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Original article

Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage

Premalatha Muniandy[a], Amal Bakr Shori[b,*], Ahmad Salihin Baba[a]

[a] Biomolecular Research Group, Division of Biochemistry, Institute of Biological Sciences, Faculty of Science,University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah 21589, Saudi Arabia

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ABSTRACT

The present study investigated the effect of green, white or black tea (*Camellia sinensis*) on the fermentation of milk and antioxidant potential of yogurt during 21 days of storage at 4 °C. All yogurts were analyzed for total phenolic content (TPC), identification of phenolic compounds and antioxidant potential using diphenyl picrylhydrazyl radical scavenging (DPPH), ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) assays. Green tea yogurt showed the highest phenolic content ($p < 0.05$) followed by white tea yogurt and black tea yogurt. LCMS/MS analysis revealed the absence of several phenolic compounds in tea yogurts, despite their presence in tea water extracts, as well as the presence of new phenolic compounds. All tea yogurts showed higher ($p < 0.05$) FRAP and FIC values than respective control during 21 days of storage. However, BTY showed the lowest values of DPPH scavenging activity and FRAP during storage period. In addition, the antioxidant activity for all tea yogurts remained almost constant over storage period. In conclusion, green, white and black tea can be successfully employed to improve the antioxidant properties of yogurt and provide sustained antioxidants during storage.

1. Introduction

Free radicals such as reactive oxygen species (ROS) are continually produced in our body as a by-product of many metabolic processes. Under normal conditions, the body has its own antioxidant defense system comprising of several enzymes such as catalase, superoxide dismutase and glutathione peroxidase to detoxify these free radicals (Scheibmeir et al., 2005). Dietary antioxidants such as vitamins C, E and A also play a crucial role in fighting these free radicals. However, when there is an over-production of these free-radicals leading to an imbalance between the generation and elimination of free radicals in the body, a situation known as oxidative stress occurs. This in turn results in oxidative damage to cellular components and biomolecules, thus marks the onset of many degenerative diseases related to aging such as cardiovascular disease, diabetes, cancer and neurodegenerative diseases (Aruoma, 1998).

Since antioxidants are vital for their role to delay or inhibit oxidation of cellular components, adequate intake of these compounds in the diet will be beneficial to protect against oxidative damages to the cell. However, the use of synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) are still under evaluation in many countries due to their potential health hazard (Wang, Jónsdóttir, & Ólafsdóttir, 2009). In this regards, extracts of many medicinal plants or herbs rich in phenolic compounds are increasingly used either as additive in food or consumed directly as a natural source of antioxidant (Wong, Li, Cheng, & Chen, 2006).

* Corresponding author.
E-mail address: shori_7506@hotmail.com (A.B. Shori).

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Yogurt is a coagulated milk product obtained from fermentation process carried out by the combined activity of two lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbereuckii* subsp. *bulgaricus*. Yogurt is traditionally consumed as a healthy food due to its nutritional properties and its health benefits can be further enhanced by incorporating probiotic strains of lactic acid bacteria (Shah, 2007). Regular consumption of yogurt with live cultures and probiotic strains is said to be effective in reducing serum cholesterol levels, lactose digestion in case of lactose intolerance, bowel syndromes, gut infections and inflammation, diarrhea and colon cancer (Vasiljevic & Shah, 2007). Yogurt is a rich source of bioactive peptides that form during fermentation and have antioxidant activity. Farvin, Baron, Nina Skall, and Jacobsen (2010) suggested that high oxidative stability of yogurt is associated with antioxidant peptides released during the fermentation of milk by lactic acid bacteria. Moreover, the addition of 4% whey protein concentrate (WPC) in yogurt found to increase the DPPH scavenging activity, Fe^{2+} chelating activity and hydrogen peroxide (H_2O_2) scavenging activity (Unal & Akalın, 2012). Similarly, Unal, El, Akalin, and Dinkci (2013) reported that the fortification of probiotic yogurt with WPC increased DPPH scavenging activity. In addition, the mixture of sodium calcium caseinate with WPC significantly ($p < 0.05$) increased Fe^{2+} chelating activity.

Tea (*Camellia sinensis*) is a common beverage consumed worldwide. Tea can be divided into three types based on the method of processing the leaves, namely the non-fermented green and white teas, partially fermented oolong tea and fermented black tea (Horzic et al., 2009). Tea has varying chemical compositions attributed to the processing steps (Almajano, Carbo, Jimenez, & Gordon, 2008). Wang, Provan, and Helliwell (2000) have reported tea to be a rich source of flavanols and flavonols. Catechins are the primary flavanols in tea that contribute 30–42% of the dry weight in green tea leaves. There are six major forms of catechins found in fresh tea leaves namely (−)epicatechin (EC), (−)epicatechin-3-gallate (ECG), (−)epigallocatechin (EGC), (−)epigallocatechin-3-gallate (EGCG), (+)catechin and (+)gallocatechin (GC). Among all the catechin derivatives found in tea leaves, EGCG was reported to be the most abundant form of catechin contributing about 50–80% of total catechins found in green tea leaves (Sang, Lambert, Ho, & Yang, 2011). The catechin content in black tea is reduced to 10–12% due to fermentation process as a result of polymerisation into theaflavin and thearubigins (Dufresne & Farnworth, 2001). Flavonols present in tea contribute to almost 5–10% of dry weight in green tea and 6–8% of dry weight in black teas (Dufresne & Farnworth, 2001) which include quarcetin, kaemperol and myrecitin.

Tea polyphenols have great medicinal and health benefits and they are potent source of antioxidants (Sharangi, 2009). Thus, it is important to establish the differences in the types of tea used on the effects of microbial metabolism and the changes of antioxidant activity in yogurt. Food industry is seeking to improve the quality characteristics of yogurt products with high level of antioxidant activity. Preparation of yogurt with antioxidant properties has a promising potential for utilization as functional product. The addition of different types of tea extracts into yogurt may improve the antioxidant activity of yogurt. Therefore, the objectives of this study were to compare the total phenolic content and antioxidant activity of green, white and black teas. In addition, the changes of total phenolic content and antioxidant potential in probiotic yogurt due to addition of tea and their stability during 21 days of refrigerated storage were evaluated. Identification the major phenolic compounds present in tea extracts and the changes in the composition of these phenolic compounds in yogurt were also studied.

2. Materials and methods

2.1. Materials

Pasteurized whole milk (Dutch Lady, Malaysia) was used for making yogurt. The three types of ground tea leaves used in this study were Long Jing green tea, Shou Mei white tea (China origin; Purple Cane Enterprise, Malaysia) and black tea (Malaysia origin; Lipton, Malaysia) purchased from a local hypermarket. Commercially available direct vat set (Chris-Hansen, Denmark) starter culture powder used in yogurt preparation consist of a mixture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12, *Lactobacillus casei* LC-01, *S. thermophilus* Th-4 and *Lactobacillus delbrueckii* ssp. *bulgaricus* in the ratio of 4:4:1:1:1. All chemicals and reagents used in this study were purchased from Sigma–Aldrich Chemical Co., USA and John Kollin Chemicals, UK.

2.2. Preparation of tea water extract

The strength of the tea infusions used for analysis was 2% (w/v). Boiled hot water (85 °C; 100 ml) was poured into a beaker containing tea (2 g). The beaker was covered using aluminum foil and the tea was brewed for 10 min (Najgebauer-Lejko, Sady, Grega, & Walczycka, 2011). The brewed tea was filtered using fine tea strainer and the filtrate was cooled to ambient temperature. The tea infusates (tea water extracts) were then centrifuged (5000 × *g*, 4 °C, 10 min) and the harvested supernatants were refrigerated (4 °C) and used for analysis within 1–2 weeks of preparation.

2.3. Preparation of yogurt

Green tea yogurt (GTY), white tea yogurt (WTY) and black tea yogurt (BTY) were prepared according to the method described by Jaziri, Slama, Mhadhbi, Urdaci, and Hamdi (2009) with slight modifications. Pasteurized whole milk (100 ml) was warmed to 85 °C. Treated milk was mixed with 2% (w/v) of green, white or black teas (2 g/100 ml) corresponding to the strength of a "normal cup of tea" (Yam, Shah, & Hamilton-Miller, 1997). The teas were allowed to infuse into the milk for 10 min followed by filtration through sterile fine tea strainer to remove visible particles. The resulting tea-milk infusions (90 ml) were aliquoted into sterile disposable plastic containers placed in an incubator (45 °C). This followed by addition of 10 ml (10% w/v) starter culture (1 L of whole milk incubated with DVS starter culture powder for 12 h) into the milk-tea infusion. Plain yogurt (PY) was prepared in the same manner as previously described without tea (control). All inoculated milk and milk-tea infusates were placed in an incubator at 42 °C until the pH values reached 4.5 (Shori, Baba, & Chuah, 2013b). The yogurts were then refrigerated (4 °C) up to 21 days. Samples of each yogurt type were removed from the fridge the following day (day 1) and on days 7, 14 and 21 of storage for further analysis.

2.4. Preparation of yogurt water extract

Water extraction of yogurt was carried out as described by Shori and Baba (2013a). Plain- and tea-yogurts (10 g) were weighed into plastic centrifuge tubes. The yogurts were then homogenised (Polytron, at highest setting for 10 s) with sterile distilled water (2.5 ml). pH of the yogurts was determined using a pH meter and the yogurts were subsequently acidified to pH 4.0 by adding HCl (0.1 M). The acidified yogurts were then incubated for 10 min in a water bath (45 °C) followed by centrifugation (5000 × *g*, 4 °C, 10 min). The pH of the resulting supernatant was then adjusted to 7.0 using NaOH (0.1 M) followed by another step of centrifugation (5000 × *g*, 4 °C, 10 min). The clear supernatant obtained was stored

in a freezer (−20 °C) and used for analysis within 1–2 weeks of preparation.

2.5. Determination of total phenolic content (TPC)

TPC assay was carried out according to the method described by Najgebauer-Lejko et al. (2011). Samples of yogurt water extracts, tea extracts or standard solutions of gallic acid (100 μl) were mixed with distilled water (7.9 ml) and 2 M Folin–Ciocalteu reagent (0.5 ml). After thorough mixing, the mixture was allowed to stand at room temperature for 5 min. Sodium carbonate solution (1.5 ml, 20% w/v) was added into the mixture and following a brief mixing, the mixture was left standing in the dark for 2 h at room temperature. Absorbance at 765 nm was measured against distilled water as blank using a spectrophotometer (Genesys 10UV). The results were converted into total phenolic content using the gallic acid calibration curve constructed using various concentrations of gallic acid (10–60 μg/g) in 95% ethanol and run each time assay was carried out. The absorbance values were expressed as μg gallic acid equivalent per milliliter (μg GAE/ml).

2.6. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging assay was carried out according to the procedure described by Najgebauer-Lejko et al. (2011) with slight modification. Samples of yogurt water extracts or tea extracts (100 μl) were mixed with 3.0 ml of 0.1 mM DPPH reagent (39.4 mg of DPPH in 1 L of methanol) followed by incubation in the dark at room temperature for 2 h. Absorbance (Abs) at 515 nm was measured against distilled water as blank using a spectrophotometer (Genesys 10UV). Control was a mixture of methanol (100 μl) and DPPH reagent (3.9 ml). The radical scavenging activity was calculated as below:

$$\text{Scavenging activity } (\%) = 1 - \left[\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right] \times 100\% \quad (1)$$

2.7. Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was carried out following the procedure described by Benzie and Strain (1996) with slight modification. A working FRAP reagent was prepared by mixing 300 mM acetate buffer, 8 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) reagent and 20 mM $FeCl_3$ solutions at a ratio of 10:1:1. Freshly prepared working FRAP reagent (3.6 ml) was added into a test tube containing 400 μl of yogurt water extract, tea extract or standard iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) solution and the contents were mixed thoroughly. The mixtures were then incubated at 37 °C in a water bath for 10 min. Absorbance at 593 nm was measured against distilled water as blank using a spectrophotometer (Genesys 10UV). The results were calculated from a standard scale of $FeSO_4{\cdot}7H_2O$ (0.3–1.0 mM) and expressed as mmol Fe^{2+} equivalent/l (mmol Fe^{2+}E/L).

2.8. Ferrous ion chelating (FIC) assay

FIC assay was carried out following the procedure described by Chan, Lim, and Chew (2007). The iron(II) sulphate hydrate ($FeSO_4{\cdot}xH_2O$) solution (2 mM) and ferrozine solution (5 mM) were both diluted 20 times prior to use in the assay. Diluted $FeSO_4{\cdot}xH_2O$ solution (1 ml) was added to samples of yogurt water extracts or tea extracts (1 ml) and mixed well. Diluted ferrozine solution (1 ml) was then added to the mixture followed by incubation for 10 min at room temperature. Absorbance at 562 nm was measured against distilled water as blank using a spectrophotometer (Genesys 10UV). Control was a mixture of $FeSO_4{\cdot}xH_2O$ solution (1 ml), ferrozine solution (1 ml) and distilled water (1 ml). The FIC ability of samples was calculated using the above-mentioned equation (Eq. (1)).

2.9. LCMS/MS analysis of phenolic compounds

The samples of yogurt water extracts on day 1 of storage and tea extracts were analyzed for key phenolic compounds using LCMS/MS. A full scan of samples with MS/MS data collection was performed using AB Sciex 3200Q Trap LCMS/MS system. Analysis of the compounds was carried out using the negative ionization mode (m/z M − H^+). Chromatographic separation of the compounds in the samples was carried out using a Phenomenex Aqua C18 column (50 mm × 2 mm × 5 μM). Samples were filtered with nylon membrane filter (0.22 μm) and 20 μl was injected into the column. For the separation, a gradient run programme was carried out using mobile phase A (water with 0.1% formic acid and 5 mM ammonium formate) and mobile phase B (acetonitrile with 0.1% formic acid and 5 mM ammonium formate). The total run time was about 16 min and the gradient profile was set as follows: 10% A to 90% B from 0.01 min to 8.0 min, held for 3 min and back to 10% A in 0.1 min, followed by re-equilibration for 5 min. Identities of the compounds were obtained by matching the molecular ion fragments (m/z) obtained from the analysis with literature data as well as using the advanced chemometrics mass fragmentations predictive software.

2.10. Statistical analysis

All experiments were performed in three separate batches and in duplicates. Results for each analysis were expressed as mean ± standard deviation. All data were subjected to a two way analysis of variance (ANOVA) and the significance of differences between means was determined on the basis of Duncan test at significance level of $p < 0.05$. The statistical analysis was carried out using software IBM SPSS statistics for windows, released 2011, version 20.0. Armonk, NY: IBM Corp.

3. Results and discussion

3.1. Total phenolic content in yogurt as affected by the presence of tea extract

The TPC in water extracts of green, white and black tea is summarized in Table 1. Based on the present results, green tea extract (GTE) showed the highest phenolic content (3220.15 ± 37.80 μg GAE/ml) followed by white tea extracts (WTE; 2811.26 ± 44.74 μg GAE/ml) and black tea extract (BTE, 2504.59 ± 24.48 μg GAE/ml). The inclusion of green, white or black tea into milk prior to bacterial fermentation significantly increased

Table 1
Total phenolic content of tea extracts.

Extract	TPC (μg GAE/ml)
GTE	3220.15 ± 37.80^{a}
WTE	2811.26 ± 44.74^{b}
BTE	2504.59 ± 24.48^{c}

The TPC values of tea extracts in gallic acid equivalent (GAE) are based on the infusion of 2% (w/v) dried ground tea leaves typically used in a cup of tea.
Values are means of three replicates ± standard deviation. GTE, Green tea extract; WTE, White tea extract; BTE, Black tea extract. Means with superscripts having different letters are significantly ($p < 0.05$) different.

($p < 0.05$) TPC compared to milk alone (Table 2). This could be attributed to the high content of flavonoids in tea leaves (Komes, Karlović, Kovačević Ganič, Horžić, & Glavaš, 2007; Rusak, Komes, Likic, Horzic, & Kovac, 2008). However, a significant decline in TPC was noticed in all tea extracts (TEs) upon inclusion in milk by 83.7%, 86% and 88.2% for GTE, WTE and BTE respectively (Tables 1 and 2). This was likely related to milk–polyphenol interaction as reported in previous studies (Dubeau, Samson, & Tajmir-Riahi, 2010). 'The binding capacity between the tea polyphenols and protein increases as the number of hydroxyl (OH) groups increase in phenolic compounds in the order of C ~ EC > EGC > EGCG (Hasni et al., 2011).

An increase in TPC was observed for all yogurts following fermentation particularly for PY and GTY (increments of 10.56 μg GAE/ml and 46.86 μg GAE/ml respectively; $p < 0.05$; Table 2). Proteolysis of milk proteins may release amino acids with phenolic side chains such as tyrosine, which could contribute to the increase in TPC (Korhonen, 2009). In addition, microbial metabolism of phenolic compounds in tea yogurt as well as production of new phenolic acids during acidification may result in an increase of phenolic groups as the ring structure is broken down (Blum, 1998). There was a significant reduction ($p < 0.05$) in TPC by 85.93 μg GAE/ml, 56.3 μg GAE/ml and 37.22 μg GAE/ml for GTY, WTY and BTY respectively after 2 weeks of storage. However, GTY showed the highest TPC among others which could be due to the abundant presence of catechin and epicatechin in green tea (Komes et al., 2007).

3.2. Identification of phenolic compounds in tea yogurt

A summary on the comparison of the presence and/or absence of phenolic compounds in tea and tea-yogurt extracts are shown in Table 3.The phenolic composition of GTE and WTE are remarkably similar with some exceptions to flavonol glycoside compositions as reported in earlier studies (Gondoin, Grussu, Stewart, & McDougall, 2010). The present results indicated that flavonol glycosides such as kaempferol-3-rutinoside and quarcetin-rhamnosylgalactoside or rutinoside were present in WTE but absent in GTE. In addition, catechin derivatives such as gallocatechin (GC) and epigallocatechin (EGC) were not detected in WTE but were present in GTE (Table 3). Despite having many physiological roles in plant survival including ultraviolet B protection, disease resistance and defense against predation, the contents of these flavonoids compounds in plants do reduce as plant matures, thus explaining the differences in phenolic compound observed between GTE and WTE (Song, Kelman, Johns, & Wright, 2012).

Gallo-flavanols have high oxidative potential and tendency to dimerise readily into theaflavins and thearubigins (Wang et al., 2000) compared to catechol-flavanols. Thus, the absence of gallocatechin (GC), epigallocatechin (EGC) and gallocatechin gallate (GCG) and the subsequent presence of theaflavin-3-*O*-gallate observed in BTE could be direct consequences of oxidative fermentation during the manufacturing process. Similarly, several flavonol glycosides such as myrecitin-3-*O*-glucoside, kaempferol-3-*O*-galactosyl-rhamnosyl-glucoside and kaempferol-rhamnose-hexose-rhamnose were absent in BTE but present in GTE. This could also be attributed to oxidative fermentation (Kim et al., 2011).

Fermentation of milk by yogurt bacteria yielded interesting observations with regard to formation and degradation of polyphenolic compounds. Most of the catechin and its derivatives present in tea extracts were not detected in tea yogurt samples (Table 3). In addition several flavonol glycosides such as myrecitin-3-*O*-glucoside, kaempferol-3-rutinoside and kaempferol-3-*O*-glucoside were detected in WTE but not in WTY. New compounds were also detected in tea yogurt, for instance kaempferol-3-rutinoside and quinic acid conjugate were absent in GTE but present in GTY. There were two unidentified peaks found in PY. This may be compounds derived from metabolism of milk protein by yogurt bacteria since one of the peaks (MW = 723 g/mol) was detected in all tea yogurt samples (Table 3). The differences in phenolic compounds between tea extracts and their respective tea yogurt plus the presence of several new peaks in each tea yogurt suggest gradual degradation of phenolic compounds (Wegrzyn et al., 2008) or milk–polyphenol interaction (Hasni et al., 2011). In addition, some considerations may also be given to the possibility of phenolic compounds being metabolized by yogurt bacteria (Tabasco et al., 2011). However, yogurt bacteria (*S. thermophilus* and *L. bulgaricus*) have been reported unable to metabolized green tea phenolic compounds such as catechin gallate, epigallocatechin, catechin, epigallocatechin gallate, gallocatechin gallate and epicatechin gallate (Jaziri et al., 2009). The bacterial species and strain in addition to chemical structure and concentration of the polyphenols play a significant role in 'metabolism of the phenolic compounds (Tabasco et al., 2011). A number of *Lactobacillus* spp. are found to be able to metabolize phenolic compounds in food (Rodríguez et al., 2009).

3.3. Antioxidant potential of tea-yogurt

Phenolic compounds could act as antioxidants via their ability to donate hydrogen or electron which resulted in the termination of a chain reaction or by chelating transition metal ions hence, terminating the Fenton reaction (Rice-Evans, Sampson, Bramley, & Holloway, 1997). An antioxidant compound that directly scavenge free radical is termed as primary antioxidant while compounds that prevent the formation of free radical via Fenton reaction is known as secondary antioxidant (Chan, Lim, Chong, Tan, & Wong, 2010). Both DPPH and FRAP measure the primary antioxidant properties of the sample while FIC assay measure the secondary antioxidant property of the sample (Deetae, Parichanon, Trakunleewatthana, Chanseetis, & Lertsiri, 2012). Since total antioxidant

Table 2
The changes in total phenolic content of milk mixture with tea before fermentation and in tea yogurt during refrigerated storage.

Storage day	TPC (μg GAE/ml)			
	PY	GTY	WTY	BTY
BF	118 ± 3.33^{aA}	479.48 ± 8.34^{aB}	366.89 ± 6.19^{abC}	278.37 ± 20.16^{abD}
Day 1	128.56 ± 1.47^{bA}	526.34 ± 16.02^{bB}	394.12 ± 17.56^{bC}	294.67 ± 11.82^{bD}
Day 7	126.70 ± 4.72^{bA}	467.08 ± 19.44^{aB}	367.26 ± 21.60^{abC}	268.93 ± 18.95^{abD}
Day 14	122.82 ± 3.06^{abA}	440.41 ± 2.74^{cB}	337.82 ± 15.38^{cC}	257.45 ± 17.78^{aD}
Day 21	135.04 ± 5.94^{cA}	465.23 ± 6.31^{aB}	345.60 ± 18.86^{acC}	269.30 ± 13.83^{abD}

Values are means of three replicates ± standard deviation. PY, Plain yogurt (control); GTY, Green tea yogurt; WTY, White tea yogurt; BTY, Black tea yogurt. BF, Before fermentation, refers to mixture of milk with/without tea prior to the initiation of the fermentation by starter culture (10% v/v).
[abcd] Means with superscripts having different letters in the same column are significantly ($p < 0.05$) different.
[ABCD] Means with superscripts having different letters in the same row are significantly ($p < 0.05$) different.

Table 3
Identity of phenolic compounds present in tea extracts and tea yogurt extracts.

Compound	MW (g/mol)	MS	MS/MS fragmentation	GTE	WTE	BTE	PY	GTY	WTY	BTY
Gallic acid	170	169	125, 67	√	√	√			√	√
Quinic acid	192	191	93, 85	√	√	√				
Catechin	290	289	245, 203, 123, 109	√	√	√				
Epicatechin (EC)	290	289	245, 123, 109	√	√	√		√		
Epigallocatechin (EGC)	305	304	167, 137, 125	√						
Gallocatechin (GC)	306	305	219, 137, 125, 109	√						
Unknown	332	331	215, 185					√		
p-Coumaroylquinic acid	338	337	191, 173, 163, 119, 93	√		√		√		√
Quinic acid conjugate	338	337	191, 173					√		
5-Caffeoylquinic acid	344	343	191, 93, 85	√	√	√				
Chlorogenic acid	354	353	191, 135			√				
Unknown	378	377	161						√	√
Unknown	432	431	89				√			
Epicatechin gallate (ECG)	442	441	289, 169, 125	√	√	√				
Kaempferol-3-*O*-glucoside	448	447	300, 284, 255, 227	√	√	√				
Unknown	452	451	225						√	
Epigallocatechin gallate (EGCG)	458	457	169, 125	√	√	√				
Gallocatechin gallate (GCG)	458	457	305, 219, 169, 125	√	√					
Quarcetin-3-glucoside	464	463	300, 271, 255			√				
Myricitin-3-*O*-glucoside	480	479	316, 287, 271	√	√					
Unknown	498	497	451, 225							√
Arabinosyl-glucosyl apigenin	564	563	383, 353, 297	√	√	√		√	√	√
Procyanidin B1	578	577	407, 289, 245, 161, 125	√						
Unknown	590	589	257							√
Dicaffeoquinic acid conjugate	594	593	383, 353	√	√	√				
Kaempferol-3-rutinoside	594	593	285, 255		√	√		√		√
Quarcetin-rhamnosylgalactoside or rutinoside	610	609	300, 271, 255		√	√				√
Strictinin	634	633	301, 275		√					
Theaflavin-3-*O*-gallate	716	715	563, 545, 389, 281, 269, 253, 241, 169			√				
Unknown	720	719	546, 528, 318						√	
Unknown	724	723	677, 451, 225				√	√	√	√
Kaempferol-rhamnose-hexose-rhamnose	740	739	285	√				√		
Kaempferol-3-*O*-galactosyl-rhamnosyl-glucoside or galactoside	756	755	285	√	√			√	√	
Prodelphinidin A-2 3′-*O*-gallate	760	759	607, 589, 425, 169			√				
Unknown	769	768	619, 195					√		√
Unknown	901	900	883, 865					√	√	√
Unknown	997	996	439						√	√

The symbol "√" indicates the presence of the compound in the respective extracts.
MW, Molecular weight of the compound; MS, *m*/*z* (mass to charge ratio).
Only major MS/MS fragments for each phenolic compound are shown in the table.

capacity of food samples may have resulted by multiple reaction mechanisms, a single antioxidant assay may not reflect the total antioxidant capacity (Du, Li, Ma, & Liang, 2009). Hence, this explains the relevance of assessing the antioxidant potential of tea yogurts in this study using three different methods. The changes in the DPPH radical scavenging activity, FRAP values and FIC ability of tea extracts are presented in Table 4. The present study showed that water extracts of green, white and black tea showed high free radical scavenging potential (~98%; Table 4). There were significant differences in FRAP values between GTE and BTE (27.15 ± 1.58 mmol Fe^{2+} E/l and 18.35 ± 1.16 mmol Fe^{2+} E/L, respectively; Table 4). On the other hand, FIC values of the three tea extracts were similar (~84%). The inclusion of tea extracts into milk significantly increased ($p < 0.05$) free radical scavenging activity (88% – 92%) compared to milk alone (5.84%; Fig. 1). Fermentation of milk had no effect on antioxidant capacity of PY (3–7%). However, the presence of tea extracts appeared to increase ($p < 0.05$) DPPH radical scavenging activity in yogurt (83–97%) during 21 days of storage. On the other hand, the inclusion of tea extracts into milk reduced ($p < 0.05$) FRAP values to 3.71 ± 0.48 mmol Fe^{2+} E/L, 2.85 ± 0.13 mmol Fe^{2+} E/L and 2.14 ± 0.38 mmol Fe^{2+} E/L for GTY, WTY and BTY respectively compared to tea extracts (Table 4). However, FIC values showed small increase ($p > 0.05$) upon inclusion of tea extract into milk (Fig. 3). All tea yogurt samples showed higher ($p < 0.05$) FRAP and FIC values than respective control during 21 days of storage (Figs. 2 and 3). The fluctuations in values of FRAP or FIC of all tea yogurts remained insignificant ($p > 0.05$) throughout the storage period. Based on DPPH radical scavenging activity and FRAP, BTY showed the lowest antioxidant activity during 21 days of storage.

Table 4
Antioxidant potential of tea extracts measured by DPPH radical scavenging activity, FRAP and FIC ability.

Extract	Antioxidant potential		
	DPPH radical scavenging activity (%)	FRAP (mmol Fe^{2+} E/L)	FIC ability (%)
GTE	98.53 ± 0.06^{a}	27.15 ± 1.58^{a}	84.4 ± 0.34^{a}
WTE	98.11 ± 0.17^{a}	22.47 ± 1.51^{ab}	84.07 ± 0.31^{a}
BTE	97.71 ± 0.17^{a}	18.35 ± 1.16^{b}	84.83 ± 0.16^{a}

The DPPH radical scavenging activity, FRAP and FIC ability of tea extracts are based on the infusion of 2% (w/v) dried ground tea leaves typically used in a cup of tea.
Values are means of three replicates ± standard deviation. GTE, Green tea extract; WTE, White tea extract; BTE, Black tea extract. Means with superscripts having different letters in the same column are significantly ($p < 0.05$) different.

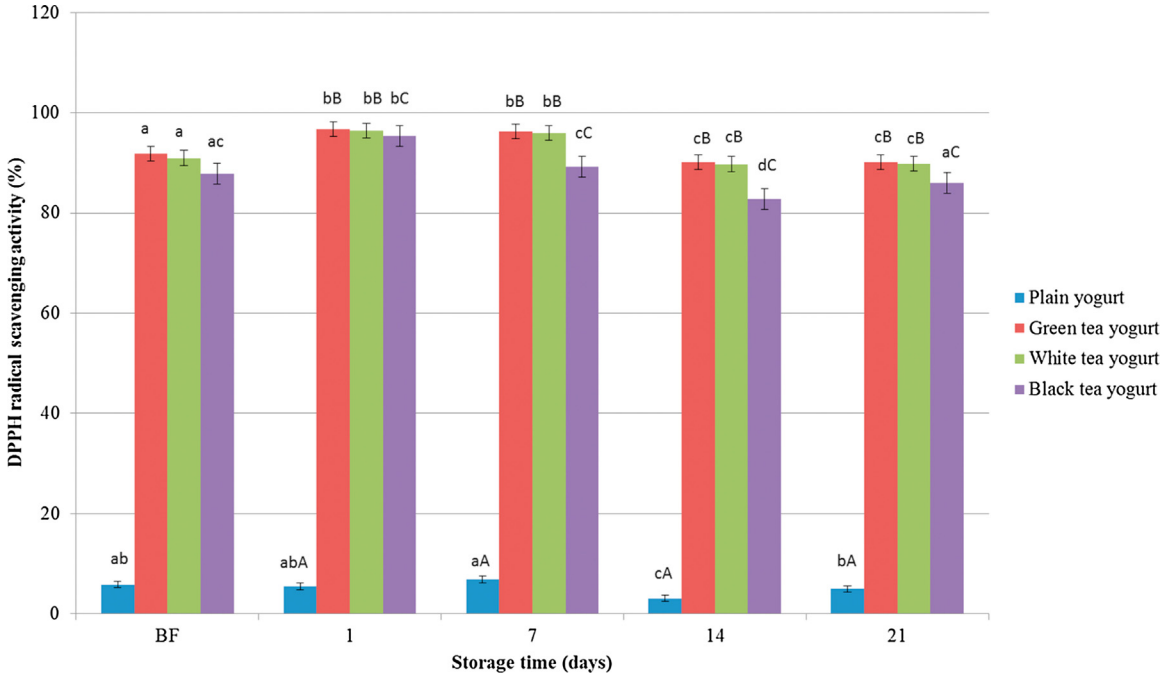

Fig 1. DPPH radical scavenging activity of milk + tea before fermentation (BF) and tea yogurt during 21 days of refrigerated storage at 4 °C. Error bars represent a pooled standard deviation of the mean ($n = 3$). [abcd] Means with superscripts having different letters in the same column are significantly ($p < 0.05$) different. [ABCD] Means with superscripts having different letters in the same row are significantly ($p < 0.05$) different.

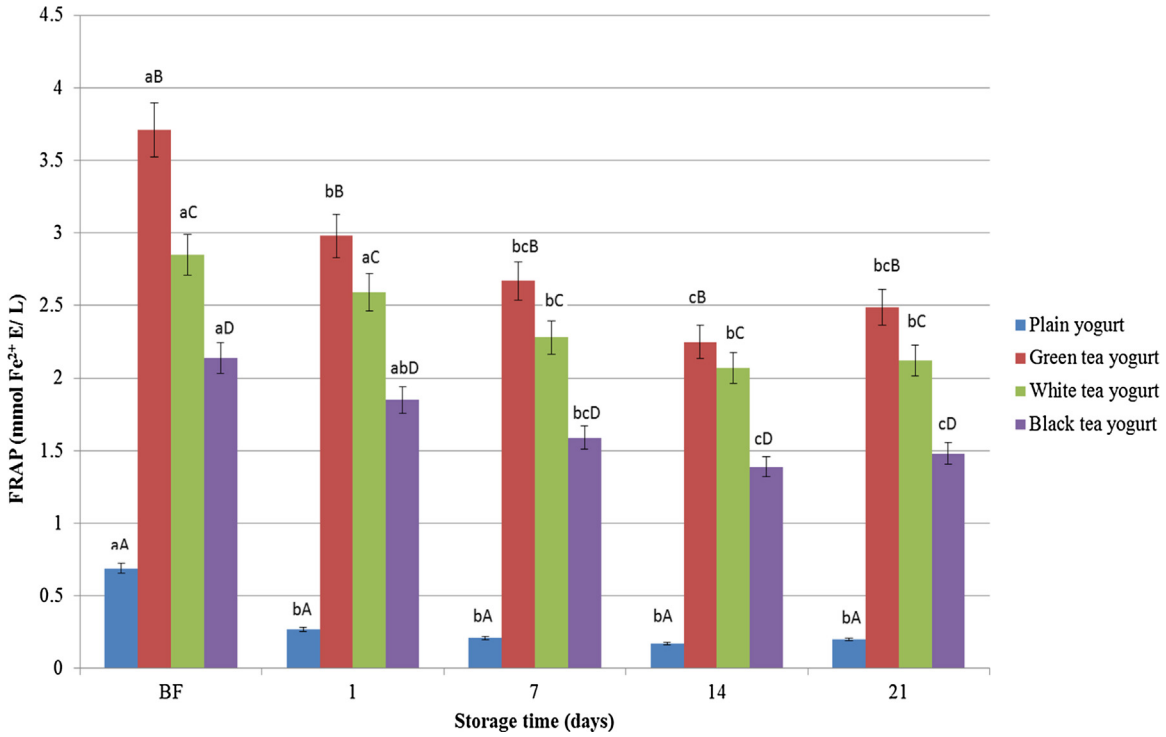

Fig. 2. FRAP of milk + tea before fermentation (BF) and tea yogurt during 21 days of refrigerated storage at 4 °C. Error bars represent a pooled standard deviation of the mean ($n = 3$). [abcd] Means with superscripts having different letters in the same column are significantly ($p < 0.05$) different. [ABCD] Means with superscripts having different letters in the same row are significantly ($p < 0.05$) different.

Several studies have reported that the presence of phenolic content of plant materials such as garlic, cinnamon, neem, soybean, chickpea and goji berry in yogurt could be associated with high antioxidant activity (Shori & Baba, 2011b; Baba et al., 2014; Shori & Baba, 2013a). Tea catechins and some low molecular polyphenols could contribute to the high antioxidant potential of the tea extracts (Zhu, Hackman, Ensunsa, Holt, & Keen, 2002) which accordingly explained high antioxidant activity in tea yogurt. High correlations between TPC and both DPPH radical scavenging activity and FRAP were observed in the three types of tea yogurt (data not shown). In addition, the proteolysis by lactic acid bacteria may release bioactive peptides with antioxidant

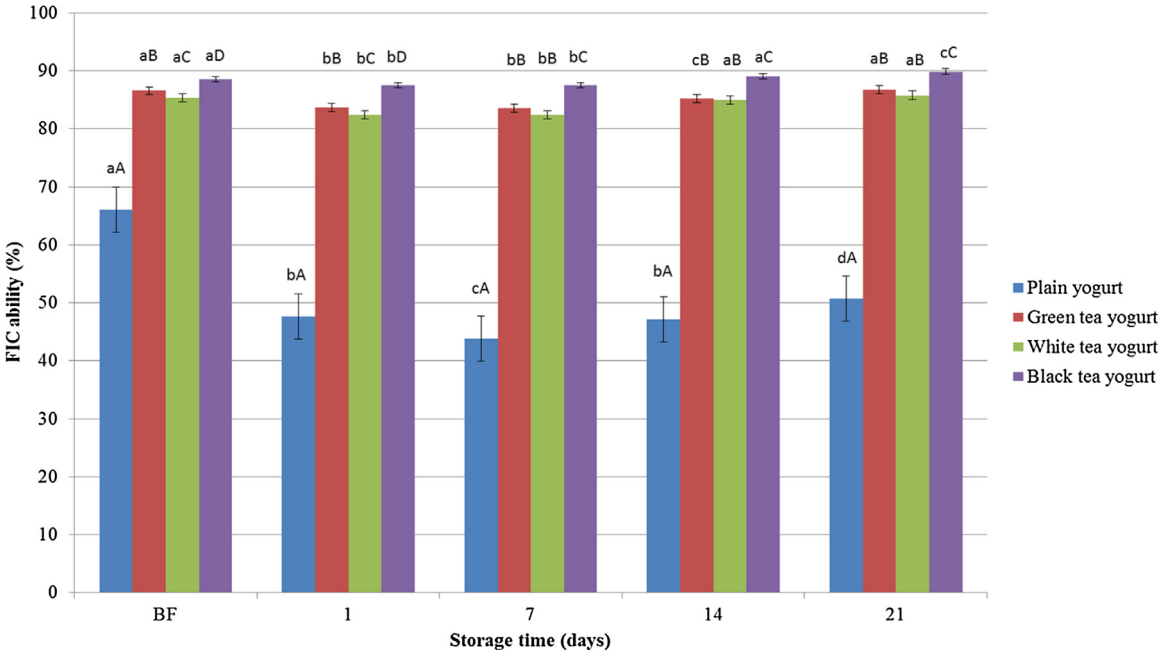

Fig. 3. FIC ability of milk + tea before fermentation (BF) and tea yogurt during 21 days of refrigerated storage at 4 °C. Error bars represent a pooled standard deviation of the mean ($n = 3$). [abcd] Means with superscripts having different letters in the same column are significantly ($p < 0.05$) different. [ABCD] Means with superscripts having different letters in the same row are significantly ($p < 0.05$) different.

activity in fermented milk products (Farvin et al., 2010). For instance κ-casein-peptides with DPPH radical scavenging activity were produced in milk fermented by *Lactobacillus delbreuckii* subsp. *bulgaricus* (Kudoh, Matsuda, Igoshi, & Oki, 2001). In the present study, higher viability of *Lactobacillus* spp was recorded in tea yogurt compared to PY (data not shown) which may be associated with higher bioactive peptides with antioxidant properties. The DPPH radical scavenging activity of all the three types of tea yogurts was significantly higher (83–97%; Fig. 1) than other yogurts in the presence of garlic (24–37%; Shori & Baba, 2011a), cinnamon (26–53%; Shori & Baba, 2011a), neem (24–53%; Shori & Baba, 2013a), soybean (35–61%; Shori, 2013a), chickpea (37–60%; Shori, 2013b) and goji berry (35–41%; Baba et al., 2014). The reduction in antioxidant activities in all tea-milk mixtures compared to their respective tea extracts could be related to milk–polyphenol interaction led to a decrease in antioxidant capacity (Arts et al., 2002). Milk–polyphenol interaction which is common in proline rich milk proteins (casein) due to the strong affinity of the proline groups towards the hydroxyl groups present in the phenolic compounds (Arts et al., 2002). This leads to precipitation of the phenolic compound and may decrease the antioxidant potential (Yuksel, Avci, & Erdem, 2010). Low antioxidant activity (DPPH scavenging activity and FRAP) of BTY compared to GTY and WTY likely occurred due to oxidative degradation and polymerization of phenolic compounds (catechins into larger theaflavin and thearubigin) during fermentation in the manufacturing process of black tea (Dufresne & Farnworth, 2001).

4. Conclusion

The present study demonstrated that, green, white and black tea extracts were effectively employed for production of polyphenol fortified yogurt with antioxidant properties. Green tea extract has the highest content of polyphenols and antioxidant activity followed by white and black tea extracts respectively. These tea extracts positively affected the phenolic content in yogurt during 21 days of refrigerated storage. LCMS/MS analysis revealed the absence of several phenolic compounds in tea yogurts, despite their presence in tea water extracts, as well as the presence of new phenolic compounds. However, all tea yogurts showed high antioxidant activity compared to plain yogurt during 21 days of storage. In addition, black tea yogurt showed the lowest antioxidant activity among the others. Since tea is a natural herbal product with therapeutic and nutritional properties; green, white and black teas are all strongly recommended as a novel ingredient to enhance yogurt's antioxidant properties as a new functional food. Further study will be needed to examine the consumer sensory evaluation and viability of probiotics of all the three types of tea yogurt.

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