1**). This
271 variation in the recovery of the different components can be due to the different binding affinity
272 between the individual phenolic components and milk proteins (Hasni et al., 2011). In a recent
273 study, Helal, et al., (2014) found that kaempferol had the highest binding affinity with milk
274 caseins, while syringic acid showed the lowest binding affinity.

275 The ABTS and DPPH scavenging activities of plain yogurt and supplemented samples are
276 shown in **Figure 3**. Fortified yogurt exhibited significantly higher radical scavenging activity
277 than the plain yogurt both in the ABTS and in DPPH assay ($P<0.05$). The radical scavenging
278 activity of plain yogurt is mainly due to the formation of bioactive peptides with radical
279 scavenging activity because of the proteolytic activity of the starter lactobacilli used in yogurt
280 production (Rutella et al., 2016). The ABTS and DPPH scavenging activities in the supernatant
281 of cinnamon-fortified yogurt is less than 32% and 43% of that theoretically expected
282 (considering the sum of the contribution of plain yogurt and cinnamon-water extract),
283 respectively. Similar results were previously obtained, where the antioxidant activity of yogurt
284 fortified with strawberry was reduced due to the polyphenol-protein interaction (Oliveira et al.,
285 2015).

286

287 **3.3 Effect of *in-vitro* digestion on total phenolic compounds, individual phytochemicals and** 288 **antioxidant activity in the supernatant of cinnamon water extract and yogurts**

289 The changes in total phenolic content in the formulated samples during the *in-vitro* digestion are
290 shown in **Figure 2**. In the cinnamon water extract, a significant decrease ($P<0.05$) in total
291 polyphenols, from 76.6 ± 4.2 to 57.0 ± 1.3 mg of catechin/ 100 g of cinnamon water extract, was
292 found after peptic digestion. The subsequent incubation in the pancreatic fluid did not influence
293 the total polyphenols concentration ($P>0.05$). At the end of the pancreatic digestion, the
294 bioaccessibility index (calculated as the percentage ratio between the post-pancreatic
295 concentration and the total polyphenol concentration before the digestion) of total phenolic
296 compounds in cinnamon water extract was 79.8%. The bioaccessibility index of total phenolic
297 compounds measured after 120 min of simulated gastro-pancreatic digestion is in agreement
298 with that previously determined by Helal et al. (2014) after *in-vitro* digestion of a cinnamon
299 beverage. The formation of insoluble complexes between tannins and pepsin was the

300 explanation of the decrease in total polyphenols found in the Helal et al. (2014) study after
301 gastric digestion. Therefore, we measured the amount of tannins in the cinnamon water extract
302 during *in-vitro* digestion. Results showed that the tannins concentration decreased from $62.1 \pm$
303 1.8 mg catechin/100 g of water extract (before the digestion) to 42.8 ± 1.1 mg catechin/100 g of
304 water extract after the gastric phase of digestion. The decrease in tannins content after gastric
305 digestion was 19.3 mg catechin/100 g of cinnamon water extract, which is quite similar to the
306 decrease recorded in total phenolic compounds after gastric digestion of the cinnamon water
307 extract (**Figure 2**). No further changes in the concentration of tannins were found after
308 incubation with the pancreatic fluid.

309 In the cinnamon-fortified yogurt, after the peptic stage of digestion a significant increase
310 ($P < 0.05$) in the total polyphenols concentration was observed. A further, not significant increase
311 ($P > 0.05$) was recorded at the end of the pancreatic phase of the digestion. The amount of
312 phenolic compounds in cinnamon-fortified yogurt after gastro-intestinal digestion was $92.5 \pm$
313 3.3 mg of catechin/100 g of yogurt, which resulted in a value of 66.4 mg of catechin/100 g of
314 yogurt when corrected for the contribution of plain yogurt (26.1 ± 1.5 mg of catechin/100 g of
315 yogurt). The total polyphenols bioaccessibility index for the cinnamon-fortified yogurt was
316 calculated as percentage ratio between the post-pancreatic concentration corrected for the
317 contribution of plain yogurt and the total polyphenol concentration in the cinnamon water
318 extract before the digestion. The bioaccessibility index in the cinnamon-fortified yogurt was
319 86.7% , which was significantly higher ($P < 0.05$) than that calculated for the cinnamon water
320 extract. The protective effect of yogurt matrix can be due to the initial binding between milk
321 proteins and tannins, which make them no longer available for the interaction with pepsin. As
322 the digestion proceeds, milk proteins are hydrolysed and tannins can be released from milk
323 proteins resulting in an increased total polyphenols bioaccessibility. Sucrose addition to
324 cinnamon-fortified yogurt did not induce any significant effect on bioaccessibility of

polyphenols (**Figure 2**). These results clearly showed that yogurt matrix enhanced the gastro-intestinal stability and the bioaccessibility of cinnamon polyphenols.

The behaviour of monomeric phenolic compounds in fortified yogurt and cinnamon water extract during the *in-vitro* digestion was investigated and the results shown in **Table 2**. Different behaviour of identified monomeric phenolic during *in-vitro* digestion was observed. In the cinnamon water extract, most of the phenolic compounds showed high stability during the peptic stage of digestion with the exception of coumaric and syringic acids. The passage to the pancreatic phase of digestion caused a significant decrease in the concentration of the different phenolic compounds (**Table 2**). Syringic acid showed the highest loss with a bioaccessibility index of 24.9% after the two stages of digestion. Similar behaviour was observed in the case of quercetin, which showed a bioaccessibility index of 33.3%. Other authors have already reported the high instability of these compounds. For example, Boyer, Brown, & Liu (2005) found a loss of 53.5% of quercetin after *in-vitro* simulated digestion of onion whereas Helal et al. (2014) found a decrease of 78% of syringic acid after digestion of a cinnamon tea. Quercetin-3-rhamnoside was found to be more stable than the corresponding aglycone (**Table 2**). The presence of the sugar moiety may increase the stability of the phenolic compounds as suggested by Boyer et al. (2005). Coumaric acid content decrease of about 50% during digestion. Similar behavior of coumaric acid during *in-vitro* digestion was already reported by other authors using different food sources and cooking methods (Helal et al., 2014; Juaniz et al., 2017).

Ferulic acid and kaempferol showed the lowest decrease during pancreatic stage with a bioaccessibility index of 89.3% and 84.5%, respectively. These results confirmed previously reported data (Helal et al., 2014; Zaupa et al., 2014). Similarly, cinnamaldehyde was found to be especially stable under *in-vitro* digestive condition as already suggested by Helal et al. (2014).

In-vitro gastro-intestinal digestion of the cinnamon-fortified yogurt resulted in a significant higher concentration of phenolic acids and flavonols at the end of the pancreatic phase of

350 digestion compared to the digested cinnamon water extract (**Table 2**). As reported above, the
351 presence of yogurt matrix determined an initial low recovery yield of the individual phenolic.
352 However, as the digestion proceeded, low molecular weight phenolic compounds were released
353 from the food matrix to the gastro-intestinal fluids. The hydrolysis of caseins during digestion,
354 especially during the pancreatic phase, allowed the release of the bound compounds, resulting in
355 a higher bioaccessibility index respect to the cinnamon water extract. Previous studies showed
356 that the presence of dairy matrices significantly improved the total polyphenols recovery during
357 the digestion, as the interaction between polyphenols and milk proteins exhibited a protective
358 effect (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). This interaction may provide a
359 physical trapping and increase the polyphenols stability during the digestion (Hasni et al., 2011).
360 During *in-vitro* digestion of the cinnamon water extract, cinnamaldehyde was quite stable with a
361 bioaccessibility index of 90.6%. In the case of cinnamon-fortified yogurt, the cinnamaldehyde
362 concentration significantly increased during peptic digestion ($P<0.05$). A further but not
363 significant increase was found also at the end of the pancreatic digestion. However, differently
364 from the monomeric phenolic compounds, the bioaccessibility index of cinnamaldehyde was
365 lower ($P<0.05$) in the cinnamon-fortified yogurt compared to the cinnamon water extract (**Table**
366 **2**). The presence of sucrose had no significant effect on phenolic acids, flavonols and
367 cinnamaldehyde bioaccessibility (**Table 2**).

368 Changes in radical scavenging activity were also evaluated during the *in-vitro* digestion, and the
369 data are presented in **Figure 3**. The radical scavenging activity of plain yogurt progressively
370 increased in both the assays during digestion as a result of the further release of antioxidant
371 peptides and amino acids encrypted in the milk proteins sequences (Tagliazucchi et al., 2016).
372 On the contrary, no significant changes in the radical scavenging activity of the cinnamon water
373 extract were found during the *in-vitro* digestion with both the assays. At the end of the
374 pancreatic digestion, the cinnamon-fortified yogurt showed the highest radical scavenging

375 activity values with both the assays. The presence of sucrose had no significant effect on radical
376 scavenging activity values (**Table 2**).

377 **4. Conclusions**

378 Cinnamon powder was successfully employed for the production of cinnamon-fortified yogurt.
379 The supplemented samples contained cinnamon polyphenols in amounts lower than those
380 present in the cinnamon water extract but contained more total phenolics and exhibited higher
381 radical scavenging activity compared to plain yogurt. Indeed, the presence of yogurt matrix
382 greatly improved the total phenolic as well as the individual phenolic recovery at the end of the
383 digestion in comparison with the cinnamon water extract. In addition to the known health
384 benefits of fermented milk, cinnamon-fortified yogurt showed high polyphenols and
385 cinnamaldehyde content with high bioaccessibility after the simulated gastro-pancreatic
386 digestion and may therefore be considered as an important source of dietary bioaccessible
387 polyphenols. For its greater radical scavenging activity the cinnamon-fortified yogurt can be
388 considered a good candidate for the protection of the gastro-intestinal tract from free radical
389 injury.

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Conflicts of interest: none

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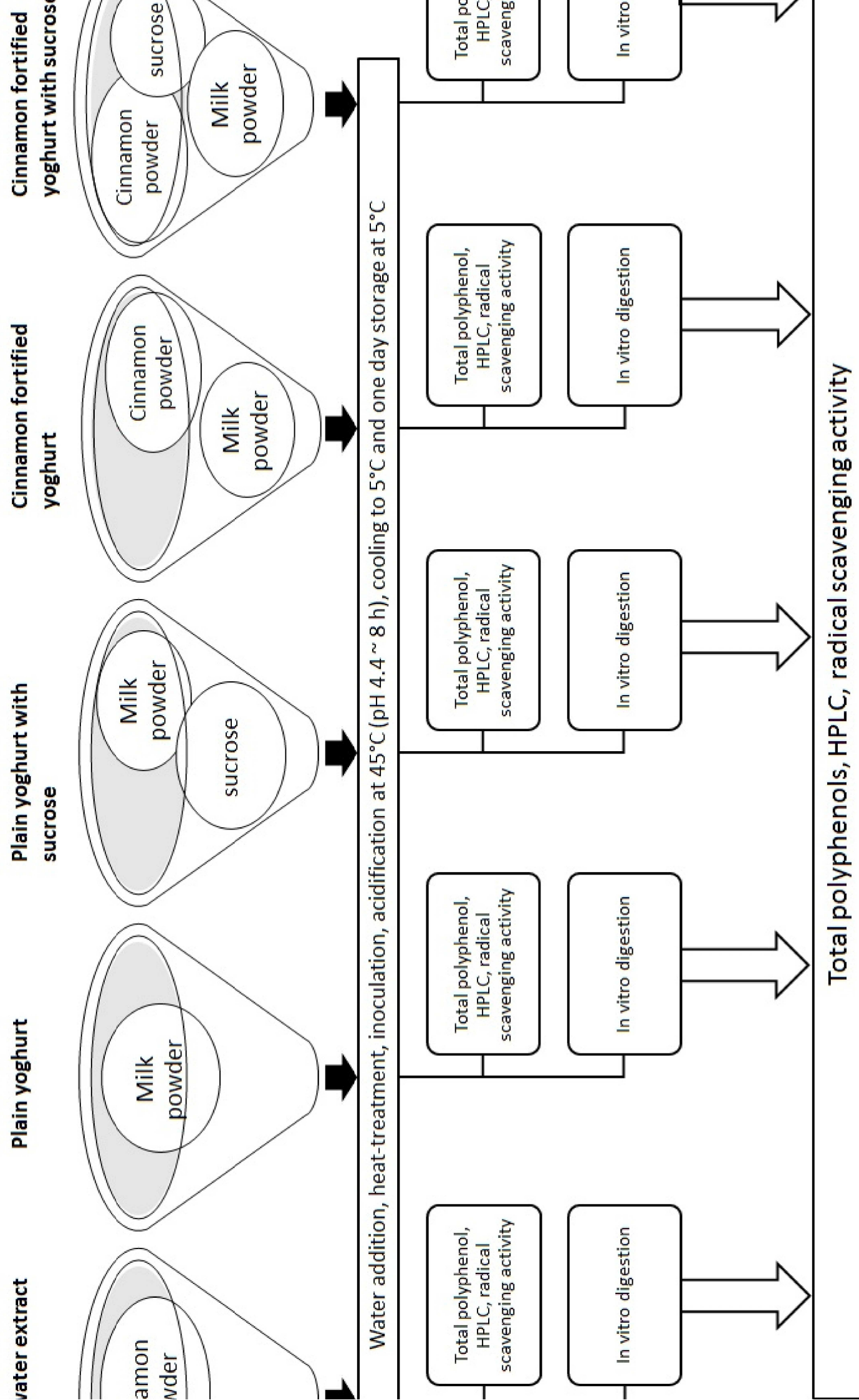
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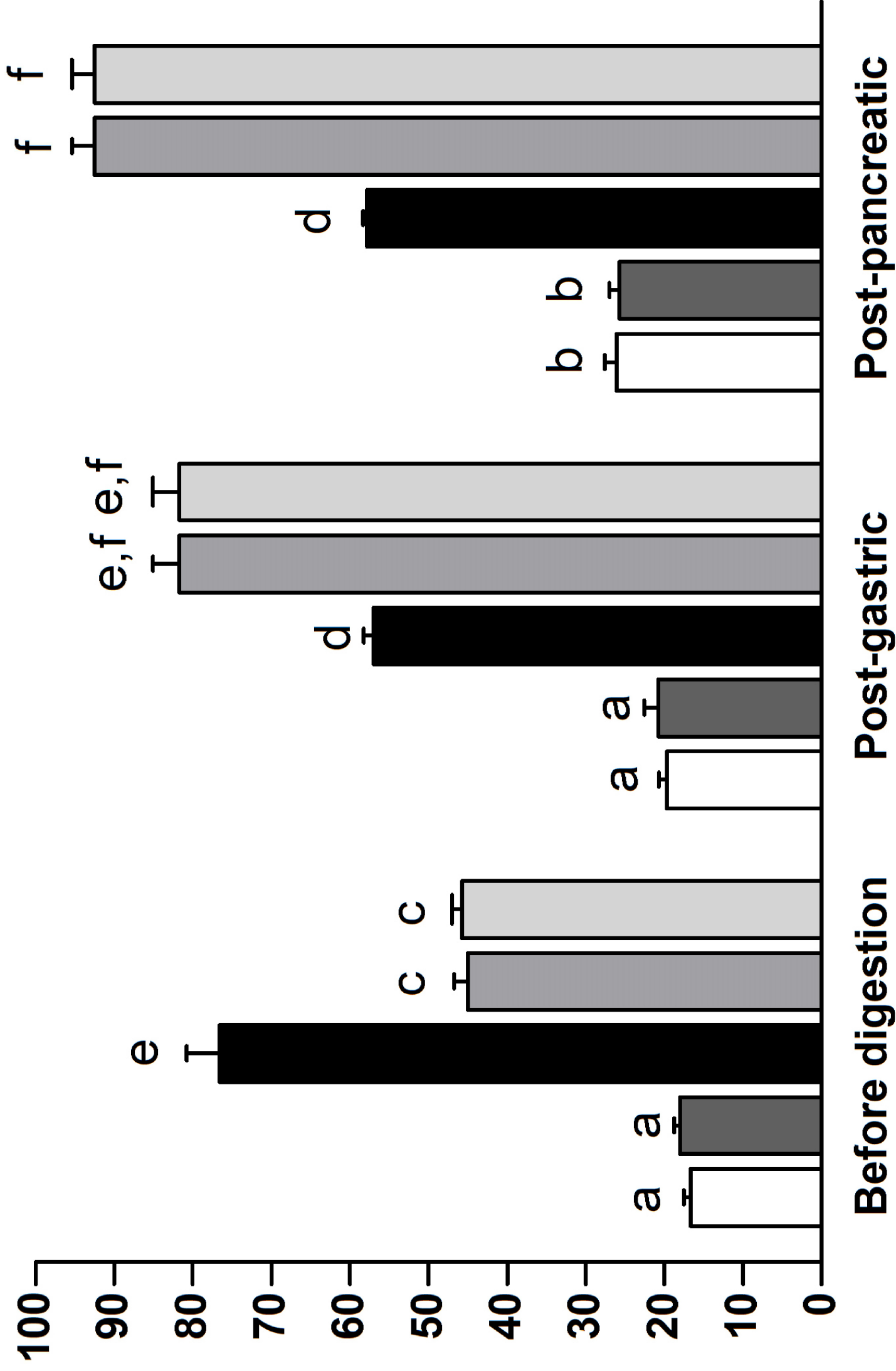
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Figure 1. Experimental strategy for the preparation and characterization of cinnamon-fortified yogurt. This figure details the experimental steps performed for preparing and characterizing cinnamon-fortified yogurt. Milk was formulated starting from full cream milk powder and added at 12% (w/v) concentration. Cinnamon powder was added at 1.5% (w/v) concentration. Sucrose was added at 7.5% (w/v) concentration. Cinnamon water extract was formulated in the same way as the cinnamon-fortified yogurt omitting milk powder from the preparation. After water addition, all the treatments were heat-treated at 95°C for 5 min followed by cooling to 45°C and then inoculated with starter culture and incubated at 45°C until the pH reached 4.4 (~8 h). Abbreviations: HPLC, high performance liquid chromatography.

Figure 2. Total phenolic compounds content measured in the supernatants before and during *in-vitro* digestion. Plain yogurt (□); plain yogurt with sucrose (▤); cinnamon water extract (■); cinnamon fortified yogurt (▥) and cinnamon-fortified yogurt with sucrose (▧). Note that the amount of phenolic compounds in cinnamon water extract (black columns) is referred to 100 g of cinnamon water extract. Values are means of three independent digestions ± standard deviation (SD). Different letters indicate significantly different values ($P < 0.05$).

Figure 3. Radical scavenging properties of yogurts submitted to *in-vitro* digestion. Plain yogurt (□); plain yogurt with sucrose (▤); cinnamon water extract (■); cinnamon-fortified yogurt (▥) and cinnamon fortified yogurt with sucrose (▧). Both ABTS (A) and DPPH (B) results are shown. Note that the radical scavenging activity in cinnamon water extract (black columns) is referred to 100 g of cinnamon water extract. Values are means of three independent digestions ± standard deviation (SD). Different letters indicate significantly different values ($P < 0.05$).





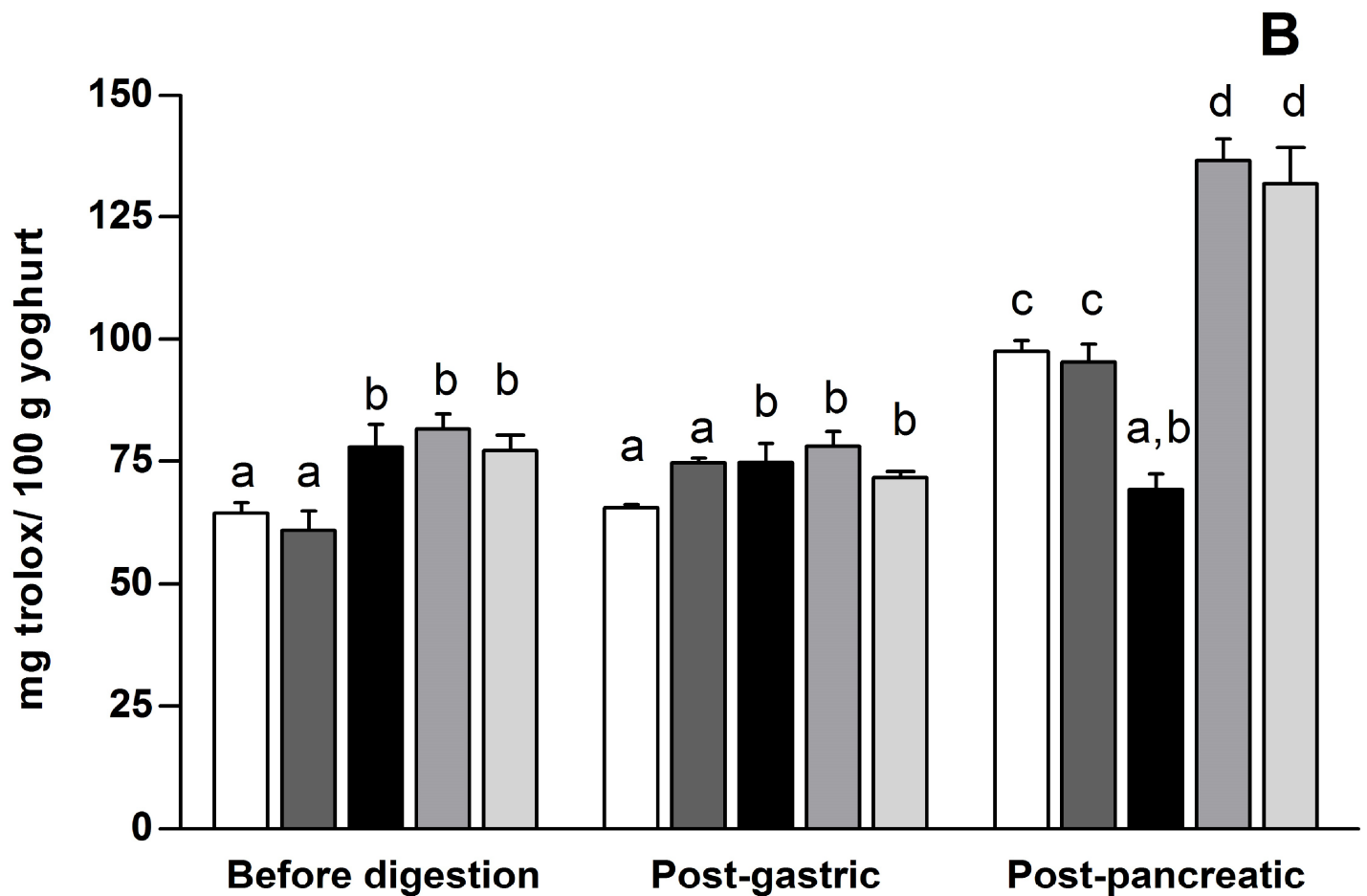
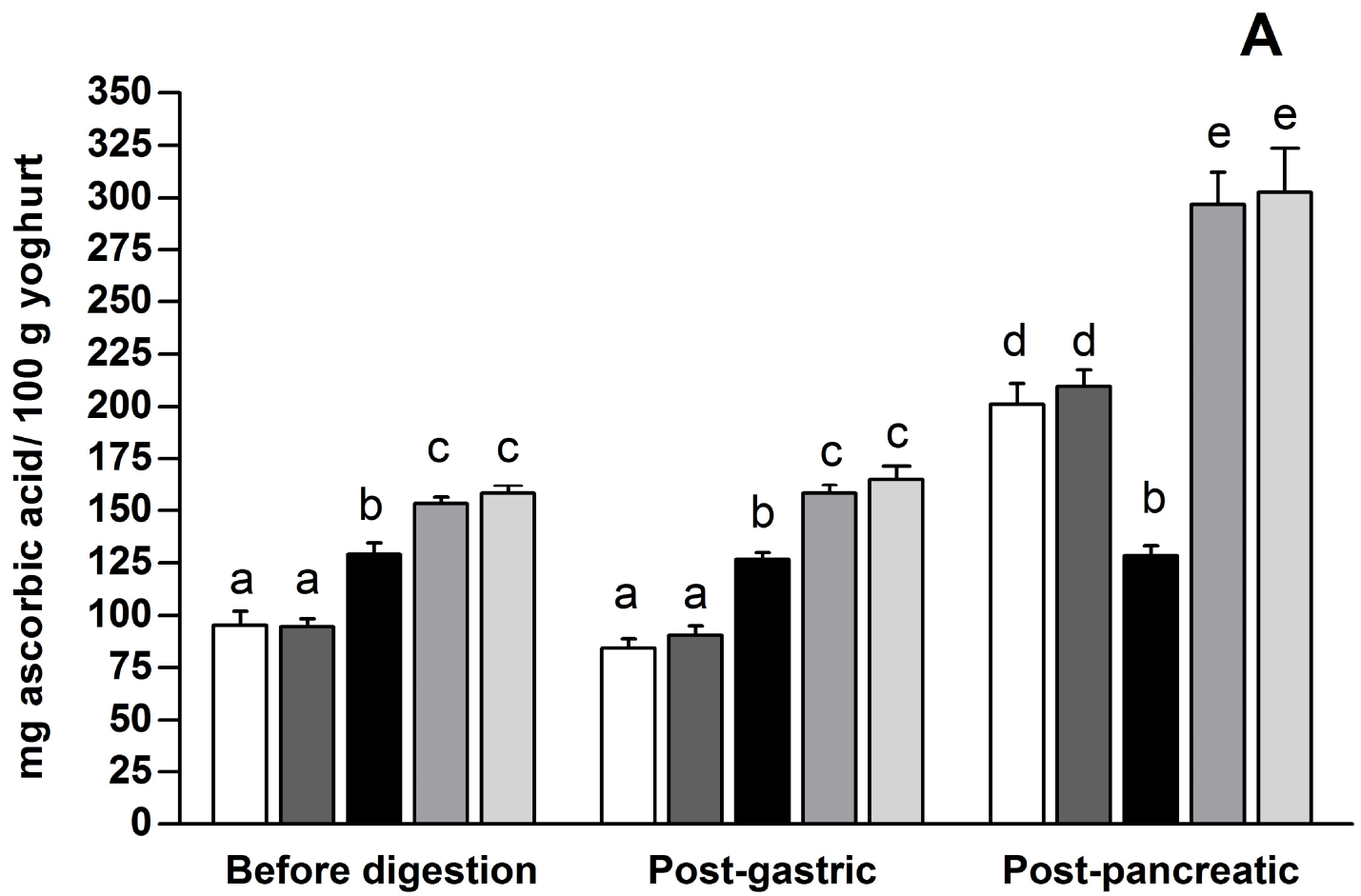


Table 1. Monomeric phenolic compounds and cinnamaldehyde content in cinnamon water extract and cinnamon-fortified yoghurts supernatant determined by HPLC. Results are expressed as µg or mg of individual compound in 100 g of water extract or yoghurt.

	<i>Cinnamon water extract</i>	<i>Cinnamon- fortified yoghurt</i>	<i>Cinnamon-fortified yoghurt with sucrose</i>	<i>Recovery (%)^a</i>
<i>Phenolic acids</i>				
Coumaric acid (µg/100g)	2493.0 ± 15.6 ^a	966.5 ± 34.6 ^b	946.2 ± 19.1 ^b	38.8
Syringic acid (µg/100g)	484.0 ± 8.5 ^a	279.0 ± 4.2 ^b	265.0 ± 17.0 ^b	57.6
Ferulic acid (µg/100g)	153.1 ± 3.2 ^a	82.7 ± 3.3 ^b	85.1 ± 4.9 ^b	54.0
<i>Flavonols</i>				
Quercetin (µg/100g)	29.8 ± 1.1 ^a	16.6 ± 1.1 ^b	16.3 ± 0.3 ^b	55.7
Quercetin-3-rhamnoside (µg/100g)	41.3 ± 1.8 ^a	21.9 ± 1.5 ^b	20.7 ± 1.3 ^{ab}	53.0
Kaempferol (µg/100g)	20.0 ± 0.2 ^a	4.2 ± 0.1 ^b	4.0 ± 0.3 ^b	21.0
Cinnamaldehyde (mg/100g)	53.3 ± 3.3 ^a	18.5 ± 1.9 ^b	18.7 ± 0.6 ^b	34.7

^aThe recovery yield was defined as the percentage ratio between the concentration in the cinnamon-fortified yogurt and the concentration in the cinnamon water extract.

Values represent means ± standard deviation of triplicate determination; different superscript letters within the same row indicate that the values are significantly different (P < 0.05).

Table 2. Effect of *in vitro* digestion on cinnamon monomeric phenolic compounds and cinnamaldehyde in cinnamon water extract and cinnamon-fortified yoghurts. Results are expressed as µg or mg of individual compound in 100g of water extract or yoghurt.

	Monomeric phenolic compounds and cinnamaldehyde						
	Coumaric acid µg/100g	Syringic acid µg/100g	Quercetin-3- rhamnoside µg/100g	Quercetin µg/100g	Kaempferol µg/100g	Ferulic acid µg/100g	Cinnamaldehyde mg/100g
<i>Cinnamon water extract</i>							
Before digestion	2493.0 ± 15.6 ^c	484.0 ± 8.5 ^c	41.3 ± 1.8 ^c	29.8 ± 1.1 ^c	20.0 ± 0.2 ^d	153.1 ± 3.2 ^d	53.3 ± 3.3 ^e
Post peptic	2345.0 ± 77.8 ^d	371.8 ± 28.0 ^d	41.0 ± 2.6 ^c	30.1 ± 2.2 ^c	20.8 ± 1.6 ^d	143.3 ± 3.3 ^{c,d}	51.5 ± 1.3 ^{d,e}
Post pancreatic	1267.5 ± 38.9 ^b	120.8 ± 6.0 ^a	18.0 ± 1.0 ^a	9.9 ± 0.6 ^a	16.9 ± 0.6 ^c	136.7 ± 5.2 ^c	48.3 ± 1.6 ^d
BI%*	50.8	24.9	43.6	33.3	84.5	89.3	90.6
<i>Cinnamon-fortified yoghurt</i>							
Before digestion	966.5 ± 34.6 ^a	279.0 ± 4.2 ^c	21.9 ± 1.5 ^b	16.6 ± 1.1 ^b	4.2 ± 0.1 ^a	82.7 ± 3.3 ^a	18.5 ± 1.9 ^a
Post peptic	995.0 ± 15.6 ^a	242.0 ± 14.1 ^b	21.5 ± 0.7 ^{a,b}	16.9 ± 0.8 ^b	6.3 ± 0.8 ^b	105.4 ± 6.6 ^b	24.2 ± 1.2 ^{b,c}
Post pancreatic	1514.0 ± 22.6 ^c	291.5 ± 14.8 ^c	23.1 ± 1.2 ^b	15.9 ± 0.4 ^b	19.6 ± 0.8 ^d	149.3 ± 1.3 ^d	27.4 ± 1.6 ^c
BI%*	60.7	60.2	55.8	53.4	98.0	97.5	51.5
<i>Cinnamon-fortified yoghurt with sucrose</i>							
Before digestion	946.2 ± 19.1 ^a	265.0 ± 17 ^{b,c}	20.7 ± 1.3 ^{a,b}	16.3 ± 0.3 ^b	4.0 ± 0.3 ^a	85.1 ± 4.9 ^a	18.7 ± 0.6 ^a
Post peptic	981.0 ± 12.7 ^a	234.5 ± 20.5 ^b	20.9 ± 0.9 ^{a,b}	16.8 ± 0.5 ^b	6.4 ± 0.4 ^b	103.9 ± 5.8 ^b	23.3 ± 1.1 ^b
Post pancreatic	1486.5 ± 32.2 ^c	295.5 ± 12.7 ^c	22.8 ± 0.1 ^b	16.1 ± 0.5 ^b	19.2 ± 0.5 ^d	148.0 ± 4.2 ^d	26.9 ± 1.6 ^{b,c}
BI%*	59.6	61.0	55.2	53.9	96.0	96.7	50.4

*Bioaccessibility index (BI%) of monomeric component is the percentage ratio between the post pancreatic concentration and the concentration before the digestion in the cinnamon water extract.

Data are means ± SD (n=3).

^{a-c}Significant differences within the same column are shown by different letters (Tukey's test, $P < 0.05$).