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Original Article

Boost anti-oxidant activity of yogurt with extract and hydrolysate of cinnamon residues

Pei-ling Tang[a,b,c], Er-wei Hao[a,c], Jia-gang Deng[a,c], Xiao-tao Hou[a,c,*], Zuo-hui Zhang[a,c], Jin-ling Xie[a,c]

[a] Guangxi Key Laboratory of Efficacy Study on Chinese Materia Medica, Guangxi University of Chinese Medicine, Nanning 530200, China
[b] Department of Bioscience, Faculty of Applied Sciences, Tunku Abdul Rahman University College, Jalan Genting Kelang, Setapak, W. P. Kuala Lumpur 53300, Malaysia
[c] Collaborative Innovation Center for Research on Functional Ingredients of Agricultural Residues, Guangxi University of Chinese Medicine, Nanning 530200, China

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ABSTRACT

Objective: This study was conducted to explore the potential use of cinnamon residues (twigs and leaves) in boosting the anti-oxidant activity of yogurt.
Methods: The cinnamon bark was used as the benchmark. The extracts of cinnamon bark (BW), twigs residue (TW), and leaves residue (LW) were prepared by using water, whereas the hydrolysates of cinnamon bark (BE), twigs residue (TE) and leaves residue (LE) were prepared via cellulase hydrolysis. The extracts and hydrolysates were then co-fermented respectively with the skimmed milk to produce yogurt.
Results: Results obtained indicated that BW and TE yogurt possessed the highest anti-oxidant activity. *In vitro* digestion improved the anti-oxidant activity of yogurt significantly ($P < 0.05$). DPPH activity of the LW yogurt was improved drastically after *in vitro* digestion. Although the total phenolic content (TPC) and total flavonoids content (TFC) of LW were lower than BW, the anti-oxidant activity of LW yogurt was not significantly different ($P < 0.05$) with the BW yogurt after digestion.
Conclusion: This study suggested that the anti-oxidant activity of the cinnamon yogurt was influenced by complex protein-phenolic interactions.

1. Introduction

China is one of the main yogurt producers worldwide. In order to fulfill consumer demands on natural products, both food scientists and entrepreneurs are unceasingly innovating varieties of yogurts. Recently, herbal extracts have been gained attention. Many researches had concluded that bioactivity and functionality of yogurt can be enhanced through fortification with herbal extracts such as cinnamon, clove, lemongrass etc. (Granato et al., 2018).

Cinnamomum cassia Presl is the main cinnamon species widely cultivated in Guangxi, Guangdong, Yunnan, Hainan, and Guizhou Provinces in China. Furthermore, it is also distributed in India, Vietnam, Sri Lanka, Madagascar, and Seychelles (He, Zeng, Jiang & Tu, 2016; Li et al., 2013). It is commonly used as spice and flavoring agent in cooking, as well as drug in traditional Chinese medicine. The barks and leaves are commonly used to treat broad range of diseases and known to exhibit anti-oxidant, anti-bacterial, anti-fungal, anti-diabetic, anti-inflammatory, nematicidal, insecticidal, and anti-cancer effects (Vasconcelos, Croda & Simionatto, 2018). In traditional Chinese medicine, the dried cinnamon twigs are used to relieve dyspepsia, gastritis, blood circulation disturbances and inflammatory diseases (Liu et al., 2018). In the study by Helal and Tagliazucchi (2018), the bioactivity and bioaccessibility of yogurt had been successfully enhanced by adding cinnamon bark powder. The post-digestive phenolics recovery and radical scavenging activity of the produced cinnamon-fortified yogurt were significantly higher than the cinnamon aqueous extract and plain yogurt alone. Their finding recommended that yogurt is an ideal polyphenols protective carrier along the human digestion tract.

Various researches have been conducted to isolate bioactive compounds from different parts of *C. cassia*, such as barks, twigs and leaves. These researches suggested that a variety of phytochemicals were present in different parts of *C. cassia* (He et al., 2016; Li et al., 2013; Liu et al., 2018). Nonetheless, based on our literature review, most *C. cassia* researches were focused on the investigation of its medicinal efficacy and bioactivity. Sharma, Chauhan and Singh (2018) proved the anti-arthritic properties of *C. cassia* barks extract, while Lee et al. (2018) and

* Corresponding author at: Guangxi Key Laboratory of Efficacy Study on Chinese Materia Medica, Guangxi University of Chinese Medicine, Nanning 530200, China
E-mail address: xthou@126.com (X.-t. Hou).

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Lin et al. (2017) proposed that *C. cassia* was a potential anti-cancer drug. Moreover, Shin et al. (2017) also suggested that *C. cassia* extract exhibited protective effects against gout and septic. In the study by Yan et al. (2015), sesquiterpenoids of *C. cassia* was proven effective for the treatment of diabetic nephropathy; While Goswami, Inamdar, Jamwal and Dethe (2014) proved the use of methanol extract of *C. cassia* barks to improve sexual function. Moreover, Kang, Racicot, Pilkenton and Apostolidis (2014) proved the anti-hyperglycemic activity of *C. cassia* barks. Furthermore, majority of the research topics were concentrated on its essential oil (Chang, Cheng, Wang, Chou & Shih, 2016; Jeon, Lee, Yang & Lee, 2017; Jeyaratnam et al., 2016; Kim, Kim, Koh, Clark & Ahn, 2006; Sun et al., 2017, 2016; Tao et al., 2016). Currently, cinnamon essential oil is widely used in food and cosmetic industries. Nonetheless, intensive research is still on-going to broaden its application in active food packaging, pharmaceuticals, nanomaterials etc. (Ribeiro-Santos et al., 2017). According to Zhai, Liu, Wang, Wu and Kluenter (2018), about 10% of the 3000 known essential oils in the world are commercially important. Besides anise, oregano, garlic, thyme, black pepper and turmeric, cinnamon is one of the most representative essential oil in the commercial market. The recent renewed growth of global essential oil market is expected to reach 11.67 billion USD by 2022.

Therefore, we strongly believed that the demands of cinnamon essential oil and its related products will continuously increase in the near future. Meanwhile, we can foresee that massive accumulation of cinnamon residues (twigs and leaves) will be the upcoming waste management issue in the related industry following high demands of cinnamon essential oil in the market. Up until recently, there is no report or research being published on the uses of cinnamon residues left behind after essential oil production. In order to widen the applications of this valuable herb, we are the pioneer to explore the potential of cinnamon twigs and leaves residue for yogurt fermentation. The cinnamon bark was used as the benchmark in this study. Initially, the hydrolysates of the cinnamons (bark, twig residue and leaf residue) were prepared through cellulase hydrolysis. While, the aqueous extracts of the cinnamons (bark, twig residue and leaf residue) were produced under the same condition of cellulase hydrolysis in the absence of cellulase enzyme. Yogurt fermentation then proceeded in the skimmed milk containing cinnamons aqueous extracts and hydrolysates respectively. The total phenolic content (TPC), total flavonoids content (TFC), and anti-oxidant activity (DPPH) of different type of yogurts were determined before and after simulated *in vitro* gastro-pancreatic digestion.

2. Materials and methods

2.1. Materials

The dried *C. cassia* barks, twigs residues and leaves residues were supplied by Guangxi Gengyuan Flavor and Fragrance Co., Ltd. The twigs and leaves residues were the by-product remained after steam distillation process in the cinnamon essential oil production. The dried barks, twigs residue and leaves residue were ground into particle size distribution in the range of 0.18–2.00 mm, 0.85–2.00 mm and 0.85 mm less than 5.00 mm, respectively. The commercial *Lactobacillus* yogurt starter containing *L. bulgaricus, Streptococcus thermophilus, L. acidophilus, L. plantarum*, and *L. casei* was manufactured by Beijing Chuanxiu Technology Ltd. All chemicals and enzymes used were analytical grade.

2.2. Methods

2.2.1. Total compositional analysis

Total composition of cinnamon bark, twigs residue and leaves residue were determined according to NREL standard protocol (National Renewable Energy Laboratory, USA) with slight modification. The moisture content was determined by drying the samples at 105 °C for 15 h in an oven (Sluiter et al., 2008a). Total ash content was determined by igniting the samples at 550 °C for 15 h in a furnace (Sluiter et al. 2008b). For total extractives analysis, the samples were refluxed with distilled water for 6 h, followed by absolute ethanol for 6 h in Soxhet extractor (Sluiter, Ruiz, Scarlata, Sluiter & Templeton, 2008c). The extractive free samples were then hydrolyzed in 72% sulfuric acid at 30 °C for 1 h, followed by hydrolysis with 4% sulfuric acid at 121 °C for 1 h. The solid residues remained after acid hydrolysis was filtered and recorded as total Klason lignin (Sluiter et al., 2012). Total carbohydrate was estimated based on the total reducing sugars content in the hydrolysate. Total reducing sugars content was determined according to DNS (2,3-dinitrosalicyclic acid) method. About 0.1 mL of sample/glucose standard was mixed with 0.3 mL DNS reagent. The mixture was then heated in boiling water bath for 5 min. The absorbance was measured at 540 nm by using PerkinElmer EnVision Manager software of EnVision® Xcite microplate reader (PerkinElmer, USA). Total reducing sugars content was quantified by interpolate the glucose calibration curve.

2.2.2. Preparation of cinnamon bark (BE), twigs residue (TE) and leaves residue (LE) hydrolysate

About 10% substrate (cinnamon bark, twigs residue, leaves residue) was immersed in purified water. The pH of suspension was adjusted with 0.1 mol/L hydrochloric acid to 4.8 at room temperature. The suspension was pretreated at 130 °C for 30 min in an autoclave, followed by enzymatic hydrolysis with food grade cellulase enzyme (Ningxia Heshibi Biotechnology Co., Ltd.) at 50 °C and 180 rpm in a stirred-tank reactor. The enzyme activity was (197.5 ± 2.1) FPU/g. The bark, twigs residue and leaves residue were hydrolyzed at enzyme loading of 1: 0.12, 1:0.1, and 1:0.08 (substrate: enzyme) respectively for 74 h.

2.2.3. Preparation of cinnamon bark (BW), twigs residue (TW) and leaves residue (LW) extract

About 10% substrate (cinnamon bark, twigs residue, leaves residue) was immersed in purified water at pH 4.8 at room temperature. The suspension was then pretreated at 130 °C for 30 min in an autoclave, followed by agitation at 180 rpm and 50 °C for 74 h in a stirred-tank reactor.

2.2.4. Yogurt fermentation

About 13 g skimmed milk powder (Nouriz, New Zealand) was dissolved in 100 mL purified water (control), cinnamon aqueous extracts and cinnamon hydrolysates respectively to prepare skimmed milk at 4.2% protein content. The milk was pasteurized at 95 °C for 10 min in a water bath. After cooled down to 42 °C, about 2 g of yogurt starter was added and mixed well. The fermentation was proceeded at 42 °C for 9 h. Fermentation was terminated by kept the yogurt at 4 °C for at least 12 h prior to analysis (Gao, Yu, He, Tang & Zeng, 2018).

2.2.5. Yogurt characterization

The pH of yogurt was determined by using pH meter (Leici PHS-3C, Shanghai) while the titratable acidity was determined according to method of Gao et al. (2018). The titratable acidity was expressed as g lactic acid/100 g yogurt (or % lactic acid).

Prior analysis, the yogurt samples were centrifuged at 10 000 rpm for 3 min to separate the casein curd. The supernatant was collected for the subsequent analysis. Total phenolic content (TPC) and total flavonoids content (TFC) were determined according to method of Samantha, Shyamsundarachary, Srinivas and Swamy (2012) with slight modification. Briefly, about 790 μL of

purified water was added into 10 μL sample/gallic acid standard solution, followed by the additional of 50 μL 0.1 N Folin-Ciocalteu reagent and mixed well. After 1 min, about 150 μL of 20% aqueous sodium carbonate was added. The mixture was kept in dark at room temperature for 2 h. The absorbance of mixture was measured spectrophotometrically at wavelength 760 nm by using PerkinElmer EnVision Manager software of EnVision® Xcite microplate reader (PerkinElmer, USA). For TFC assay, about 0.5 mL sample/catechin standard solution was mixed with 0.15 mL 5% sodium nitrate and 2 mL water. After kept at room temperature for 6 min, about 0.15 mL of 10% aluminum chloride was added, followed by the addition of 2 mL 4% NaOH in the subsequent 6 min. The mixture was kept at room temperature for 15 min and its absorbance was measured at wavelength 510 nm.

The DPPH (1,1-diphenyl-2-picrylhydrazyl) activity was determined according to method of Helal and Tagliazucchi (2018) with modification. Briefly, about 50 μL sample was mixed with 600 μL of 60 μmol/L DPPH in methanol. After stand in dark at room temperature for 20 min, the absorbance was measured at wavelength 517 nm. The radical scavenging activity (%) was calculated as inhibition percentage (%) = $[(ABS_{control}\text{-}ABS_{sample})/ABS_{control}] \times 100$.

In order to investigate the effect of digestion on the produced yogurts, gastro-pancreatic digestion was simulated according to Jin, Yu, Wang, Yan and Zou (2016) protocol with slight modification. Briefly, about 10 mL of yogurt sample was mixed with 5 mL 35 mmol/L sodium chloride. The mixture was homogenized and the pH was adjusted to 2 with HCl. The mixture was digested with pepsin (~3.7 KU/mg, Sigma, U.S.) at ratio 1:100 (enzyme: substrate) and 37 °C for 2 h. Once the digestion is completed, the pH was adjusted to 7 by using NaOH. The hydrolysate was further digested with pancreatic enzymes consisted of 1:100 trypsin (~2 KU/mg), 1:100 chymotrypsin (~40 U/mg), 1:500 elastase (3.8 U/mg) and 1:100 carboxypeptidase A (~57 U/mg). The digestion was proceeded for 2 h at 37 °C. The hydrolysate was then heated to 50 °C for 5 min to terminate the enzymes activity. TPC, TFC and DPPH activity of the hydrolysates were determined according to the procedures as described above.

2.2.6. Statistical analysis

All experiments were in triplicates and the data was expressed as mean ± standard deviation. The statistical software SPSS 20.0 (SPSS Inc., Chicago, USA) was used to conduct analysis of variance (ANOVA) and post-hoc Duncan's test. The differences were significant when $P < 0.05$ at 95% confidence level.

3. Results and discussion

3.1. Total composition of cinnamon bark, twigs residue, and leaves residue

Table 1 showed the composition of cinnamon bark, twigs residue and leaves residue. The results revealed that cinnamon bark contains higher ash [(5.66 ±< 0.00)%] and carbohydrates [(42.44 ± 0.85)%] content than twigs and leaves residue. According to Ribeiro-Santos et al. (2017), cinnamon bark contained lower ash (2.4%–2.89%) than the twigs (3.6%–3.9%). This variation can be explained by the discrepancy of macro- and micro-nutrients of cinnamon in different growth condition, harvest time, climate, origin, geographic parameters etc. Nonetheless, our results of carbohydrates were in agreement with Ribeiro-Santos et al. (2017). The carbohydrates content of bark and twig was reported at 47.25% and 28.1%–36.6%, respectively. Moreover, the twigs residue had also been found to contain the highest Klason lignin, followed by the leaves residue and lastly the bark. This finding was also in accordance with the study by Ribeiro-Santos et al. (2017), where the cinnamon bark and twig had been found contain about 33% and 43%–53% fiber respectively. High lignin content unveiled that cinnamon twigs and leaves residues are high potential substrate for bioactive aromatic compounds recovery in the downstream process. Cinnamon is well recognized with its broad variety of bioactive compounds such as rutin, protocatechuic acid, cinnamic acid, ferulic acid, epicatechin, coumaric acid, syringic acid etc., which are also the building units and derivatives of lignin polymer (Ribeiro-Santos et al., 2017; Xu et al., 2018). So far, there was no literature reporting the macronutrients composition of cinnamon leaves. Therefore, no composition reference available for the cinnamon leaves residue.

Table 1
Total composition of cinnamon bark, cinnamon twigs residue and cinnamon leaves residue (mean ± SD, $n = 3$).

Compositions	Cinnamon bark/%	Cinnamon twigs residue/%	Cinnamon leaves residue/%
Moisture	11.67 ± 1.53	13.01 ± 0.83	13.69 ± 0.55
Ash	5.66 ±< 0.00	2.21 ± 0.10	2.98 ± 0.71
Total extractives	21.36 ± 1.48	18.70 ± 0.72	21.15 ± 0.78
Klason lignin	34.70 ± 1.59	45.81 ± 2.17	38.97 ± 0.47
Carbohydrates	42.44 ± 0.85	33.55 ± 0.50	38.15 ± 2.03

3.2. Characterization of cinnamons aqueous extracts and hydrolysates

Table 2 summarized the TPC, TFC and DPPH activity of the aqueous extracts (BW, TW, LW) and hydrolysates (BE, TE, LE) of cinnamon bark, twigs residue, and leaves residue. The results revealed that BW contains the highest TPC, TFC, and DPPH activity. Moreover, TPC and TFC of TW were no significant differences ($P > 0.05$) with TE. The similar results had also been observed for LW and LE. These findings suggested that enzymatic hydrolysis did not exert significant effect ($P > 0.05$) on the recovery of TPC and TFC from the twigs and leaves residues. Nonetheless, enzymatic hydrolysis was significantly reduced ($P < 0.05$) the TPC and TFC of the cinnamon bark. This result disclosed that the effect of enzymatic hydrolysis on different cinnamon substrates was distinct. However, different results had been observed in the DPPH test. TE and BW possessed higher DPPH activity than TW and BE respectively, whereas the DPPH activity of LW and LE were no significant differences ($P > 0.05$).These findings suggested that TPC and TFC were not the only factors that influenced the DPPH activity of cinnamon aqueous extracts and hydrolysates. According to Sharma, Kumar and Rao (2008), phenolic compounds can interact with sugar, consequently diminished their radical scavenging activity. Similar findings have also been reported by Nakilcioglu-Tas (2018), Wipatanawin, Phongsawanit, Maneeratprasert, Lertsiri and Deetae (2015) and Korir, Wachira, Wanyoko, Ngure and Khalid (2014). According to Korir et al. (2014), glucose is likely to interact with the phenolics to form complex

Table 2
TPC, TFC, and DPPH anti-oxidant activity of different cinnamon aqueous extracts and hydrolysates (mean ± SD, $n = 3$).

Samples	TPC/(mg GAE·mL^{-1})	TFC/(mg·mL^{-1})	DPPH/(% inhibition)
BW	0.54 ± 0.04^{b}	6.05 ± 0.13^{d}	21.98 ± 2.07^{b}
BE	0.45 ± 0.02^{b}	2.37 ± 0.05^{c}	15.39 ± 2.60^{a}
TW	0.27 ±< 0.00^{a}	0.64 ± 0.02^{a}	14.29 ± 1.04^{a}
TE	0.28 ± 0.01^{a}	0.60 ± 0.01^{a}	19.23 ± 0.26^{b}
LW	0.30 ± 0.01^{a}	1.11 ± 0.03^{b}	19.05 ± 0.73^{ab}
LE	0.38 ± 0.02^{ab}	1.04 ± 0.09^{b}	16.36 ± 1.18^{a}

*a-d: Different alphabets in the same column indicated that there are significant differences among the samples ($P < 0.05$).
*BW: Cinnamon bark aqueous extract; BE: Cinnamon bark hydrolysate; TW: Twigs residue aqueous extract; TE: Twigs residue hydrolysate; LW: Leaves residue aqueous extract; LE: Leaves residue hydrolysate. Same as below:

compounds, ultimately altering the biological activity of the phenolics. HPLC sugar analysis of the cinnamon extracts and hydrolysates indicated that BE contained the highest glucose content (22.15 ± 0.61 mg/mL), followed by TE [(7.18 ± 0.31) mg/mL] and LE [(7.97 ± 0.15) mg/mL]. There was no glucose being detected in BW, TW and LW. Therefore, we strongly believed that low DPPH activity of BE was caused by the antagonistic effect of high glucose content in BE. Furthermore, Muhammad, Praseptiagga, Walle and Dewettinck (2017) have also proved that there are possibly synergistic or antagonistic interactions between the anti-oxidant compounds. This explained the reason why TE had higher DPPH activity than TW, even though their TPC and TFC level were no significant differences ($P > 0.05$). According to Xue et al. (2017), cellulase hydrolysis can increase the release of varieties phenolic compounds, however most of them are in inactive conjugated form. Therefore, this study was further designed to investigate whether the co-fermentation of cinnamon aqueous extracts and hydrolysates could improve the anti-oxidant activity of yogurt.

3.3. Characterization of yogurt

3.3.1. PH and titratable acidity of yogurt

Table 3 indicated PH and titratable acidity of yogurt produced from different cinnamon aqueous extracts and hydrolysates. The pH and titratable acidity of different yogurt were ranged from 5.05 to 6.84 and 0.49% to 1.07% respectively. The pH and titratable acidity of BE, LW and LE yogurt were no significant differences ($P > 0.05$) with the control. This result indicated that these extracts/hydrolysates did not interfere the lactic acid production during probiotic fermentation. Through observation, the BE, LW and LE yogurt were in semi-solid curd, however, BW, TW and TE yogurt remained as thicken fluid. Cinnamon, just like other medicinal plants exhibited antimicrobial property due to its wide range of secondary metabolites, such as *trans*-cinnamaldehyde, cinnamate, cinnamic acid, camphor, isoamyl benzoate etc. Nonetheless, these compounds are varying depending on the parts of cinnamon (Vasconcelos et al., 2018; Li et al. 2013). Up to recent, investigation on the effects of cinnamon extracts/hydrolysates on probiotic fermentation is limited. Fazilah, Ariff, Khayat, Rios-Solis and Halim (2018) revealed that the appearance, probiotics growth, PH and titratable acidity of functional yogurt will be influenced by the polyphenols content. Therefore, we believed that the secondary metabolites in BW, TW, and TE had exhibited stronger inhibition on the lactic acid production by probiotics during fermentation, compared to BE, LW, and LE.

Table 3
pH and titratable acidity of yogurts produced from different cinnamon aqueous extracts and hydrolysates (mean ± SD, $n = 3$).

Samples	pH	Titratable acidity /%
Control	5.34 ± 0.14^{c}	0.92 ± 0.09^{c}
BW	6.46 ± 0.18^{b}	0.61 ± 0.07^{b}
BE	5.24 ± 0.06^{c}	1.01 ± 0.07^{c}
TW	6.84 ± 0.02^{a}	0.49 ± 0.02^{a}
TE	6.49 ± 0.03^{b}	0.61 ± 0.03^{b}
LW	5.05 ± 0.03^{c}	1.07 ± 0.01^{c}
LE	5.38 ± 0.13^{c}	0.98 ± 0.01^{c}

*a-c: Different alphabets in the same column indicated that there are significant differences among the samples ($P < 0.05$).

3.3.2. *Simulated* in vitro *gastro-pancreatic digestibility of yogurt*

Table 4 demonstrated the TPC, TFC, and DPPH activity of different yogurts before and after simulated *in vitro* gastro-pancreatic digestion. The results revealed that TPC, TFC and DPPH activity of BW yogurt was the highest. These results were within our expectation as BW contained the highest TPC, TFC, and DPPH activity among the tested samples. TPC and TFC of yogurts containing cinnamon extracts/hydrolysates were higher than control, except BE yogurt. In spite of that, there was also no detectable TFC found in the control and BE yogurt. By referring to results in Table 2, TPC and TFC of BE were higher than TW, TE, LW and LE. Yet, the availability of TPC and TFC in the BE yogurt was lower. This observation most probably caused by the complex interactions between milk protein and phenolic compounds. Protein-phenolic interactions resulted in changes in both protein and phenolic availability in the food matrix (Ozdal, Capanoglu & Altay, 2013).

Furthermore, results of DPPH analysis indicated that only BW and TE yogurts exhibited higher free radicals scavenging activity than control. Although TPC and TFC of TE yogurt were comparatively lower than BW yogurt, yet its DPPH activity was not significantly different ($P < 0.05$) with the BW yogurt. In spite of that, LW yogurt had also been found exhibited the lowest DPPH activity. This finding further showed that DPPH activity of yogurts was not merely influenced by the TPC and TFC. According to Li et al. (2018) and Ozdal et al. (2013), the strength of protein-phenolic interactions is compound-specific. Different antioxidant compound exhibited different antioxidant activity. Furthermore, anti-oxidant activity of phenolic compounds is significantly influenced by the protein-phenolic interactions (Alu'datt et al., 2016). In spite of that, Bouarab-Chibane et al. (2018) also disclosed that protein-phenolic interaction is correlated with the binding affinity of phenolics to protein. Moreover, Von Staszewski, Pilosof and Jagus (2011) found that the anti-oxidant and antimicrobial activities of green tea infusions had been masked by the addition of whey protein. Besides, the study by Ryan and Petit (2010) also reported that the anti-oxidant activity of tea was significantly reduced following the addition of milk. Therefore, we strongly believed that the complex milk protein-phenolic interactions are the

Table 4
TPC, TFC, and DPPH anti-oxidant activity of different yogurts before and after simulated *in vitro* gastro-pancreatic digestion (mean ± SD, $n = 3$).

Samples	TPC/(mg GAE·mL^{-1})		TFC/(mg·mL^{-1})		DPPH/(% inhibition)	
	Before digestion	After digestion	Before digestion	After digestion	Before digestion	After digestion
Control	0.09 ± 0.01^{a}	1.11 ± 0.07^{a}	<0.00^{a}	<0.00^{a}	12.20 ± 1.86^{b}	30.99 ± 0.45^{a}
BW	0.61 ± 0.02^{d}	1.24 ± 0.02^{b}	1.71 ± 0.06^{e}	3.02 ± 0.11^{d}	17.62 ± 2.38^{c}	38.15 ± 2.51^{b}
BE	0.11 ± <0.00^{a}	1.07 ± 0.06^{a}	<0.00^{a}	0.40 ± 0.06^{b}	14.17 ± 1.84^{b}	28.39 ± 0.81^{a}
TW	0.55 ± 0.04^{c}	1.09 ± 0.04^{a}	0.68 ± 0.07^{c}	0.41 ± 0.06^{b}	12.86 ± 2.95^{b}	29.56 ± 0.81^{a}
TE	0.53 ± 0.03^{c}	1.04 ± 0.04^{a}	0.80 ± 0.09^{d}	0.61 ± 0.06^{c}	19.09 ± 3.30^{c}	29.30 ± 0.39^{a}
LW	0.25 ± 0.01^{b}	1.02 ± 0.07^{a}	0.05 ± <0.00^{b}	0.68 ± 0.17^{c}	5.99 ± 0.66^{a}	35.16 ± 2.76^{b}
LE	0.30 ± 0.04^{b}	1.03 ± 0.05^{a}	0.08 ± 0.01^{b}	0.36 ± 0.06^{b}	13.62 ± 1.44^{b}	29.04 ± 1.63^{a}

*a-e: Different alphabets in the same column indicated that there are significant differences among the samples ($P < 0.05$).

factor that leads to the uncorrelated relationship between TPC and TFC with DPPH activity of yogurts. Phenolics profile differences of different cinnamon extracts and hydrolysates contributed to distinct protein-phenolic interactions, and hence their availability and free radical scavenging activity of the phenolics (Li et al., 2018; Ozdal et al., 2013).

Interestingly, the TPC and DPPH activity of the produced yogurts were increased tremendously after *in vitro* digestion. After *in vitro* digestion, TFC of BW yogurt was the highest, followed by TE and TW yogurts. Besides, TFC which was not detected in the BE yogurt earlier on had been detected after *in-vitro* digestion. However, TFC of TW and TE yogurts was diminished after *in vitro* digestion. In spite of that, the results also showed that only TPC of BW yogurt was higher than the control after *in vitro* digestion. While the DPPH activity of BW yogurt remained the highest after *in vitro* digestion. Nevertheless, the DDPH activity of LW yogurt surprisingly improved drastically and had no significant differences ($P < 0.05$) with the BW yogurt after *in vitro* digestion. Through the Pearson correlation analysis at 95% confidence level, the DPPH activity of yogurts was found strongly correlated to TPC before *in vitro* digestion ($r = 0.624$; $P = 0.003$), yet it was strongly correlated to TFC after *in-vitro* digestion ($r = 0.686$; $P =< 0.000$). According to Pessato et al. (2018), protein-phenolic interactions resulted in casein conformation changes, and subsequently reduced the *in-vitro* anti-oxidant activity upon digestion. Stable polyphenol-protein interaction was the hypothesized leading cause (Gallo, Vinci, Graziani, Simone & Ferranti, 2013). Moreover, Corrochano et al. (2018) also demonstrated that the anti-oxidant activity of bovine whey protein had been improved after *in-vitro* digestion. The soluble peptides that released following digestion had been proved to possess prominent anti-oxidant property (Ahmed, El-Bassiony, Elmalt & Ibrahim, 2015). This explained why the DPPH activity of control was increased after *in vitro* digestion. Moreover, protein-phenolic interactions presumably influence the efficacy of cinnamon extracts/hydrolysates as the anti-oxidant agent to boost the anti-oxidant property of yogurt. Protein-polyphenols interaction is characterized by the non-covalent binding between the amino acid residues of milk protein and polyphenols. The nature and strength of the interactions differ among varieties of phenolics and peptides (Gallo et al., 2013). This explained the reason why DPPH activity of the yogurts was changed after *in vitro* digestion.

4. Conclusion

This study demonstrated that co-fermentation of BW and TE successfully boosted the anti-oxidant property of yogurt. However, the anti-oxidant activity of TE yogurt was diminished after *in vitro* digestion. Surprisingly, the anti-oxidant activity of LW yogurt which was initially low but it had increased drastically after *in vitro* digestion. Nonetheless, the anti-oxidant activity of BW yogurt which is the experiment benchmark remained the highest even after *in vitro* digestion. Although both TE and LW contained lower TPC and TFC than BW, the produced yogurts possessed equivalent anti-oxidant property as BW yogurt. These findings disclosed that complex protein-phenolic interaction was the core factor that influences anti-oxidant property of the yogurt. Overall, the results proved that cinnamon residues (twigs and leaves) are the potential substrate to boost the functionality of yogurt. Notwithstanding, further study should be conducted to clearly elucidate the relation of anti-oxidant activity of yogurt with the protein-phenolic interaction.

Declaration of Competing Interest

The authors have no conflict of interest to disclose.

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