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Interactions between milk fat globules and green tea catechins

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ABSTRACT

The determination of putative chemical interactions between the milk fat globule membrane and green tea catechins provided useful information about the role of milk fat globules (MFGs) in high-fat dairy systems, such as cheese, and containing bioactive compounds, such as tea catechins. Catechins from green tea (125–1000 ppm), including (+)-catechin, (−)-epigallocatechin gallate, and green tea extract were added to washed MFGs to examine possible interactions. The addition of catechins gave a significant change in the size and ζ-potential of MFGs. The recovery of different catechins from the milk fat globule suspensions was found to vary, suggesting selective association with the milk fat globule membranes. The interactions were further investigated using transmission electron microscopy and Fourier transform infra-red spectroscopy. It is suggested that catechins are localised in association with milk fat globule membrane domains as they contain both hydrophobic and hydrophilic moieties with potential points of molecular interaction.

1. Introduction

Dietary catechins are polyphenolic compounds that are found in plants, such as tea, especially green tea, and have been shown to have strong antioxidant activities with some efficacy for improving human health (Johnson, Bryant, & Huntley, 2012; Oze et al., 2014). Consumption of tea has been reported to reduce the risk of major chronic diseases, such as cancer, coronary heart disease and stroke (Oze et al., 2014). These putative health benefits have resulted in attention being given to green tea catechins as food additives to improve the antioxidant properties and shelf-life of some foods, such as dairy products (Giroux et al., 2013; Hala et al., 2010).

Due to the potential for interactions between tea catechins and milk proteins (Ye, Fan, Xu, & Liang, 2013; Yuksel, Avci, & Erdem, 2010), creating functional dairy foods, containing tea catechins, that maintain antioxidant activity, is a challenge unless new technologies, such as nanoencapsulation to protect catechins from interacting with milk components, are applied (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014). Han et al. (2011) and Huvaere et al. (2011) reported that the addition of some polyphenols, including catechins, affected the composition of curds and cheese due to possible interactions between milk components and catechins. These catechins may interact non-covalently with proteins, such as through hydrophobic association (Yuksel et al., 2010). Most authors on this topic have reported interactions between catechins and milk proteins, and a few studies have speculated that there may be interactions between catechins and milk fat globules (Langley-Evans, 2000b; Ye et al., 2013). Bovine milk contains around 3–4% protein and about 3–8% fat, which exists in the form of an oil-in-water emulsion of milk fat globules (MFGs; MacGibbon & Taylor, 2006) stabilized by a membrane known as the milk fat globule membrane (MFGM). The MFGM is a biofunctional multilayer system that coats the triacylglycerol inner core of MFGs (Keenan & Mather, 2006). MFGs have a wide size distribution and composition, not only amongst different animal breeds, but also within the same breed and at different stages of lactation of a single cow (Walstra, Wouters, & Geurts, 2006). In addition, MFG composition may be affected by climate (Walstra et al., 2006), processes, such as homogenisation and storage, (Darling & Butcher, 1978), and also the methods of isolation of MFGs prior to analysis (Mulder & Walstra, 1974).

The aim of this investigation was to investigate the putative interactions between milk fat globules and green tea catechins in relation to the role of MFGs in dairy systems containing these catechins. This is relevant when considering high-fat products, such as cheese, as potential vehicles for polyphenolic compound delivery in the human diet.

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2. Materials and methods

2.1. Milk, reagents, and chemicals

Bovine (Jersey) raw milk samples were obtained from a local dairy farm (Port Chalmers, New Zealand), collected in the morning between 8:30 and 10:30 directly after milking. The herd was pasture-fed and milked by in-line vacuum milking equipment. Milk from Jersey cows has a relatively high fat content (5.2%), as well as a larger volume-based diameter of milk fat globules (4.5 μm) compared with milk produced by most other breeds (Mulder & Walstra, 1974). To minimise the effects of environmental factors on composition and structure of milk fat globules (Walstra et al., 2006), raw milk was cooled down to room temperature, immediately after milking, with no further mechanical disturbance.

(+)-Catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), Trolox, 2,4,6-tris (2-pyridyl)-s-triazine, and gallic acid monohydrate were purchased from Sigma Aldrich (Auckland, New Zealand). (−)-Epigallocatechin gallate (EGCG) was supplied by Sapphire BioScience (Auckland, New Zealand). Green tea extract (GTE) was obtained from Invita (Auckland, New Zealand). Fluorescein and 2,2′-azobis (2-amidinopropane) dihydrochloride reagent were from Eastman Kodak (Kingsport, TN, USA) and Cayman chemical companies (Ann Arbor, MI, USA) respectively. Folin–Ciocalteu's phenol reagent was obtained from Merck (Darmstadt, Germany). Methanol (HPLC grade) was from Thermo Fisher Scientific (Auckland, New Zealand). All other chemicals used were of analytical-reagent quality.

2.2. Milk fat preparation

Unpasteurised cream was obtained by centrifugation of raw milk at 1030×g, 10 min, and 20 °C following the method of Gallier, Gordon, Jiménez-Flores, and Everett (2011). The raw cream was then washed three times with deionized water (1030×g for 10 min, at 20 °C) (Kathriarachchi, Leus, & Everett, 2014). The top layer was collected and re-dispersed in ultra-filtered monophosphate buffer (0.05 M, pH: 6.8) to form a dispersion containing 4% fat content (w/v) and left for 1 h at 20–25 °C.

2.3. Addition of green tea polyphenols

Stock solutions (1 g l^{-1}) of C, EGCG, and GTE as a source of EGCG and other catechins, were prepared in acetate buffer (0.25 M; pH 3.8) along with a blank of the same buffer. The catechins, at four different concentrations (125, 250, 500, and 1000 ppm) were added to milk fat dispersions in dark brown bottles (three replicates) and mixed. The suspensions were held at 31 °C for 30 min and then 38 °C for 1 h, with random shaking, followed by centrifugation for 10 min (1030×g, 20 °C). Both the supernatant and subnatant were collected for further analysis. The supernatant was carefully collected from above the subnatant using a clean spatula. The subnatant was transferred into a 50 ml syringe and filtered through a 0.45 μm membrane at room temperature.

2.4. Particle size, specific surface area, and ζ-potential measurements

Particle size (diameter) distribution and specific surface area (S.S.A.) on both a volume and surface area weighted basis were measured by laser diffraction with a red wavelength diode laser and a blue light emitting diode (Horiba model LA-950; Irvine, CA, USA). The mean diameter was calculated as the average of the means from three replicates. Sample refractive indices of 1.460 and 1.470 were used for the diode laser and light-emitting diode, respectively. The continuous medium was deionized water. All measurements were carried out in triplicate. Mean particle size (peak of frequency distribution) and S.S.A. were calculated using the Horiba diffraction software. A Zetasizer Nano (ZS90; Malvern Instruments Ltd., Worcestershire, UK) was used for determination of the ζ-potential of milk fat globules with three replicates. The samples were diluted in acetate buffer (0.25 M; pH 3.8) before measuring ζ-potential. For the relatively large milk fat globule size and ionic strength, the Smoluchowski approximation is considered appropriate for calculating the ζ-potential.

2.5. Total phenolic content

To calculate the recovery of added catechin from milk fat suspensions, the quantity of catechins in the subnatant was first determined. The filtered (0.45 μm) subnatants were analysed for total phenolic content (TPC) using the Folin–Ciocalteu phenol reagent, following the method described previously (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2013).

2.6. Ferric reducing antioxidant power assay

Antioxidant activity by the ferric reducing antioxidant power (FRAP) assay was carried out using the method reported previously (Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2015; Rashidinejad et al., 2013). A linear standard regression was plotted with different concentrations (100–1000 μM) of $FeSO_4$ and used to express the results as mmol $FeSO_4$ equivalents (mmol kg^{-1}).

2.7. Oxygen radical absorbance capacity assay

The oxygen radical absorbance capacity fluorescein ($ORAC_{FL}$) assay was carried out in a microplate reader (BioTek), as reported previously (Rashidinejad et al., 2013, 2015). The area under the curve was calculated (Rashidinejad et al. (2013)) and the final ORAC results expressed as μmol of Trolox equivalents (Teq) per gram of fresh sample (Teq, μmol g^{-1}).

2.8. High performance liquid chromatography recovery of green tea catechins

Catechin concentrations were measured (in triplicate) by high performance liquid chromatography (HPLC) on a system equipped with a diode array detector (Agilent Technologies 1200 Series, Diegem, Belgium) following the isocratic method of Li, Martini, Wu, and Wen (2012). The mobile phase was 0.1% trifluoroacetic acid in deionized water (pH 2.0) and methanol (HPLC grade, Fisher) at a volume ratio of 75:25 with a flow rate of 0.8 ml min^{-1}. The sample injection volume was 20 μl where different concentrations of C, EGCG, EC, EGC, and ECG dissolved in acetate buffer (0.25 M; pH 3.8) were used as the standards. Chromatographic peaks of analytes were determined at 280 nm and identified by comparing the retention times with those of the external standards of the corresponding catechins. Peak integration using the external standard method was employed for quantification. Consequently, recovery of the catechins was calculated based on the initial concentration of the related standards and the concentration of each catechin detected in subnatants. For determining the correlation between the recovery of catechins in GTE, measured by HPLC, and the results of other analyses (TPC, FRAP, and ORAC), the most abundant green tea catechin in GTE (i.e. EGCG) was considered.

2.9. Transmission electron microscopy

Transmission electron microscopy (TEM) was carried out on the supernatant (containing fat globules) of the samples by a freeze