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Antioxidant activity and recovery of green tea catechins in full-fat cheese following gastrointestinal simulated digestion

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ABSTRACT

Green tea extract (GTE) was incorporated into full-fat cheeses at 250, 500, and 1000 ppm to investigate the effect of green tea catechins on antioxidant properties and microstructure of cheese, and recovery of catechins. Cheeses were ripened for 90 days at 8 °C followed by simulated gastrointestinal digestion. The composition on Day 0, and pH, total phenolic content (TPC), and antioxidant activity (AA) of the cheeses on Days 0, 30, and 90, were determined. The concentrations of the major GTE catechins in the curd and from the digesta of mature cheeses were determined using HPLC. Addition of GTE significantly ($p \leq 0.05$) decreased the pH of whey and curd during cheese manufacture and ripening, however there was no significant ($p > 0.05$) effect on moisture, protein, or fat contents. Addition of GTE increased TPC and AA at all concentrations, but in a non-linear manner. Individual catechins were selectively retained in the curd, whereas different portions were recovered from the cheese digesta. Transmission electron microscopy showed that the ripened control cheese had a regular distribution of milk fat globules entrapped in a homogeneous structure of casein proteins, which was disrupted by GTE. Interactions between green-tea catechins and cheese fat were confirmed by Fourier transform infrared spectroscopy.

1. Introduction

Green tea (*Camellia sinensis*) is well known for various health benefits associated with risk reduction of a wide range of chronic diseases, such as cancer, diabetes, and cardiovascular diseases (Hollman and Katan, 1999; Iwasaki et al., 2014; Johnson et al., 2012; Onakpoya et al., 2014; Oze et al., 2014; Velayutham et al., 2008; Weisburger et al., 1997). Polyphenolic compounds, namely the catechins, (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG) are thought responsible for such beneficial effects. These green tea antioxidants, well-studied for biological activities, such as inhibition of oxidative enzymes, inhibition of cancer-related transcriptional factors, reactive oxygen scavenging, and redox active metal chelation (Higdon and Frei, 2003; Nichenametla et al., 2006; Rathore and Wang, 2012; Sarkar and Bhaduri, 2001), account for approximately 80% of the total phenolic content of brewed green tea (Green et al., 2007). Consumption of the equivalent of 6–10 cups daily of green tea has been suggested by scientists to provide health benefits; however this large amount of tea is not realistic to consume. Thus, there has been growing interest to use green tea catechins as additives in food products that are consumed regularly as part of a daily diet. Cheese, as a compact nutrient food product, is consumed widely in many countries and could be considered as a potential delivery vehicle for green tea catechins because of its nutritional value and long shelf-life. Incorporating an extract from green tea containing all beneficial catechins will afford a cheap source of potent antioxidants to be incorporated into a food matrix, such as cheese, to create functional foods. This will be effective, providing there are no detrimental effects on the structure of the food or loss of antioxidant activity of the catechins due to potential interactions between green tea catechins and cheese components such as proteins (Brown and Wright, 1963; Corredig and Dalgleish, 1996; Haratifar and Corredig, 2014; Ozdal et al., 2013; Rashidinejad et al., 2015b; Ye et al., 2013; Yuksel et al., 2010).

Previously, interactions between full-fat milk and green tea extract, thought to involve milk fat globules (MFG), were observed in our laboratory (Rashidinejad et al., 2016). Initially, to test the possible interactions between catechins and milk proteins we incorporated C, as a representative green tea catechin, at different concentrations (125, 250, and 500 ppm) into a low-fat cheese

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(Rashidinejad et al., 2013), ripened for 90 days, and examined the behaviour of such a low-fat cheese fortified with C under a simulated gastrointestinal digestion system (Rashidinejad et al., 2015a). Although C was able to significantly increase the phenolic content and antioxidant activity of the hard low-fat cheese ($p \leq 0.05$) digested in the simulated gastrointestinal system, interactions between this phenolic compound and milk proteins were speculated to occur. There are also other catechins in green tea (e.g., EGCG) with higher affinity to milk proteins (Lamothe et al., 2014; Sirk et al., 2008) and interactions with the lipid components are also likely (Rashidinejad et al., 2016, 2015a,b).

In a study carried out by Giroux et al. (2013), the effect of green tea extract (GTE) on the physicochemical and organoleptic characteristics of Cheddar-type cheese, made on a pilot scale and stored for 29 days, was evaluated. Although it was found that the addition of 2 g kg^{-1} GTE could significantly ($p < 0.05$) decrease the moisture content of cheese by 1.9%, there was no effect of this extract found on the composition (protein, fat, and calcium contents) of the cheese. These researchers did not investigate interactions between green tea catechins and milk components, nor the recovery of different catechins in GTE from the cheese. GTE was not analysed for catechin composition, so the results could be apparent values. In addition, the cheese was not ripened for longer than 29 days.

The aim of the present investigation was to examine the impact of GTE, as a natural and inexpensive source of green tea catechins, on antioxidant properties, composition, and microstructure of full-fat cheese ripened for 90 days. Furthermore, this study aimed to measure the retention of different catechins in GTE in cheese curd, as well as measuring the recovery of these catechins after *in vitro* digestion. The results obtained from this experiment will lead to better knowledge about the behaviour of GTE in a cheese matrix containing a high amount of fat, and accordingly, provide more information about putative associations between green tea catechins and MFGs. A future extension of this work is to develop a functional food with additional benefits to human health.

2. Materials and methods

2.1. Milk, reagents, and chemicals

Bovine pasteurized standard full-fat (3.3% fat) milk was purchased from a supermarket in Dunedin, New Zealand. (+)-Catechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, Trolox, 2,4,6-tris (2-pyridyl)-*s*-triazine (TPTZ), and gallic acid monohydrate were purchased from Sigma–Aldrich (Auckland, New Zealand). (−)-Epigallocatechin gallate was supplied by Sapphire BioScience (Auckland, New Zealand). Green tea extract was obtained from Invita (Auckland, New Zealand). Folin-Ciocalteu's phenol reagent was obtained from Merck (Darmstadt, Germany). Fluorescein and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) reagent were from Eastman Kodak (Kingsport, TN) and Cayman Chemical (Ann Arbor, MI), respectively. Methanol (HPLC grade) was from Thermo Fisher Scientific (Auckland, New Zealand). All other chemicals used were of analytical-reagent quality.

2.2. Cheese manufacture

Full-fat hard cheese was manufactured from pasteurized milk (3.3% fat) in 500-mL vats with the same method developed previously in our laboratory (Rashidinejad et al., 2013). Cheese from each vat was weighed, divided into three parts (to be analyzed on Day 0, 30, and 90) and vacuum packed in foil pouches (Audiovac, Audion, Weesp, The Netherlands). The Day 0 sample was transferred to a −80 °C freezer immediately after manufacture and the other two samples were stored at 8 ± 2 °C to be analyzed on Days 30 and 90. For different analyses during the study, subsamples were withdrawn randomly at different locations within each cheese sample. GTE was dissolved in acetate buffer (0.25 M; pH 3.8) to make a stock solution and then added to the milk at three concentrations of 250, 500, or 1000 ppm.

2.3. Experimental design

To examine the effect of GTE on the phenolic properties and antioxidant activities of the full-fat hard cheese, the experiment was carried out in a completely randomized design, similar to the study published previously (Rashidinejad et al., 2013), applying the same statistical model. The experiment comprised four different treatments, each treatment replicated four times in random order. In each cheese making session, four vats of cheese were made adding up to a total of 16 vats of cheese over four sessions. The treatments included: (1) control without GTE; (2) cheese made from milk containing 250 ppm GTE; (3) cheese made from milk containing 500 ppm GTE; (4) cheese made from milk containing 1000 ppm GTE.

2.4. Composition, yield, and pH

The pH of both whey and cheese samples was measured either during cheese manufacture or through the ripening period. The pH of whey samples was measured by direct insertion of a pH probe. For measuring the pH of cheese samples, 4–5 g of cheese were first mixed with the same weight of distilled water and then ground in a mortar and pestle to obtain a slurry. The pH of the slurry was measured in the same way as for the whey samples. Moisture, fat, and protein content of cheese samples on Day 0 were also determined using standard methods for dairy products (AOAC, 1984). The method and equation previously published (Rashidinejad et al., 2015a) were used to calculate cheese yield according to the cheese weight and initial weight of milk.

2.5. Extraction of phenolic compounds from cheese

Previously it was reported that gastrointestinal simulated digestion of cheese samples was a better extraction method compared to methanol extraction (Rashidinejad et al., 2015a). Accordingly, to extract both endogenous and incorporated phenolic compounds from full-fat cheese samples in the current study, the cheese was digested in the same simulated digestion model detailed in that paper. Briefly, a two-stage digestion system including gastric digestion and intestinal digestion was used. The digestion lasted for 6 h (2 h in the gastric section and 4 h in the intestinal section) with the pH maintained at 1.2 and 6.8 for the gastric and intestinal sections, respectively. The digestion fluids were pre-warmed (37 °C) before being added to the samples and the temperature was kept at 37 °C during the experiment. The digested cheese samples were filtered through a 0.45-μm membrane at 37 °C and the filtrates were kept at −80 °C for further analyses. Three replications were examined for each cheese sample.

2.6. Total extractable phenolic content

The filtered digesta obtained from simulated digestion of cheese samples were used for determination of the extractable phenolic compounds using the Folin–Ciocalteu assay adapted for a 96-well microplate reader (KC4 Multi-Mode; BioTek, Winooski, VT) as described previously (Rashidinejad et al., 2013). Correspondingly, the total phenolic content (TPC) was expressed as gallic acid