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Effect of simulated processing on the antioxidant capacity and *in vitro* protein digestion of fruit juice-milk beverage model systems

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ABSTRACT

The effects of simulated processing (pH adjustment and thermal treatment) on the antioxidant capacity and *in vitro* protein digestion of fruit juice-milk beverage (FJMB) models consisting of whey protein (WP), and chlorogenic acid (CHA) or catechin (CAT) were investigated. Results indicated that CAT was more susceptible to processing than CHA, and showed a significant ($p < 0.05$) decrease in ABTS and FRAP after sterilization (121 °C/10 min) and pH adjustment to 6.8. WP addition had different effects (none, masking, synergetic effect) on the antioxidant activity of FJMB. Pasteurization (63 °C/30 min) and pH adjustment (pH 3.7 or pH 6.8) had either non-significant or slight effects on FJMB's antioxidant capacity, while sterilization significantly ($p < 0.05$) increased or decreased its ABTS and FRAP depending on the different models. *In vitro* digestion of WP in FJMB was obviously ($p < 0.05$) inhibited by phenolics to varying degrees, and little influenced ($p > 0.05$) by pasteurization, whereas sterilization initially accelerated WP digestion but did not change its overall digestibility.

1. Introduction

In recent years, new functional beverages containing fruit juices and milk have received considerable attention due to the nutritional value of milk protein and the health effects of fruit juice (Cilla et al., 2012; Salvia-Trujillo, Peña, Rojas-Graü, & Martín-Belloso, 2011). Mixed fruit juice and milk beverages are rich in phenolics, vitamins, minerals and fiber, and have become some of the most widely consumed functional foods. Their market potential is currently growing in response to the consumer demand for highly nutritious health foods (Sharma, 2005; Zulueta, Esteve, & Frígola, 2007). The health benefits of fruit juices are mainly attributable to their abundant phenolic compounds including flavonoids such as catechin, quecetin and anthocyanins, and phenolic acids such as chlorogenic acid, caffeic acid, coumaric acid and gallic acid (Mullen, Marks, & Crozier, 2007; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). These possess numerous biological properties such as antioxidant and free radical scavenging activities which can prevent cancers, cardiovascular disease, inflammation, Alzheimer's disease, diabetes and other oxidative stress-induced diseases (Gong, Huang, & Zhang, 2012; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013).

The efficacy of phenolic compounds in fruit juice may be influenced by milk protein. A number of studies on tea, coffee, cocoa and fruit beverages have shown that the addition of milk or milk protein can affect the antioxidant capacity of phenolics due to the polyphenol–protein interactions, but the results are equivocal. Some studies have reported that adding milk to tea decreases the free radical scavenging ability of tea phenolics (Arts et al., 2002; Dubeau, Samson, & Tajmir-Riahi, 2010). Other studies have shown that milk protein was able to bind with coffee polyphenols and inhibit the antioxidant activity of coffee beverages (Niseteo, Komes, Belščak-Cvitanovic, Horzic, & Budec, 2012). Tadapaneni et al. (2012) and Zulueta, Esteve, and Frígola (2009) found that the addition of milk to orange juice and strawberry-based beverages significantly lowers the *in vitro* antioxidant activity of the fruit juice. However, similar studies show that milk or milk proteins had no significant effects on the antioxidant power of polyphenols in tea, coffee and cocoa (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006; Keogh, McInerney, & Clifton, 2007; Van het Hof, Kivits, Weststrate, & Tijburg, 1998). Research suggests that the contradictory results regarding the effect of milk on the antioxidant ability of polyphenol-rich beverages are due to the different antioxidant assay methods used in the experiments (e.g., ABTS,

Abbreviations: WP, whey protein; CHA, chlorogenic acid; CAT, catechin; FJMB, fruit juice-milk beverage; ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing antioxidant power; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TPTZ, 2,4,6-tris (2-pyridyl)-s-triazine; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TEAC, Trolox equivalent antioxidant capacity; DH, degree of hydrolysis; β-CN, β-caseins; β-LG, β-lactoglobulin.

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FRAP, DPPH, or ORAC assay) (Dubeau et al., 2010; Zulueta et al., 2009).

Previous studies have shown that the effect of milk on the antioxidant capacity of tea is associated with the interaction of the tea polyphenols and milk proteins. α-, β-caseins (α-, β-CN) and β-lactoglobulin (β-LG), the major proline-rich proteins in milk, can interact with tea catechins (C, EC, EGC, EGCG) through non-covalent bonds (hydrophobic attraction and hydrogen bonding) and form protein–polyphenol complexes (Hasni et al., 2011; Kanakis et al., 2011). This binding can affect the antioxidant activity of tea catechins by influencing their electron donating ability and decreasing the number of free hydroxyl groups available for oxidation (Arts et al., 2002; Bourassa, Côté, Hutchandani, Samson, & Tajmir-Riahi, 2013). The strong interactions between tea polyphenols and milk proteins can also induce secondary and tertiary structural changes in β-CN and β-LG, which are closely related to the effect of milk on the antioxidant properties of tea polyphenols (Bandyopadhyay, Ghosh, & Ghosh, 2012; Hasni et al., 2011; Kanakis et al., 2011). However, interactions with the polyphenols can also affect the digestibility of food proteins in the gastrointestinal tract. Świeca, Gawlik-Dziki, Dziki, Baraniak and Czyż (2013) observed a reduction in bread protein digestibility *in vitro* from 78.4% to 55% after the bread dough was fortified with 4% onion skin rich in flavonoid compounds. Stojadinovic et al. (2013) reported that non-covalent interactions between β-LG and polyphenol extracts of tea, coffee and cocoa were negatively correlated with the protein's susceptibility to digestion, with stronger polyphenol-β-LG interactions delaying the pepsin and pancreatin *in vitro* digestion of β-LG. Lysozyme presented different results during *in vitro* digestion, after modification by the covalent attachment of phenolic compounds (such as chlorogenic acid, dihydroxybenzenes, ferulic and gallic acid), resulting in the inhibition of peptic digestion of the derivatized lysozyme, whereas its tryptic, chymotryptic and pancreatic hydrolysis were enhanced (Rawel, Kroll, & Rohn, 2001).

Thermal processing and pH adjustment are commonly used unit operations in the food industry. For example, thermal processing is the most widely used preservation method in industrial beverage production (Andrés, Villanueva, Mateos-Aparicio, & Tenorio, 2014). However, thermal treatment can cause organoleptic and nutritional loss, and change the levels of ascorbic acid, phenolic compounds and carotenoids, thereby leading to a decreased antioxidant capacity and other bioactivities. Previous studies have also shown that food processing conditions (e.g., pH, heating temperature) can affect the polyphenol–protein interactions (Bandyopadhyay et al., 2012; Tadapaneni et al., 2012). Hence, it is necessary to assess changes in the antioxidant capacity and protein nutrition of FJMB subjected to food processing, and their correlation with the corresponding polyphenol–protein interactions in FJMB models.

To the best of our knowledge, there have been relatively few similar studies on FJMB models compared to the large body of research on polyphenol–protein interactions and its effect on the antioxidant activity of tea-milk beverages. In particular, there is little available information concerning the polyphenol–protein interaction, antioxidant capacity and milk protein digestion of FJMB systems under food processing conditions. Therefore, the objective of this study was to investigate the effects of pH adjustment and thermal treatment on the antioxidant activity and protein *in vitro* digestion in the FJMB model.

2. Materials and methods

2.1. Materials and chemicals

Whey protein (WP) with a protein content of 90% (w/w, dry basis) was purchased from Davisco Foods International Inc. (Eden Prairie, MN, USA). Standards of chlorogenic acid (CHA) and catechin (CAT) were obtained from Shanxi Sciphar Industry Co., Ltd. (Xian, Shanxi, China). 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), 2,4,6-trinitrobenzenesulfonic acid (TNBS), pepsin and pancreatin were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals, which were of analytical grade, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Distilled deionized water was used throughout the experiments.

2.2. Preparation of FJMB models

WP is the main protein component of milk, and CHA and CAT are the main phenolic compounds in fruit juice. Therefore, these compounds were selected to make up the FJMB models. WP and phenolic compounds (CHA, CAT) were separately dissolved in deionized water, then mixed together by stirring uniformly. The final FJMB model solutions contained WP (0.2% or 0.6%, w/v) and one of the two phenolic compounds (0.01% or 0.025%, w/v). WP solution (0.2% or 0.6%, w/v) without the addition of phenolics and phenolic solutions (0.01% or 0.025%, w/v%) without WP were used as controls.

2.3. Simulated processing of the FJMB

To simulate the pH adjustment and thermal processing of the FJMB, the pH values of the mixture solutions of WP and the phenolic compounds (CHA or CAT) and their controls were adjusted to 3.7 or 6.8 with 0.1 M HCl and 0.1 M NaOH, respectively. pH 6.8 was used for the neutral formulated FJMB product, while pH 3.7 was used for the acidic formulated FJMB product. The solutions were then subjected to heat treatment at 63 °C for 30 min, or at 121 °C for 10 min, separately. After treatment, all of the samples were quickly cooled to room temperature and kept for further analysis.

2.4. ABTS free radical scavenging assay

The ABTS free radical scavenging capacity of the test samples was measured according to the method described by Re et al. (1999) with slight modification. The ABTS solution was prepared by mixing ABTS stock solution (7 mM) with 140 mM potassium persulfate at a ratio of 500:88 (v:v), and then stored for 12–16 h in the dark at room temperature. The ABTS solution was diluted with 60% (v/v) ethanol to an absorbance of 0.70 ± 0.02 at 734 nm before use, and 0.1 ml of the test sample was then mixed with 3.9 ml of the diluted ABTS solution. The mixture was vortexed for 15 s and kept at 30 °C for 10 min, and the absorbance was recorded at 734 nm on a UV-2800H spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). The blank was made from 0.1 ml of 60% (v/v) ethanol and 3.9 ml of diluted ABTS solution. A calibration curve was plotted of absorbance reduction and concentration of the Trolox standard. The ABTS scavenging capacity of the test samples was expressed as Trolox equivalent antioxidant capacity (TEAC) in millimole per liter of sample (mM TEAC/L).

2.5. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the methods reported by Benzie and Strain (1996) with some modification. The FRAP reagent was freshly prepared by mixing 10 mM TPTZ in 40 mM hydrochloric acid, 20 mM $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v) and stored for 1 h at 37 °C. The FRAP reagent (3 ml) and deionized water (0.3 ml) were

added to 0.1 ml diluted test samples, and mixed at 37 °C for 30 min. The absorbance at 593 nm of the mixture was recorded using a UV-2800H spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). Trolox standard solutions (0–600 μM) were used to determine the calibration curves. The ferric reducing activity of the test samples is expressed as TEAC in millimole per liter of sample (mM TEAC/L).

2.6. In vitro simulated gastrointestinal digestion

In vitro gastrointestinal digestion was carried out according to the procedure described by Lo, Farnworth, and Li-Chan (2006). The 0.6% WP solution containing the phenolic compound (0.01% or 0.1%, wt%) was adjusted to pH 2.0 with 1 M HCl, and pepsin (2%, w/w, protein basis) was added. The mixture solution was incubated at 37 °C for 1 h in a shaking water bath. Then, the pH was adjusted to 5.3 with 0.9 M $NaHCO_3$, and pancreatin (2%, w/w, protein basis) was added, and the pH was further adjusted to 7.5 using 1 M NaOH. The digestion solution was incubated in a shaking water bath at 37 °C for another 2 h. During digestion, aliquots (1 ml) of WP digest were collected at 0, 30, 60, 120 and 180 min, and then submerged in a boiling water bath for 10 min to inactivate the pepsin and pancreatin. The aliquots were then cooled and stored at −25 °C for further analysis.

2.7. Determination of the digestibility of WP

The *in vitro* digestibility of WP in the gastrointestinal tract can be determined by measuring the degree of hydrolysis (DH) of protein. The DH of WP was determined using the TNBS method of Adler-Nissen (1979). The aliquots from the hydrolysate sample collected from the digestion process were centrifuged at 11000*g* for 15 min, and 0.25 ml of the supernatant was mixed with 2 ml of 0.2 M sodium phosphate buffer (pH 8.2). After the addition of 2 ml of 0.01% (w/v) TNBS, the solution was incubated at 50 °C for 30 min in a covered water bath (to prevent light). 2 ml of 0.1 M Na_2SO_3 was then added to terminate the reaction. The samples were allowed to cool to room temperature, and the absorbance was read at 420 nm using a UV-2800H spectrophotometer (Unico Instrument Co., Ltd., Shanghai, China). L-leucine (0–2.5 mM) was used to generate a standard curve. The DH value was calculated according to the following equation:

$$DH\ (\%) = 100 \times (AN_2 - AN_1)/Npb,$$

where AN_1, AN_2 is the amino nitrogen content of the protein substrate before and after hydrolysis (mg/g protein), respectively, and Npb is the nitrogen content of the peptide bonds in the protein substrate (mg/g protein). An Npb value of 123.3 was used for the WP. The values of AN_1 and AN_2 were obtained by reference to the L-Leucine standard curve.

2.8. Statistical analysis

All experiments were replicated three times and triplicate sample analyses were performed on each replication. Data are expressed as the mean ± standard deviation (SD) ($n = 9$). Statistical analysis was carried out using the general linear model procedure, using Statistix software 9.0 (Analytical Software, Tallahassee, FL, USA). Significant differences ($p < 0.05$) between means were identified by the least significance difference (LSD) procedure.

3. Results and discussion

3.1. Effect of processing on the antioxidant capacity of individual phenolic compounds in the FJMB model without WP

Two different antioxidant assays (ABTS and FRAP) were used to measure the antioxidant capacity of the FJMB models subjected to different processing methods. The ABTS radical scavenging capacities of each phenolic compound under pH adjustment and thermal treatment are shown in Table 1. The effect of pH on the ABTS scavenging capacity of the individual phenolics was dependent on their concentrations. At a concentration of 0.025% of fruit phenolics, no significant changes ($p > 0.05$) in the ABTS values of CHA and CAT were observed, after they were subjected to pH adjustment (6.8 or 3.7). At the lower concentration of 0.01%, the ABTS scavenging capacity of CHA significantly ($p < 0.05$) increased after pH adjustment to 3.7, compared to the control (no treatment) and pH 6.8. In contrast, the scavenging capacity of CAT reduced significantly ($p < 0.05$) after pH adjustment to both 3.7 and 6.8. However, under

Table 1
ABTS radical scavenging capacities (mM TE/L) of FJMB models under the analog processing conditions.[A]

Samples		No treatment	pH adjustment		Thermal processing			
					63 °C/30 min		121 °C/10 min	
			pH 6.8	pH 3.7	pH 6.8	pH 3.7	pH 6.8	pH 3.7
0.01% CHA		0.38 ± 0.02^{d}	0.39 ± 0.03^{cd}	0.43 ± 0.01^{ab}	0.37 ± 0.02^{d}	0.41 ± 0.00^{bc}	0.38 ± 0.01^{d}	0.45 ± 0.01^{a}
0.025% CHA		0.91 ± 0.02^{bc}	0.91 ± 0.05^{bc}	0.91 ± 0.04^{bc}	0.88 ± 0.06^{c}	0.98 ± 0.06^{ab}	0.91 ± 0.03^{bc}	1.00 ± 0.02^{a}
0.01% CAT		1.39 ± 0.02^{a}	1.34 ± 0.00^{b}	1.35 ± 0.02^{b}	1.23 ± 0.04^{c}	1.36 ± 0.01^{ab}	1.13 ± 0.00^{d}	1.22 ± 0.01^{c}
0.025% CAT		3.21 ± 0.07^{a}	3.08 ± 0.00^{ab}	3.17 ± 0.09^{a}	2.91 ± 0.23^{bc}	3.17 ± 0.18^{a}	2.82 ± 0.00^{c}	3.08 ± 0.00^{ab}
0.2% WP + 0.01% CHA	Mix	0.50 ± 0.01^{fg}	0.49 ± 0.01^{g}	0.53 ± 0.02^{efg}	0.49 ± 0.01^{g}	0.54 ± 0.00^{efg}	0.89 ± 0.06^{ab}	0.90 ± 0.04^{ab}
	Sum	0.58 ± 0.04^{cde}	0.56 ± 0.05^{de}	0.61 ± 0.03^{cd}	0.55 ± 0.02^{ef}	0.63 ± 0.01^{c}	0.87 ± 0.02^{b}	0.94 ± 0.03^{a}
0.2% WP + 0.025% CHA	Mix	1.05 ± 0.03^{de}	1.05 ± 0.02^{de}	1.01 ± 0.07^{e}	1.04 ± 0.01^{de}	1.11 ± 0.02^{d}	1.47 ± 0.08^{ab}	1.48 ± 0.06^{ab}
	Sum	1.10 ± 0.03^{d}	1.09 ± 0.07^{de}	1.10 ± 0.06^{d}	1.06 ± 0.06^{de}	1.20 ± 0.06^{c}	1.40 ± 0.04^{b}	1.50 ± 0.04^{a}
0.6% WP + 0.01% CHA	Mix	0.68 ± 0.02^{g}	0.65 ± 0.00^{g}	0.68 ± 0.02^{g}	0.66 ± 0.00^{g}	0.75 ± 0.00^{f}	1.26 ± 0.02^{c}	1.34 ± 0.02^{b}
	Sum	0.78 ± 0.04^{ef}	0.75 ± 0.06^{f}	0.81 ± 0.04^{e}	0.76 ± 0.02^{ef}	0.89 ± 0.00^{d}	1.35 ± 0.02^{ab}	1.40 ± 0.08^{a}
0.6% WP + 0.025% CHA	Mix	1.49 ± 0.06^{def}	1.39 ± 0.02^{g}	1.51 ± 0.02^{de}	1.42 ± 0.04^{efg}	1.62 ± 0.00^{c}	2.11 ± 0.07^{b}	2.34 ± 0.07^{a}
	Sum	1.42 ± 0.04^{efg}	1.39 ± 0.08^{g}	1.41 ± 0.07^{fg}	1.38 ± 0.08^{g}	1.57 ± 0.09^{cd}	2.13 ± 0.05^{b}	2.27 ± 0.05^{a}
0.2% WP + 0.01% CAT	Mix	1.45 ± 0.02^{hi}	1.43 ± 0.03^{ij}	1.51 ± 0.07^{fgh}	1.38 ± 0.05^{j}	1.51 ± 0.01^{fgh}	1.65 ± 0.01^{bc}	1.77 ± 0.04^{a}
	Sum	1.60 ± 0.04^{cde}	1.54 ± 0.03^{efg}	1.57 ± 0.04^{def}	1.49 ± 0.05^{ghi}	1.61 ± 0.03^{bcd}	1.58 ± 0.04^{de}	1.67 ± 0.03^{b}
0.2% WP + 0.025% CAT	Mix	3.26 ± 0.03^{cdef}	3.23 ± 0.03^{ef}	3.32 ± 0.07^{bcdef}	3.17 ± 0.08^{f}	3.24 ± 0.11^{def}	3.41 ± 0.16^{abcd}	3.58 ± 0.07^{a}
	Sum	3.43 ± 0.10^{abc}	3.28 ± 0.05^{cdef}	3.39 ± 0.09^{bcde}	3.17 ± 0.21^{f}	3.42 ± 0.16^{abc}	3.26 ± 0.05^{cdef}	3.47 ± 0.09^{ab}
0.6% WP + 0.01% CAT	Mix	1.71 ± 0.08^{hi}	1.67 ± 0.05^{i}	1.76 ± 0.07^{fghi}	1.72 ± 0.07^{hi}	1.86 ± 0.12^{def}	2.12 ± 0.02^{c}	2.19 ± 0.18^{c}
	Sum	1.90 ± 0.04^{de}	1.81 ± 0.04^{efgh}	1.85 ± 0.05^{defg}	1.73 ± 0.06^{ghi}	1.95 ± 0.05^{d}	2.35 ± 0.04^{b}	2.48 ± 0.05^{a}
0.6% WP + 0.025% CAT	Mix	3.37 ± 0.03^{efg}	3.23 ± 0.04^{g}	3.42 ± 0.03^{ef}	3.23 ± 0.07^{g}	3.36 ± 0.07^{fg}	4.03 ± 0.06^{b}	4.12 ± 0.22^{b}
	Sum	3.72 ± 0.10^{cd}	3.55 ± 0.06^{de}	3.67 ± 0.11^{cd}	3.41 ± 0.21^{efg}	3.75 ± 0.17^{c}	4.32 ± 0.06^{a}	4.48 ± 0.11^{a}

[A] Values are expressed as the mean ± standard deviation. Different superscripts in the same row for each group sample indicate significant differences ($p < 0.05$).

thermal processing conditions, all concentrations of CHA and CAT exhibited higher ABTS values at pH 3.7 than at pH 6.8 ($p < 0.05$). In regard to the effect of thermal treatment, there were no significant changes in ABTS for CHA and CAT after thermal treatment at 63 °C for 30 min compared to the unheated samples (pH adjustment only). When heated at 121 °C for 10 min, the ABTS radical scavenging potential of CHA showed little change ($p > 0.05$), whereas it was markedly lower for CAT ($p < 0.05$).

The FRAP of the individual phenolics for the processed and unprocessed FJMB models are shown in Table 2. No marked changes ($p > 0.05$) in FRAP were detected for CHA or CAT after pH adjustment (6.8 or 3.7) compared to the controls. However, under thermal processing, CAT had a higher FRAP value at pH 3.7 than at pH 6.8 ($p < 0.05$), whereas the FRAP value of CHA showed little change ($p > 0.05$) at the different pH levels. There were also no significant differences ($p > 0.05$) in the FRAP values for CHA subjected to thermal treatment (63 °C/30 min and 121 °C/10 min) compared to the unheated samples (pH adjustment only). However, the FRAP of CAT after thermal processing was significantly ($p < 0.05$) reduced at pH 6.8, but changed little ($p > 0.05$) at pH 3.7. The difference in the results was not-significant ($p > 0.05$) for thermal processing at 63 °C/30 min and 121 °C/10 min.

In general, the above results clearly show that pH adjustment and thermal processing did not significantly affect the ABTS scavenging activity and FRAP values of CHA in the FJMB model without WP, although pH adjustment to 6.8 slightly reduced the ABTS value of CHA simultaneously subjected to thermal processing. The stable antioxidant activity of CHA may be related to its structural change under various pH values and temperatures. CHA (5-caffeoylquinic acid) in aqueous solution was reported to be isomerized to 3-caffeoylquinic acid and 4-caffeoylquinic acid after heating at different pH values, and the various biological activities (e.g., antioxidant activity) of these isomers were found to be approximately equivalent (Dawidowicz & Typek, 2011; Narita & Inouye, 2013). In this study, the antioxidant capacity of CAT was found to be susceptible to pH adjustment and thermal processing. In particular obvious decreases in the ABTS and FRAP values were observed after thermal processing at 121 °C/10 min and pH adjustment to 6.8. The decrease in antioxidant activity is consistent with previous findings that the degradation of CAT is accelerated with increasing pH and temperature due to oxidation (Li, Taylor, Ferruzzi, & Mauer, 2012).

3.2. Effect of WP addition on the antioxidant capacity of FJMB models (unprocessed and processed)

To investigate the influence of milk on the antioxidant capacity of the FJMB models and determine if there are any synergistic or antagonistic antioxidant related effects between WP and the fruit phenolics (CHA, CAT), the antioxidant capacities (ABTS and FRAP values) of mixtures of WP and phenolics were compared with the sum of the individual antioxidant capacities of WP and the phenolics. As shown in Table 1, under conditions of no treatment, pH adjustment and thermal treatment at 63 °C, the ABTS value of the mixture of WP and CAT was less than the sum of their individual capacities, indicating that WP addition markedly ($p < 0.05$) reduced the ABTS radical scavenging capacity of the FJMB model. This masking of the ABTS scavenging capacity of polyphenols was also observed in green tea combined with bovine β-CN, β-LG, and the extent of the masking was positively correlated with the interaction between the phenolics and proteins (Arts, Haenen, Voss, & Bast, 2001; Arts et al., 2002; Stojadinovic et al., 2013). However, under thermal treatment at 121 °C, the overall ABTS value slightly increased after WP addition at a lower concentration of 0.2% w/v and decreased at 0.6% w/v, showing that there was a synergistic effect in the ABTS scavenging capacities of WP and CAT at a low concentration of WP, but a masking effect at a high concentration of WP. However, the effect of WP addition on the ABTS scavenging capacity of beverages containing CHA depended on the concentration of CHA. At a concentration of 0.01%, the mixture of WP and CHA had a lower ($p < 0.05$) ABTS value than the sum of the individual capacities of each compound, revealing that WP addition caused a masking effect on the ABTS scavenging capacity, whereas there was no significant ($p > 0.05$) difference in the ABTS value in a mixture of WP and CHA, and the sum of their capacities at a higher concentration of 0.025%, implying that WP addition had little influence on the ABTS scavenging capacity of the FJMB model containing CHA.

As shown in Table 2, the fruit phenolics mainly contributed to the total FRAP of the FJMB model as WP had a very weak FRAP

Table 2
FRAP (mM TE/L) of FJMB models under the analog processing conditions.[A]

Samples		No treatment	pH adjustment		Thermal processing			
					63 °C/30 min		121 °C/10 min	
			pH 6.8	pH 3.7	pH 6.8	pH 3.7	pH 6.8	pH 3.7
0.01% CHA		0.50 ± 0.01^{a}	0.49 ± 0.02^{ab}	0.50 ± 0.01^{a}	0.46 ± 0.00^{b}	0.47 ± 0.00^{ab}	0.47 ± 0.01^{ab}	0.48 ± 0.04^{ab}
0.025% CHA		1.19 ± 0.02^{ab}	1.20 ± 0.04^{a}	1.20 ± 0.04^{a}	1.13 ± 0.07^{ab}	1.16 ± 0.06^{ab}	1.09 ± 0.08^{b}	1.22 ± 0.07^{a}
0.01% CAT		1.07 ± 0.02^{a}	1.06 ± 0.01^{a}	1.05 ± 0.01^{a}	0.97 ± 0.03^{bc}	1.06 ± 0.02^{a}	0.93 ± 0.05^{c}	1.03 ± 0.09^{ab}
0.025% CAT		2.35 ± 0.00^{a}	2.28 ± 0.00^{b}	2.33 ± 0.01^{a}	2.14 ± 0.03^{c}	2.36 ± 0.00^{a}	2.14 ± 0.02^{c}	2.35 ± 0.05^{a}
0.2% WP + 0.01% CHA	Mix	0.52 ± 0.02^{bc}	0.52 ± 0.02^{bc}	0.52 ± 0.02^{bc}	0.49 ± 0.03^{bcde}	0.50 ± 0.01^{bcde}	0.53 ± 0.05^{ab}	0.57 ± 0.03^{a}
	Sum	0.50 ± 0.01^{bcde}	0.49 ± 0.02^{bcde}	0.50 ± 0.01^{bcde}	0.46 ± 0.03^{cde}	0.48 ± 0.04^{cde}	0.47 ± 0.03^{de}	0.51 ± 0.05^{bcd}
0.2% WP + 0.025% CHA	Mix	1.24 ± 0.04^{abc}	1.22 ± 0.03^{bc}	1.21 ± 0.04^{bcd}	1.13 ± 0.04^{de}	1.17 ± 0.04^{bcde}	1.21 ± 0.01^{bcd}	1.32 ± 0.01^{a}
	Sum	1.19 ± 0.02^{bcd}	1.20 ± 0.04^{bcd}	1.20 ± 0.04^{bcd}	1.13 ± 0.07^{de}	1.16 ± 0.06^{cde}	1.09 ± 0.08^{e}	1.25 ± 0.08^{ab}
0.6% WP + 0.01% CHA	Mix	0.52 ± 0.03^{ef}	0.53 ± 0.01^{def}	0.54 ± 0.02^{de}	0.52 ± 0.01^{ef}	0.54 ± 0.01^{de}	0.61 ± 0.02^{bc}	0.68 ± 0.02^{a}
	Sum	0.52 ± 0.01^{ef}	0.51 ± 0.02^{ef}	0.52 ± 0.01^{ef}	0.49 ± 0.04^{f}	0.51 ± 0.04^{ef}	0.57 ± 0.02^{cd}	0.62 ± 0.04^{b}
0.6% WP + 0.025% CHA	Mix	1.22 ± 0.01^{cd}	1.18 ± 0.04^{de}	1.23 ± 0.01^{cd}	1.10 ± 0.02^{e}	1.20 ± 0.03^{cd}	1.28 ± 0.02^{bc}	1.55 ± 0.07^{a}
	Sum	1.21 ± 0.02^{cd}	1.22 ± 0.04^{cd}	1.22 ± 0.04^{cd}	1.16 ± 0.07^{de}	1.19 ± 0.06^{d}	1.19 ± 0.09^{d}	1.35 ± 0.07^{b}
0.2% WP + 0.01% CAT	Mix	1.04 ± 0.05^{b}	1.03 ± 0.02^{bc}	1.05 ± 0.02^{ab}	0.91 ± 0.01^{de}	0.95 ± 0.02^{d}	0.82 ± 0.03^{f}	0.85 ± 0.03^{ef}
	Sum	1.07 ± 0.02^{ab}	1.05 ± 0.01^{ab}	1.11 ± 0.01^{a}	0.97 ± 0.03^{cd}	1.06 ± 0.02^{ab}	0.93 ± 0.05^{d}	1.09 ± 0.09^{ab}
0.2% WP + 0.025% CAT	Mix	2.43 ± 0.06^{abc}	2.39 ± 0.04^{bcde}	2.46 ± 0.04^{a}	2.28 ± 0.03^{fg}	2.35 ± 0.01^{de}	2.03 ± 0.03^{i}	2.22 ± 0.07^{g}
	Sum	2.45 ± 0.04^{ab}	2.38 ± 0.01^{cde}	2.46 ± 0.05^{a}	2.34 ± 0.03^{ef}	2.46 ± 0.02^{a}	2.14 ± 0.02^{h}	2.41 ± 0.06^{abcd}
0.6% WP + 0.01% CAT	Mix	1.06 ± 0.02^{bcde}	1.03 ± 0.04^{cde}	1.02 ± 0.08^{cde}	0.93 ± 0.05^{fg}	1.01 ± 0.02^{de}	0.91 ± 0.04^{g}	1.03 ± 0.01^{cde}
	Sum	1.08 ± 0.02^{bcd}	1.07 ± 0.01^{bcde}	1.12 ± 0.01^{ab}	1.00 ± 0.03^{ef}	1.09 ± 0.02^{bc}	1.03 ± 0.05^{cde}	1.17 ± 0.10^{a}
0.6% WP + 0.025% CAT	Mix	2.34 ± 0.04^{cde}	2.27 ± 0.19^{def}	2.37 ± 0.01^{bcd}	2.21 ± 0.01^{fg}	2.36 ± 0.04^{bcd}	2.12 ± 0.06^{g}	2.22 ± 0.05^{fg}
	Sum	2.46 ± 0.03^{ab}	2.40 ± 0.03^{abc}	2.48 ± 0.03^{a}	2.37 ± 0.03^{bcd}	2.49 ± 0.02^{a}	2.25 ± 0.02^{ef}	2.49 ± 0.06^{a}

[A] Values are expressed as the mean ± standard deviation. Different superscripts in the same row for each group sample indicate significant differences ($p < 0.05$).

value. Under conditions of no treatment, pH adjustment and thermal treatment at 63 °C, no evident difference ($p > 0.05$) in FRAP was found between the mixture of WP and phenolics (CHA, CAT). The sum of their individual capacities, except for a decrease in FRAP after mixing the WP and CAT at higher concentrations (i.e., 0.6%, 0.025%, respectively), indicates that WP addition did not induce an obvious variation in FRAP for the FJMB models containing CHA or CAT. Moreover, the masking effect in FRAP only appeared when 0.6% WP was added to 0.025% CAT, whereas under thermal treatment at 121 °C, this FRAP masking effect appeared between WP and CAT at any concentration, and there was a synergistic effect in FRAP between WP and CHA.

Numerous studies have examined the effects of proteins on the antioxidant capacity of phenolic compounds, which is affected by the interaction between the proteins and phenolics. However, these studies have produced contradictory results, possibly due to the different antioxidant assaying methods used (Dubeau et al., 2010; Ozdal, Capanoglu, & Altay, 2013). In this study, WP addition was found to have no effect, an inhibitory (masking) effect and an enhancing (synergistic) effect on the antioxidant capacity in the FJMB models. The inhibitory (masking) effect of proteins on the antioxidant activity of polyphenol-rich fruit juices such as strawberry and orange juice has been observed in previous studies (Tadapaneni et al., 2012; Zulueta et al., 2009). However, few previous studies have reported an enhancing (synergistic) effect under thermal processing conditions. Moreover, the results of this study suggest that the different effects of protein on the antioxidant capacity of phenolics not only arose from the different methods of antioxidant measurement applied, but may also have depended on the various protein–phenolics interactions, which were subsequently affected by the variety and concentrations of the proteins and phenolics, and the processing conditions, in this case the pH level and temperature. Two types of interaction between phenolics and proteins, namely covalent and non-covalent interactions, usually lead to the development of protein–phenolic complexes or precipitation via multi-site interactions (several phenolics bound to one protein) or multidentate interactions (one phenolic bound to multiple sites of one or many proteins) (Arts et al., 2002; Ozdal et al., 2013). These variable interactions could account for the different effects of proteins on the antioxidant activity of phenolics (Ozdal et al., 2013).

3.3. Effect of processing on the antioxidant capacity of the FJMB models

The effects of food processing on the ABTS radical scavenging ability of the FJMB models are shown in Table 1. After pH adjustment (pH 6.8 or 3.7) and thermal processing at 63 °C/30 min, there were no significant changes ($p > 0.05$) in the ABTS values in the mixtures of WP and fruit phenolics (CHA, CAT), indicating that pH adjustment and pasteurization (63 °C/30 min) did not greatly influence the ABTS scavenging capacity of the FJMB models, except for the slight increase in ABTS, observed in the FJMB model of 0.6% WP and CHA under pasteurization at pH 3.7, compared to pH 6.8, and for the FJMB model of WP and CHA at pH 3.7 after pasteurization (63 °C/30 min) compared to the unheated samples (pH adjustment only). In contrast, the ABTS values of the FJMB models were markedly ($p < 0.05$) increased after heating at 121 °C/10 min compared to the unheated samples (pH adjustment only), indicating that sterilization processing (121 °C/10 min) enhanced the ABTS scavenging capacity of the FJMB models.

The changes in FRAP after processing are shown in Table 2. No significant differences ($p > 0.05$) in FRAP were apparent for the FJMB models after pH adjustment to 6.8 or 3.7, suggesting that pH adjustment did not significantly affect the FRAP of the FJMB models, except for those subjected to thermal processing, which showed a slightly higher FRAP value at pH 3.7 than at pH 6.8. In regard to the effect of thermal processing, after heat treatment at 63 °C/30 min, the FRAP of the FJMB models consisting of WP and CHA had changed little ($p > 0.05$), whereas it decreased significantly for the FJMB models containing WP and CAT ($p < 0.05$), with the exception of the model with high concentrations (i.e., 0.6% WP and 0.025% CAT), which did not significantly differ ($p > 0.05$) in regard to FRAP. However, after thermal processing at 121 °C/10 min, the FRAP of the FJMB models of WP and CHA increased significantly ($p < 0.05$), whereas it was reduced for the WP and CAT models.

The results of this study indicated that pH adjustment did not have a significant effect on the antioxidant capacity (ABTS and FRAP assays) of FJMB models, except for the slight increase in FRAP observed for the FJMB model that simultaneously underwent thermal treatment and adjustment to pH 3.7. Pasteurization (63 °C/30 min) did not significantly change the FRAP of the FJMB model consisting of WP and CHA, or the ABTS scavenging capacity of the FJMB models containing WP and phenolics (CHA, CAT), although it induced a slight increase in ABTS for the WP and CHA models at pH 3.7 and a slight decrease in FRAP for the WP and CAT models. Sterilization (121 °C/10 min) did enhance the ABTS scavenging capacity of the FJMB models containing WP and the phenolics (CHA or CAT) and the FRAP of the WP and CHA models, although it caused an obvious decrease in FRAP in the WP and CAT beverage models. Compared to the effect of food processing on the antioxidant capacity of the FJMB model with no WP, a similar trend was observed for the influence of pH adjustment and pasteurization (63 °C/30 min) on the antioxidant capacity of the FJMB model with WP, whereas there was a distinct difference in the effect of sterilization (121 °C/10 min) on the antioxidant activity of the FJMB models with and without WP. This could be related to the thermal behavior of WP. The denaturation temperature of β-LG, the major globular protein in WP, was previously reported to vary with pH, between 70 °C (at pH 7) and 85 °C (at pH 3) (De Wit, 2009). Thermal denaturation of β-LG at 121 °C was shown to cause structural and conformational changes in the protein, interior sulfhydryl groups and some amino acid residues (tyrosine, tryptophan, phenylalanine, cysteine, methionine) exposed at the surface, thereby increasing activities such as radical scavenging capacity (De Wit, 2009). Similarly, in this study, it was observed that a masking or synergistic effect on the antioxidant activity between WP and the phenolics in the FJMB models was altered after thermal treatment at 121 °C. For instance, the masking effect on the ABTS scavenging capacity for 0.2% WP and 0.01% CAT was transformed into a synergistic effect after sterilization at 121 °C/10 min, and at the same time, the absence of effect in regard to FRAP changed to a masking effect. These results suggest that sterilization may influence the interaction (bonding affinity) between WP and phenolics via unfolding the structure of WP, increasing the accessibility of its reactive sites (Li, Du, Jin, & Du, 2012), thus affecting the WP-phenolic complex formation and further altering their structure and antioxidant potential (Bourassa et al., 2013; Kanakis et al., 2011).

Nevertheless, the results of this study, in regard to the effect of thermal processing on the antioxidant capacity of the FJMB models, do not completely agree with some of the existing research on real FJMB systems. Tadapaneni et al. (2012) reported that heat treatment at 72 °C/20 s significantly decreased the antioxidant capacity of a strawberry-milk beverage as determined by ORAC and FRAP compared to unprocessed beverages. Barba, Cortés, Esteve, and Frígola (2012) found a significant ($p < 0.05$) overall decrease in ABTS and ORAC values for an orange juice-milk beverage after thermal treatment at 98 °C/21 s. These findings may be due to the presence of other antioxidant components, such as vitamin C, besides CHA and CAT, which would have contributed to the total antioxidant activity in the real fruit juice.

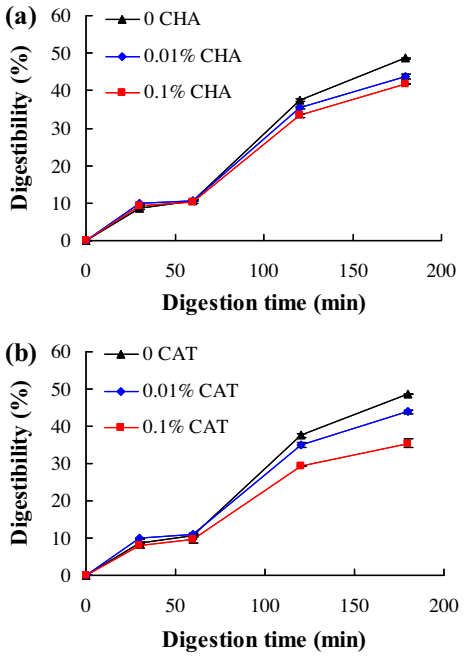

Fig. 1. The *in vitro* digestibility of WP in unprocessed FJMB models containing (a) CHA or (b) CAT.

3.4. Effect of fruit phenolics on the in vitro digestion of WP in unprocessed FJMB models

As shown in Fig. 1, simulated gastric digestion of 0.6% w/v WP showed relatively low hydrolization (DH < 11%) with pepsin at pH 2.0. A number of studies reported that β-LG, the main protein component of WP, exhibited a stable globular tertiary structure at low pH (<pH 3) and that its highly hydrophobic β-barrel made it very difficult for enzymes to access the target peptide bonds (Bateman, Ye, & Singh, 2010). In this study, no significant differences ($p > 0.05$) in the DH of WP were observed for the unprocessed FJMB models containing different concentrations of phenolics (0%, 0.01%, 0.1%), suggesting that the fruit phenolics (CHA and CAT) in the beverage models did not notably influence the *in vitro* gastric digestion of WP. During 2 h of intestinal digestion at pH 7.5, WP presented much higher hydrolysis (DH of up to 49%) by pancreatin compared to gastric digestion by pepsin. Moreover, delayed intestinal digestion of WP was observed in the presence of fruit phenolics (CHA, CAT), and the DH of the WP decreased significantly ($p < 0.05$) with the increasing CHA and CAT concentrations, meaning that CHA and CAT significantly ($p < 0.05$) inhibited WP digestion in the intestinal tract. Furthermore, with 0.1% CAT, the beverage model displayed a relatively stronger inhibitory effect on the intestinal digestion of WP compared to CHA. After the completion of the intestinal digestion, the DH of WP was reduced by 10% when mixed with 0.01% fruit phenolics (CHA, CAT), and decreased by 14% after mixing with 0.1% CHA (Fig. 1a), whereas

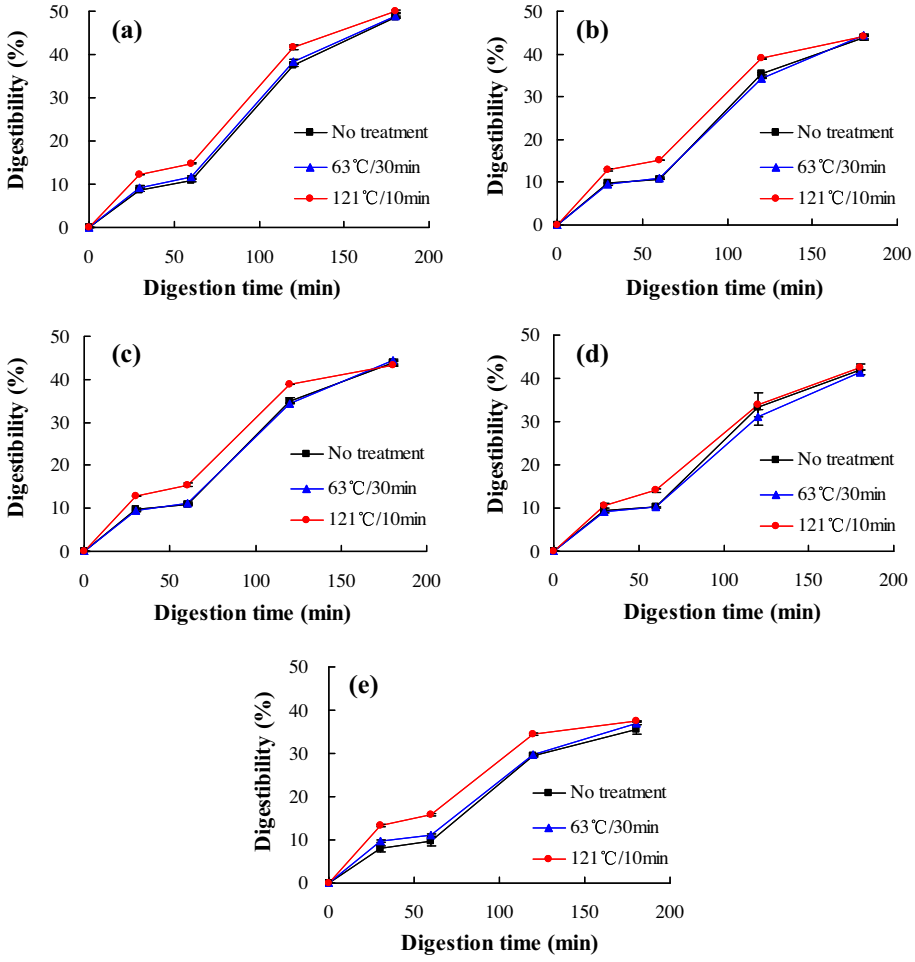

Fig. 2. The *in vitro* digestibility of WP in FJMB models (unprocessed or thermally processed) containing (a) no phenolics, (b) 0.01% CHA, (c) 0.01% CAT, (d) 0.1% CHA, (e) 0.1% CAT.

it reduced by 27% with 0.1% CAT (Fig. 1b). Some studies observed that the inhibition of proteolytic enzymes by dietary phenolics may be one of the reasons for the reduction in protein digestibility in the gastrointestinal tract (Rawel et al., 2001). However, the significant decrease in the number of free amino groups in the WP detected after the addition of CHA and CAT (data not shown), indicates that the delay in the intestinal digestion of WP may be positively correlated to the strong binding affinity of CHA and CAT to WP at neutral pH, and the subsequent formation of aggregates and precipitation. These formations could have shielded the enzyme's target sites in the proteins from pancreatin hydrolysis, thus leading to the prolonged survival of intact proteins in the intestinal fluids (Stojadinovic et al., 2013).

3.5. Effect of thermal processing on the in vitro digestion of WP in the FJMB models

FJMB models consisting of WP and fruit phenolics (CHA, CAT) with or without thermal treatment were subjected to *in vitro* digestion. As shown in Fig. 2, no statistical differences ($p > 0.05$) were found in DH for 0.6% WP in the FJMB models without thermal treatment and samples treated by heating at 63 °C/30 min during gastrointestinal digestion, suggesting that pasteurization (63 °C/30 min) did not significantly affect the digestion of WP in the FJMB model. In contrast, the DH of WP in the FJMB models subjected to heating at 121 °C/10 min increased significantly ($p < 0.05$) compared to the samples without thermal treatment and thermally treated at 63 °C/30 min during gastrointestinal digestion, suggesting that sterilization (121 °C/10 min) facilitated the digestion of WP in the gastrointestinal tract. These results were in accordance with previous findings that the *in vitro* digestion of β-LG heated at 90 °C improved compared to native samples (Pinto et al., 2014). After heating at 121 °C, globular proteins in WP denatured heavily, leading to the unfolding of protein molecules and the exposure of numerous hydrophobic amino acids (peptic cleavage sites) originally buried inside the hydrophobic core, which thus increased the susceptibility of β-LG to hydrolysis by pepsin and the duodenal enzymes (Barbé et al., 2013; Mandalari, Mackie, Rigby, Wickham, & Mills, 2009).

In addition, it is interesting to note that in the initial gastric digestion, the digestion rate of 0.6% WP in the FJMB models heated at 121 °C/10 min was evidently higher than those without treatment and heated at 63 °C/30 min. DH of WP increased by 37% for FJMB models without phenolics (Fig. 2a), 41% and 36% for FJMB models containing 0.01% and 0.1% CHA (Fig. 2b and d), 42% and 64% for models containing 0.01% and 0.1% CAT (Fig. 2c and e), respectively. However, the digestion rate was subsequently much lower in the last 60 min in the intestinal tract. After 2 h of intestinal digestion, little difference ($p > 0.05$) was observed in the DH of WP for the FJMB models heated at 121 °C/10 min, 63 °C/30 min and without thermal treatment, indicating that sterilization (121 °C/10 min) initially accelerated the digestion of WP in the FJMB models but did not change the overall digestibility (DH) of the WP.

4. Conclusions

The results of this study revealed that the antioxidant potential of FJMB models containing WP and fruit phenolics (e.g., CHA, CAT) was associated with the type of phenolic, the concentrations of WP and phenolics used, the fruit juice processing (i.e., pH adjustment, thermal treatment) and the antioxidant measuring method used (e.g., ABTS and FRAP assays). WP addition had different effects (no effect, masking, synergistic effect) on the antioxidant activity of the FJMB models with and without processing treatment, which may have been modulated by different protein–phenolic interactions. Compared with the non-significant or slight effects of pH adjustment (pH 3.7, pH 6.8) and pasteurization (63 °C/30 min) on the antioxidant capacity of the FJMB models, sterilization (121 °C/10 min) significantly ($p < 0.05$) increased the ABTS scavenging capacity of the FJMB models and the FRAP of the models including WP and CHA, but decreased the FRAP of the WP and CAT models. However, the *in vitro* digestion of WP in FJMB was inhibited by the fruit phenolics to different extents, and changed little ($p > 0.05$) after pasteurization (63 °C/30 min), whereas it initially accelerated but did not tend to change the overall digestibility (DH) of the WP when the FJMB was subjected to sterilization (121 °C/10 min). In future research, the characterization of the protein–phenolics interaction in the FJMB model under different food processing conditions and its influence on the bioactivity and nutritional properties of FJMB products should be intensively examined.

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References

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry, 27*, 1256–1262.

Andrés, V., Villanueva, M., Mateos-Aparicio, I., & Tenorio, M. (2014). Colour, bioactive compounds and antioxidant capacity of mixed beverages based on fruit juices with milk or soya. *Journal of Food and Nutrition Research, 53*, 71–80.

Arts, M. J., Haenen, G. R., Wilms, L. C., Beetstra, S., Heijnen, C., Voss, H. P., et al. (2002). Interactions between flavonoids and proteins: Effect on the total antioxidant capacity. *Journal of Agricultural and Food Chemistry, 50*, 1184–1187.

Arts, M. J., Haenen, G. R., Voss, H. P., & Bast, A. (2001). Masking of antioxidant capacity by the interaction of flavonoids with protein. *Food and Chemical Toxicology, 39*, 787–791.

Bandyopadhyay, P., Ghosh, A. K., & Ghosh, C. (2012). Recent developments on polyphenol–protein interactions: Effects on tea and coffee taste, antioxidant properties and the digestive system. *Food & Function, 3*, 592–605.

Barba, F. J., Cortés, C., Esteve, M. J., & Frígola, A. (2012). Study of antioxidant capacity and quality parameters in an orange juice–milk beverage after high-pressure processing treatment. *Food and Bioprocess Technology, 5*, 2222–2232.

Barbé, F., Ménard, O., Le Gouar, Y., Buffière, C., Famelart, M. H., Laroche, B., et al. (2013). The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids. *Food Chemistry, 136*, 1203–1212.

Bateman, L., Ye, A., & Singh, H. (2010). In Vitro digestion of β-Lactoglobulin fibrils formed by heat treatment at low pH. *Journal of Agricultural and Food Chemistry, 58*, 9800–9808.

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Bourassa, P., Côté, R., Hutchandani, S., Samson, G., & Tajmir-Riahi, H. A. (2013). The effect of milk alpha-casein on the antioxidant activity of tea polyphenols. *Journal of Photochemistry and Photobiology B: Biology, 128*, 43–49.

Cilla, A., Alegría, A., Ancos, B., Sánchez-Moreno, C., Cano, M. P., Plaza, L., et al. (2012). Bioaccessibility of tocopherols, carotenoids, and ascorbic acid from milk- and soy-based fruit beverages: Influence of food matrix and processing. *Journal of Agricultural and Food Chemistry, 60*, 7282–7290.

Dawidowicz, A. L., & Typek, R. (2011). The influence of pH on the thermal stability of 5-caffeoylquinic acids in aqueous solutions. *European Food Research and Technology, 233*, 223–232.

De Wit, J. N. (2009). Thermal behaviour of bovine β-lactoglobulin at temperatures up to 150 °C. *Trends in Food Science & Technology, 20*, 27–34.

Dubeau, S., Samson, G., & Tajmir-Riahi, H. A. (2010). Dual effect of milk on the antioxidant capacity of green, Darjeeling, and English breakfast teas. *Food Chemistry, 122*, 539–545.

Dupas, C. J., Marsset-Baglieri, A. C., Ordonaud, C. S., Ducept, F. M. G., & Maillard, M. N. (2006). Coffee antioxidant properties: Effects of milk addition and processing conditions. *Journal of Food Science, 71*, S253–S258.

Gong, L. X., Huang, L. L., & Zhang, Y. (2012). Effect of steam explosion treatment on barley bran phenolic compounds and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 60*, 7177–7184.

Hasni, I., Bourassa, P., Hamdani, S., Samson, G., Carpentier, R., & Tajmir-Riahi, H. A. (2011). Interaction of milk α- and β-caseins with tea polyphenols. *Food Chemistry, 126*, 630–639.

Kanakis, C. D., Hasni, I., Bourassa, P., Tarantilis, P. A., Polissiou, M. G., & Tajmir-Riahi, H. A. (2011). Milk β-lactoglobulin complexes with tea polyphenols. *Food Chemistry, 127*, 1046–1055.

Keogh, J. B., McInerney, J., & Clifton, P. M. (2007). The effect of milk protein on the bioavailability of cocoa polyphenols. *Journal of Food Science, 72*, S230–S233.
Li, B., Du, W., Jin, J., & Du, Q. (2012). Preservation of (-)-epigallocatechin-3-gallate antioxidant properties loaded in heat treated β-lactoglobulin nanoparticles. *Journal of Agricultural and Food Chemistry, 60*, 3477–3484.
Li, N., Taylor, L. S., Ferruzzi, M. G., & Mauer, L. J. (2012). Kinetic study of catechin stability: Effects of pH, concentration, and temperature. *Journal of Agricultural and Food Chemistry, 60*, 12531–12539.
Lo, W. M. Y., Farnworth, E. R., & Li-Chan, E. C. Y. (2006). Angiotensin I-converting enzyme inhibitory activity of soy protein digests in a dynamic model system simulating the upper gastrointestinal tract. *Journal of Food Science, 71*, S231–S237.
Mandalari, G., Mackie, A. M., Rigby, N. M., Wickham, M. S. J., & Mills, E. N. (2009). Physiological phosphatidylcholine protects bovine β-lactoglobulin from simulated gastrointestinal proteolysis. *Molecular Nutrition & Food Research, 53*, S131–S139.
Mullen, W., Marks, S. C., & Crozier, A. (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *Journal of Agricultural and Food Chemistry, 55*, 3148–3157.
Narita, Y., & Inouye, K. (2013). Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. *Journal of Agricultural and Food Chemistry, 61*, 966–972.
Niseteo, T., Komes, D., Belščak-Cvitanovic, A., Horzic, D., & Budec, M. (2012). Bioactive composition and antioxidant potential of different commonly consumed coffee brews affected by their preparation technique and milk addition. *Food Chemistry, 134*, 1870–1877.
Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein–phenolic interactions and associated changes. *Food Research International, 51*, 954–970.
Pinto, M. S., Léonil, J., Henry, G., Cauty, C., Carvalho, A. F., & Bouhallab, S. (2014). Heating and glycation of β-lactoglobulin and β-casein: Aggregation and in vitro digestion. *Food Research International, 55*, 70–76.
Rawel, H. M., Kroll, J., & Rohn, S. (2001). Reactions of phenolic substances with lysozyme-physicochemical characterisation and proteolytic digestion of the derivatives. *Food Chemistry, 72*, 59–71.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.
Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolics compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.
Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2013). Changes in vitamin C, phenolic, and carotenoid profiles throughout in vitro gastrointestinal digestion of a blended fruit juice. *Journal of Agricultural and Food Chemistry, 61*, 1859–1867.
Salvia-Trujillo, L., Peña, M. M., Rojas-Graü, M. A., & Martín-Belloso, O. (2011). Microbial and enzymatic stability of fruit juice-milk beverages treated by high intensity pulsed electric fields or heat during refrigerated storage. *Food Control, 22*, 1639–1646.
Sharma, R. (2005). Market trends and opportunities for functional dairy beverages. *Australian Journal of Dairy Technology, 60*, 195–198.
Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., et al. (2013). Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chemistry, 136*, 1263–1271.
Świeca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B., & Czyż, J. (2013). The influence of protein-flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. *Food Chemistry, 141*, 451–458.
Tadapaneni, R. K., Banaszewski, K., Patazca, E., Edirisinghe, I., Cappozzo, J., Jackson, L., et al. (2012). Effect of high-pressure processing and milk on the anthocyanin composition and antioxidant capacity of strawberry-based beverages. *Journal of Agricultural and Food Chemistry, 60*, 5795–5802.
Van het Hof, K. H., Kivits, G. A. A., Weststrate, J. A., & Tijburg, L. B. (1998). Bioavailability of catechins from tea: The effect of milk. *European Journal of Clinical Nutrition, 52*, 356–359.
Zulueta, A., Esteve, M. J., & Frígola, A. (2007). Carotenoids and color of fruit juice and milk beverage mixtures. *Journal of Food Science, 72*, C457–C463.
Zulueta, A., Esteve, M. J., & Frígola, A. (2009). ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chemistry, 114*, 310–316.