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Original article

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

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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).

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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