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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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Abstract

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

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

1. Introduction

Developing of functional foods with health promoting natural ingredients has increased in the past decade (Granato, Nunes, & Barba, 2017). The development of new products with potentially positive effect on health using traditional herbs and food, which are known to be safe from the toxicological standpoint, is generally desirable since there is an increasing interest among consumers to look for healthier and natural food (Granato et al., 2017). Traditional herbs and food used to improve the functionality of food are normally chosen because rich in phenolic compounds, which possess strong antioxidant activity and show protective effects against chronic diseases including diabetes, cardiovascular diseases and cancer (Del Rio et al., 2013). In the Middle East and Arab countries, cinnamon powder is a well-known and commonly used food and traditional herbal medicine. Cinnamon showed several beneficial health properties such as anti-tumoural, cardiovascular, cholesterol lowering, and antioxidant activities (Gruenwald, Freder, & Armbruester 2010; Hlebowicz, Darwiche, Bjorgell, & Almer, 2007; Hlebowicz et al., 2009). Cinnamon polyphenols mainly consist of condensed tannins (oligomeric and polymeric procyanidins) and monomeric phenolic compounds such as flavonols and phenolic acids (Gu et al., 2004; Helal, Tagliazucchi, Verzelloni & Conte, 2014). Cinnamaldehyde is also a major component in cinnamon bark, which exhibits several biological effects such as anti-tumoural, pro-apoptotic and anti-inflammatory activities (Chao, et al., 2008; Roussel, Hininger, Benaraba, Ziegenfuss, & Anderson, 2009).

Yogurt is the most popular fermented dairy product and is highly appreciated for its nutritional value and good digestibility (Saint-Eve, Levy, Martin, & Souchon, 2006). Recently, numerous studies underlined the health benefits of yogurt consumption in terms of enhancement of the immune system, improvement of bowel function, protection against colon cancer and *Helicobacter pylori* infection (El-Abbadi, Dao, & Meydani, 2014). The health benefits of yogurt have been ascribed to the presence of bioactive peptides and probiotics (Rutella, Tagliazucchi,

& Solieri, 2016). However, it is not considered a source of phenolic compounds and therefore traditional herbs or food such as spices, fruit juices and grape seed or extract had been used to enhance the phenolic content of yogurt (Karraslan, Ozden, Vardin, & Turkoglu, 2011; Chouchouli et al., 2013; Illupapalayam, Smith, & Gamlath, 2014; Oliveira et al., 2015). Yogurt matrix seems to be an excellent delivery vehicle for plant-derived phenolic compounds. The low pH increase the stability of phenolic compounds during storage (Chouchouli et al., 2013), whereas the presence of proteins or large peptides and fat should maintain the integrity of phenolic compounds during digestion increasing their bioaccessibility (Tagliazucchi, Helal, Verzelloni, & Conte, 2012; Lamothe, Azimy, Bazinet, Couillard, & Britten, 2014).

Bioaccessibility is defined as the amount of a specific compound solubilized in the small intestine and available for the subsequent absorption. The bioaccessibility definition comprises the release of compounds from food matrices and their stability under the gastro-intestinal condition (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). This latter point is of paramount importance since only the compounds released from the food matrix and stable in the gastro-intestinal condition are potentially bioavailable and in condition to exert their beneficial effects on the gastro-intestinal tract.

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

2. Materials and methods

2.1 Materials

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

2.2 Preparation of stirred yogurts and cinnamon water extract

Yogurt preparation and experimental strategy are summarized in **Figure 1**.

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

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

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

2.3 *In-vitro* digestion

Yogurt preparations and cinnamon water extract were subjected to *in-vitro* simulated digestion to determine the effect of digestion on phenolic content and radical scavenging activity. The recent standardized digestion method by Minekus et al. (2014) was followed with some modification as reported in Tagliazucchi, Helal, Verzelloni, Bellesia, & Conte (2016). For the gastric step, samples were diluted with simulated gastric fluid stock electrolyte solution (1:1) and homogenized for 2 min in a laboratory blender. The pH was then lowered to 2.5 with 6 mol/L HCl before the addition of 2000 U/mL of pepsin. Samples were incubated for 2 hours at 37°C. The chyme was then subjected to the pancreatic phase of digestion. Simulated intestinal fluid was added and the pH was brought to 7.5 with 20% Na_2CO_3 before adding 0.8 g/L of pancreatin and 10 mmol/L of bile salts. The digestive mixture was incubated in a shaking bath for additional 2 hours at 37°C.

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

2.4 Samples preparation for analysis

Samples from yogurt preparations, cinnamon-water extract and *in-vitro* digestions were centrifuged at 17500*g* for 10 min at 5°C to eliminate the insoluble material. The clear supernatants were then analysed for the content in total free phenolic compounds, total free tannins and individual free phytochemicals as well as for the radical scavenging activity analysis.

2.5 Determination of total phenolic content total tannins content

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

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

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

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

2.6 Radical scavenging activity analysis

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

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

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

Low molecular weight phytochemicals were identified by using high performance liquid chromatography (HPLC) as previously described by Helal et al. (2014) using a Beckman HPLC (Beckman Coulter, USA), fitted with UV absorbance detector (Perkin Elmer, USA) and equipped with a C18 column (Ascentis® C18 HPLC Column 5 µm particle size, 250×4.6 mm, Sigma-Aldrich Co. LLC). The two solvents were as follows: solvent A mixture of water-formic acid (0.1%) and solvent B acetonitrile. The gradient started at 3% B for 0.5 min then linearly ramped up to 10% B in 10 min. The mobile phase composition was raised up to 40% B in 34 min, then 100% B in 1 min and maintained for 5 min in order to wash the column. The flow rate was 1 mL/min. Peaks for samples and standards were monitored at 360 nm for flavonols and at 270 nm for phenolic acids and cinnamaldehyde. Identification and quantification of phytochemicals in samples were performed comparing to chromatographic retention times and areas of external pure standards.

2.8 Statistical analysis

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

3. Results and Discussion

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

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

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

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

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

The addition of cinnamon powder determined a significant ($P<0.01$) increase in total phenolic compounds in the supernatant of fortified yogurt in comparison with the plain yogurt supernatant (**Figure 2**, before digestion). No significant differences ($P>0.05$) were found between the plain yogurt formulated with sucrose and the plain yogurt without sucrose neither between the cinnamon formulated yogurt and the cinnamon-fortified yogurt with sucrose (**Figure 2**, before digestion). The amount of phenolic compounds in cinnamon-fortified yogurt was 45.0 ± 1.8 mg of catechin/100 g of yogurt, which resulted in a value of 28.3 mg of catechin/100 g of yogurt when corrected for the contribution of plain yogurt (16.7 ± 1.8 mg of catechin/100 g of yogurt). The Folin–Ciocalteu reactivity of plain yogurt is due to the presence of milk compounds different from polyphenols such as low molecular weight antioxidants, free amino acids, peptides and proteins. A comparison with the total phenolic compounds extracted from cinnamon with only water revealed that the amount of total phenolic found in the supernatant of the fortified yogurt was 34.7% of the theoretically expected. It is important to note that total phenolic compounds were quantified in the supernatant of yogurt samples and, in these conditions, only free or unbounded polyphenols are determined. Similarly, Oliveira et al. (2015) and Trigueros, Wojdylo, & Sendra (2014) found a decrease in total phenolic content in yogurts added of strawberry and pomegranate juice respect to the control strawberry and pomegranate juice preparations without yogurts. The low recovery of phenolic compounds in the supernatant of cinnamon-fortified yogurt can be due to the presence of milk proteins that can bind and precipitate cinnamon polyphenols. In a previous study, Helal et al. (2014) found that the addition of 25% milk to a cinnamon beverage determined a decrease of about 28% in total polyphenols content and this decrease is a result of the formation of insoluble complexes between cinnamon tannins and milk proteins. Indeed, the acidic pH, as that found in yogurt because of fermentation, may enhance the binding affinity between phenolic compounds and milk proteins. Hala Mohamed et al. (2015) found that the optimum pH of the interactions between tannins and milk caseins was at pH 5. In general, the formation of insoluble complexes between proteins and tannins is maximum at pH values near the isoelectric point of the protein (Hagerman & Butler, 1978). To gain more information, the interaction of milk proteins with cinnamon tannins was investigated by precipitation assay. Milk proteins at concentration of 3.5% (w/v) were able to precipitate 27.4 ± 0.6 mg of catechin/100 g. This amount of precipitated tannins explain more than 77% of polyphenols lost during yogurt preparation.

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

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

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

The changes in total phenolic content in the formulated samples during the *in-vitro* digestion are shown in **Figure 2**. In the cinnamon water extract, a significant decrease (P<0.05) in total polyphenols, from 76.6 ± 4.2 to 57.0 ± 1.3 mg of catechin/ 100 g of cinnamon water extract, was found after peptic digestion. The subsequent incubation in the pancreatic fluid did not influence the total polyphenols concentration (P>0.05). At the end of the pancreatic digestion, the bioaccessibility index (calculated as the percentage ratio between the post-pancreatic concentration and the total polyphenol concentration before the digestion) of total phenolic compounds in cinnamon water extract was 79.8%. The bioaccessibility index of total phenolic compounds measured after 120 min of simulated gastro-pancreatic digestion is in agreement with that previously determined by Helal et al. (2014) after *in-vitro* digestion of a cinnamon beverage. The formation of insoluble complexes between tannins and pepsin was the explanation of the decrease in total polyphenols found in the Helal et al. (2014) study after gastric digestion. Therefore, we measured the amount of tannins in the cinnamon water extract during *in-vitro* digestion. Results showed that the tannins concentration decreased from 62.1 ± 1.8 mg catechin/100 g of water extract (before the digestion) to 42.8 ± 1.1 mg catechin/100 g of water extract after the gastric phase of digestion. The decrease in tannins content after gastric digestion was 19.3 mg catechin/100 g of cinnamon water extract, which is quite similar to the decrease recorded in total phenolic compounds after gastric digestion of the cinnamon water extract (**Figure 2**). No further changes in the concentration of tannins were found after incubation with the pancreatic fluid.

In the cinnamon-fortified yogurt, after the peptic stage of digestion a significant increase ($P<0.05$) in the total polyphenols concentration was observed. A further, not significant increase ($P>0.05$) was recorded at the end of the pancreatic phase of the digestion. The amount of phenolic compounds in cinnamon-fortified yogurt after gastro-intestinal digestion was 92.5 ± 3.3 mg of catechin/100 g of yogurt, which resulted in a value of 66.4 mg of catechin/100 g of yogurt when corrected for the contribution of plain yogurt (26.1 ± 1.5 mg of catechin/100 g of yogurt). The total polyphenols bioaccessibility index for the cinnamon-fortified yogurt was calculated as percentage ratio between the post-pancreatic concentration corrected for the contribution of plain yogurt and the total polyphenol concentration in the cinnamon water extract before the digestion. The bioaccessibility index in the cinnamon-fortified yogurt was 86.7%, which was significantly higher ($P<0.05$) than that calculated for the cinnamon water extract. The protective effect of yogurt matrix can be due to the initial binding between milk proteins and tannins, which make them no longer available for the interaction with pepsin. As the digestion proceeds, milk proteins are hydrolysed and tannins can be released from milk proteins resulting in an increased total polyphenols bioaccessibility. Sucrose addition to 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 digestion compared to the digested cinnamon water extract (**Table 2**). As reported above, the presence of yogurt matrix determined an initial low recovery yield of the individual phenolic. However, as the digestion proceeded, low molecular weight phenolic compounds were released from the food matrix to the gastro-intestinal fluids. The hydrolysis of caseins during digestion, especially during the pancreatic phase, allowed the release of the bound compounds, resulting in a higher bioaccessibility index respect to the cinnamon water extract. Previous studies showed that the presence of dairy matrices significantly improved the total polyphenols recovery during the digestion, as the interaction between polyphenols and milk proteins exhibited a protective effect (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). This interaction may provide a physical trapping and increase the polyphenols stability during the digestion (Hasni et al., 2011). During *in-vitro* digestion of the cinnamon water extract, cinnamaldehyde was quite stable with a bioaccessibility index of 90.6%. In the case of cinnamon-fortified yogurt, the cinnamaldehyde concentration significantly increased during peptic digestion ($P<0.05$). A further but not significant increase was found also at the end of the pancreatic digestion. However, differently from the monomeric phenolic compounds, the bioaccessibility index of cinnamaldehyde was lower ($P<0.05$) in the cinnamon-fortified yogurt compared to the cinnamon water extract (**Table 2**). The presence of sucrose had no significant effect on phenolic acids, flavonols and cinnamaldehyde bioaccessibility (**Table 2**).

Changes in radical scavenging activity were also evaluated during the *in-vitro* digestion, and the data are presented in **Figure 3**. The radical scavenging activity of plain yogurt progressively increased in both the assays during digestion as a result of the further release of antioxidant peptides and amino acids encrypted in the milk proteins sequences (Tagliazucchi et al., 2016). On the contrary, no significant changes in the radical scavenging activity of the cinnamon water extract were found during the *in-vitro* digestion with both the assays. At the end of the pancreatic digestion, the cinnamon-fortified yogurt showed the highest radical scavenging activity values with both the assays. The presence of sucrose had no significant effect on radical scavenging activity values (**Table 2**).

4. Conclusions

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

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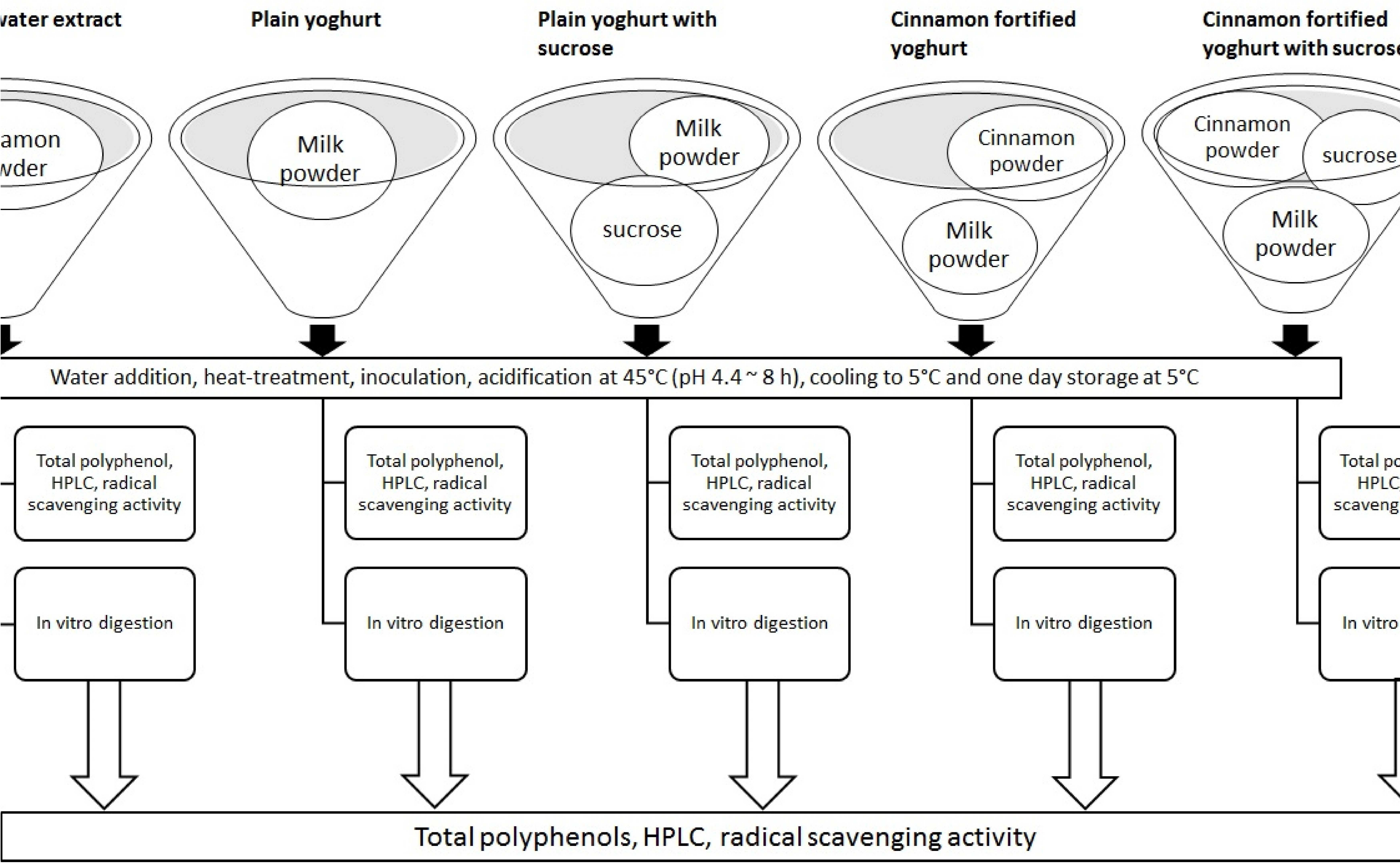
Figure captions

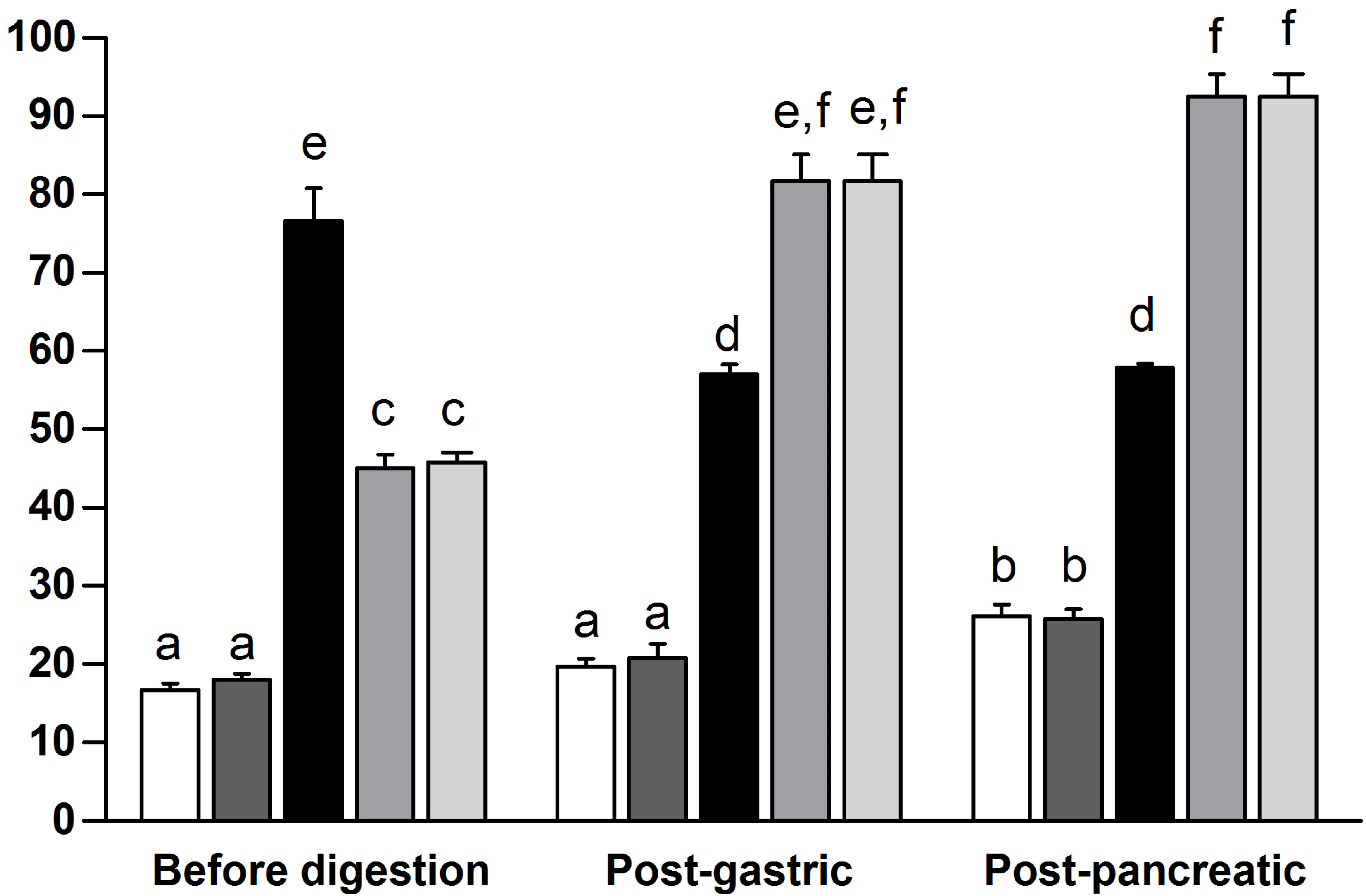
Figure 1. Experimental strategy for the preparation and characterization of cinnamon-fortified yogurt. This figure details the experimental steps performed for preparing and characterizing cinnamon-fortified yogurt. Milk was formulated starting from full cream milk powder and added at 12% (w/v) concentration. 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 ($P < 0.05$).



water extract
Plain yoghurt
Plain yoghurt with sucrose
Cinnamon fortified yoghurt
Cinnamon fortified yoghurt with sucrose
Cinnamon powder
Milk powder
sucrose
Water addition, heat-treatment, inoculation, acidification at 45°C (pH 4.4 ~ 8 h), cooling to 5°C and one day storage at 5°C
Total polyphenol, HPLC, radical scavenging activity
In vitro digestion
Total polyphenols, HPLC, radical scavenging activity


100
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Before digestion
Post-gastric
Post-pancreatic

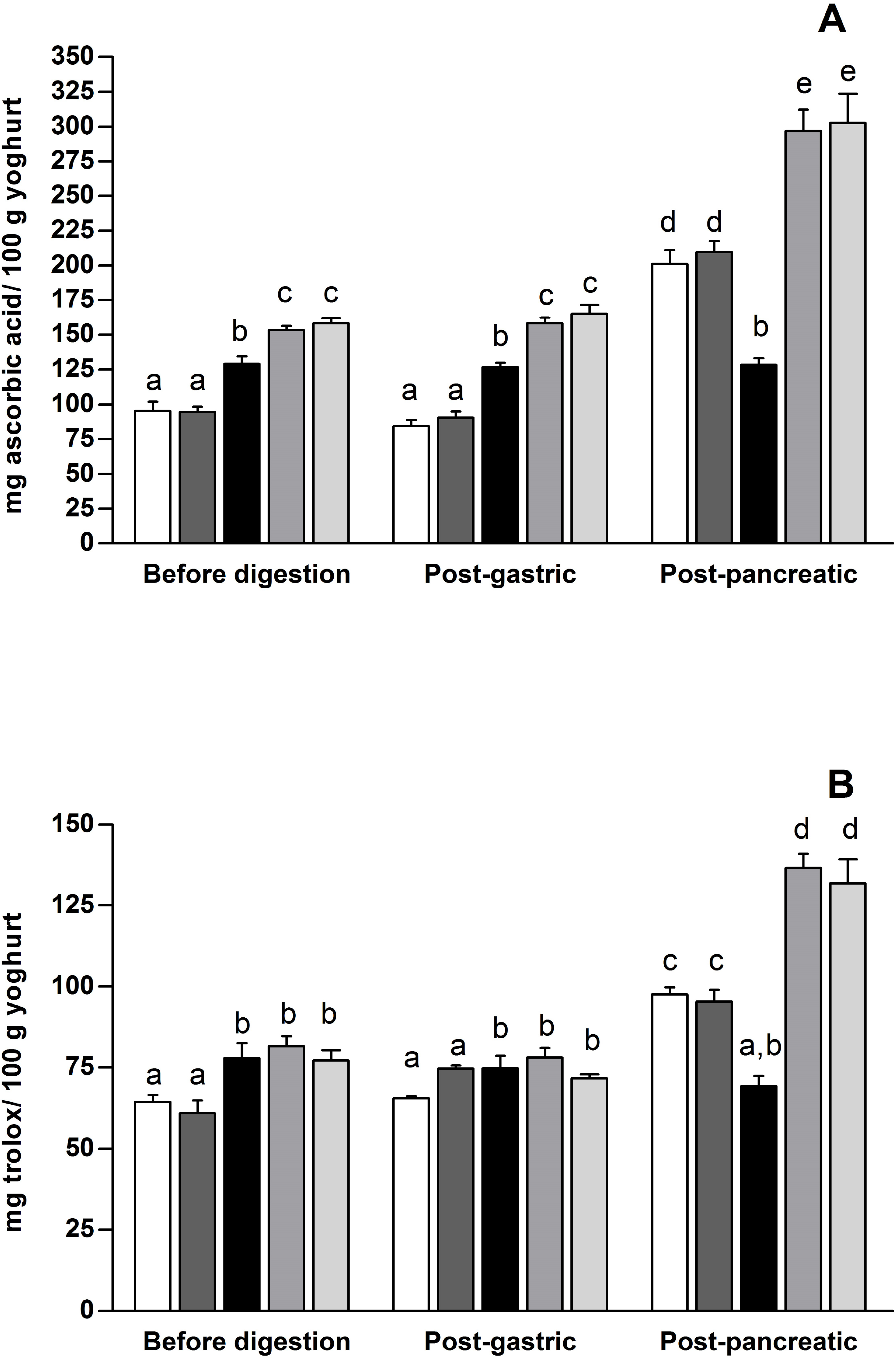
A
mg ascorbic acid/ 100 g yoghurt
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
a a b c c
a a b c c
d d b e e
Before digestion
Post-gastric
Post-pancreatic
B
mg trolox/ 100 g yoghurt
150
125
100
75
50
25
0
a a b b b
a a b b b
c c a,b d d
Before digestion
Post-gastric
Post-pancreatic

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.

	Cinnamon water extract	*Cinnamon-fortified yoghurt*	*Cinnamon-fortified yoghurt with sucrose*	*Recovery (%)[a]*
Phenolic acids				
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
Flavonols				
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

[a]The 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
Cinnamon water extract							
Before digestion	2493.0 ± 15.6^{e}	484.0 ± 8.5^{e}	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**
Cinnamon-fortified yoghurt							
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**
Cinnamon-fortified yoghurt with sucrose							
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).

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