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Interactions between tea catechins and casein micelles and their impact on renneting functionality

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ABSTRACT

Many studies have shown that tea catechins bind to milk proteins. This research focused on the association of tea polyphenols with casein micelles, and the consequences of the interactions on the renneting behaviour of skim milk. It was hypothesized that epigallocatechin-gallate (EGCG), the main catechin present in green tea, forms complexes with the casein micelles and that the association modifies the processing functionality of casein micelles. The binding of EGCG to casein micelles was quantified using HPLC. The formation of catechin–casein micelles complexes affected the rennet induced gelation of milk, and the effect was concentration dependent. Both the primary as well as the secondary stage of gelation were affected. These experiments clearly identify the need for a better understanding of the effect of tea polyphenols on the processing functionality of casein micelles, before milk products can be used as an appropriate platform for delivery of bioactive compounds.

1. Introduction

Tea (*Camellia sinensis*) polyphenols have been reported to exhibit several beneficial health effects by acting as antioxidant, anticarcinogen and cardiopreventive agents (Sharma & Rao, 2009; Wang et al., 2011; Zaveri, 2006). This is of particular interest as tea is the second most consumed beverage after water. The most abundant group of polyphenols in tea are catechins. These compounds are water-soluble, colorless compounds which contribute to astringency and bitterness in tea. Major tea catechins include epicatechin, epigallocatechin, epigallocatechin-gallate, and epicatechin-gallate in which epigallocatechin-gallate (EGCG) is the major catechin component and also the most biologically active (Du et al., 2012; Henning et al., 2003; Vaidyanathan & Walle, 2003). These natural chemicals, especially EGCG, exhibit excellent radical scavenging and hence antioxidative properties, and have been widely employed as food antioxidants (Graf, Milbury, & Blumberg, 2005).

Traditionally, in many countries, tea is consumed with the addition of milk to improve the sensory properties, i.e., to reduce the astringency sensation caused by polyphenols (Bennick, 2002; Ferruzzi, Bordenave, & Hamaker, 2012). Also, development of new products with tea ingredients formulated with milk proteins such as chai latte, and other beverages containing dairy products have expanded over the years (Ferruzzi & Green, 2006). Milk is a natural, multi-component, nutrient-rich beverage and is generally recognized as a source of beneficial substances for growth and health in children and adults (Kalkwarf, Khoury, & Lanphear, 2003; Valeille et al., 2006). For this reason, milk is an ideal platform for delivery of other bioactive compounds, to obtain a new generation of dairy products providing additional benefits to human health (Bohin, Vincken, van der Hijden, & Gruppen, 2012; Livney, 2010). Functional dairy beverages have potential to satisfy the growing market need for health and taste, but significant knowledge of the bioactive components and their interaction with dairy components is essential (Sharma, 2005). It is important to determine whether the addition of these compounds in milk may affect the processing functionality of the casein micelles. This work aims to explore some of the effects of the addition of polyphenols to milk proteins and their relevance to the design of novel dairy products containing these functional ingredients.

Strong interactions have been reported between tea catechins and purified milk proteins in model solutions. Catechins bind strongly to the caseins, but associations with the whey proteins, namely α-lactalbumin, β-lactoglobulin and bovine serum albumin have also been reported (Kartosova and Alekseeva, 2008; Li, Du, Jin, & Du, 2012; Stojadinovic et al., 2013). Sensory studies have shown that caseins, especially β-caseins, due to their strong binding ability to tea catechins, decrease the sensation of astringency caused by the tea polyphenols (Hofmann et al., 2006; Lesschaeve & Noble, 2005; Schwarz & Hofmann, 2008; Soares, Mateus, & de Freitas, 2007). It has also been suggested that since binding reduces the astringency, it may also play a role in the decreased bioavailability of EGCG (Jobstyl, Howse, Fairclough, & Williamson, 2006; Yan, Hu, & Yao, 2009). However, bioaccessibility and bioavailability of polyphenols in milk mixtures is still a source of debate. The effect of the

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complexes formed between polyphenols and milk proteins in terms of decreasing or increasing the antioxidant activity of these bioactive molecules is still controversial. It is important to state that for the antioxidant capacity of polyphenols different methods have been used which may contribute to the controversy of the subject (Dubeau, Samson, & Tajmir-Riahi, 2010).

Catechins readily interact with proteins rich in proline, with an open and flexible structure (Bandyopadhyay, Ghosh, & Ghosh, 2012; Maiti, Sundar, Ghosh, & Dasgupta, 2006; Papadopoulou & Frazier, 2004). In addition, binding is clearly affected by protein characteristics including protein structure and amino acid composition, as well as polyphenol structure (e.g., glycosylated or not) and molecular size (Frazier et al., 2009; Poncet-Legrand et al., 2006; Richard, Lefeuvre, Descendit, Quideau, & Monti, 2006).

The astringency perceived during in mouth processing of polyphenol rich foods is attributed to the interactions between the polyphenols with basic salivary proline-rich proteins (Charlton et al., 2002; Hofmann et al., 2006; O'Connell and Fox, 2001). Caseins which constitute about 80% of the proteins of bovine milk contain high numbers of proline residues evenly distributed throughout their amino acid sequences and have relatively open structural features (like the salivary proline rich proteins). For this reason, isolated caseins have often been used as model proteins in various polyphenol–protein studies (Jobstyl, O'Connell, Fairclough, & Williamson, 2004; Pascal, Poncet-Legrand, Cabane, & Vernhet, 2008; Yan et al., 2009).

The interactions between bioactive molecules and caseins have recently gained attention, and studies have been reported on binding of casein micelles with resveratrol, vitamin A, vitamin D and curcumin (a polyphenol) (Rahimi Yazdi, & Corredig, 2012; Sahu, Kasoju, & Bora, 2008; Semo, Kesselman, Danino, & Livney, 2007; Shukla, Narayanan, & Zanchi, 2009). The effect of the interactions of polyphenols with casein micelles on the processing functionality of milk proteins is not fully understood, apart from the fact that it has been recognized that the presence of polyphenols enhances the heat stability of milk (O'Connell, Fox, Tan-Kintia, & Fox, 1998).

The main objective of this research was to better understand the impact of the milk protein/polyphenol interactions on the functional properties of milk proteins.

Although many studies using several different techniques have been used to characterise the polyphenol–protein complex formation of individual milk proteins, few studies have focused on the association of polyphenols with casein micelles in milk (native form) and as well as their processing effects. The effects of the complexes formed with the casein micelles on the functional properties of caseins, in particular their rennet-induced aggregation, have yet to be reported.

2. Materials and methods

2.1. Milk preparation and interaction studies

Raw milk (Ponsonby Research Station, University of Guelph, Guelph, ON, Canada) containing sodium azide (0.02% w/v), was centrifuged at 6000*g* for 20 min at 20 °C (Model J2-21, Beckman Coulter, Mississauga, ON, Canada). The skim milk was then filtered three times through Whatman glass fibre filters (Fisher Sci.) to remove any residual fat globules. Milk permeate (milk serum) was prepared from skim milk, using a laboratory scale ultrafiltration cartridge (Millipore CDUF001LG; 10 kDa, 0.095 m^2, Fisher Scientific).

Different concentrations of EGCG (90% pure, DSM Nutritional Products, Montreal, QC, Canada) were obtained from a stock solution of EGCG in water (20 mg/ml water). When needed, dilutions were carried out using permeate, to reach high ratios of EGCG to caseins while maintaining concentrations of EGCG below those critical for solubility. The soluble fraction was separated from the casein micelles by centrifugation at 36,000*g* and at 20 °C for 45 min (Beckman Coulter, Canada, Inc., Mississauga, On, Canada), as previously described (del Angel & Dalgleish, 2006).

2.2. Quantification of polyphenol binding to casein micelles

Different concentrations of EGCG were added to skim milk, and the colloidal phase (consisting of casein micelles) was separated from the whey proteins and soluble EGCG using centrifugation (see Section 2.1). After the separation, the amount of catechin present in the centrifugal supernatant was analysed by reversed phase HPLC as previously published (Ferruzzi & Green, 2006). In brief, aliquots of whey were combined with 2% aqueous acetic acid (1:1 v/v) and centrifuged at 14,000*g* for 5 min. Supernatants were collected and filtered through a 0.45 μm PTFE filter and immediately injected. A parallel set of tea catechin standard solutions dissolved in whey (separated from skim milk by centrifugation) were also prepared for quantification. No differences were noted, when comparing samples containing isolated EGCG fractions with or without whey protein added (data not shown).

To quantify the binding of EGCG in milk, Scatchard plot was used to determine the dissociation constant (K_d) which is a useful measure to describe the strength of binding (or affinity) between proteins and their ligands (Jiang & Armstrong, 2010). Using the Scatchard equation Eq. (1) the K_d between EGCG and casein proteins was determined. Where, is the average number of ligand molecules bound per protein molecule and [L] is the free ligand concentration.

$$v/[\mathrm{L}] = 1/K_d - v/K_d \quad (1)$$

Curved Scatchard plots can be decomposed into successive linear plots, and the dissociation constant K_d can be derived from each of the slopes (Relkin, & Vermersh, 2001; Jiang & Armstrong, 2010).

In these experiments, the concentration of the tea catechin was calculated by the amount of protein present in the sample, specifically, 32 mg/mL for skim milk and 26 mg/mL for the casein micelles and expressed as mg catechin per mg protein. The average molecular weight used for caseins was 2.2 kDa and for EGCG 458.4 g/mol.

2.3. Rheological properties of rennet-induced gels

A fresh rennet solution was prepared from double strength rennet (Chymostar, Danisco, Cranberry, NJ, USA) and immediately added to milk and tea-milk samples yielding a final rennet concentration of 3.14×10^{-4} IMCU/mL. Immediately after rennet addition at 30 °C, twenty-milliliter samples were placed in concentric cylinders in a controlled-stress rheometer AR1000 (TA Instruments, New Castle, DE). The development of the gel structure was followed using 0.01 strain, 1.0 Hz frequency and temperature of 30 °C. The time of cross-over between G′ (storage modulus) and G″ (loss modulus) was considered the gelation point.

2.4. Release of casein macro-peptide (CMP) from the surface of the casein micelles

Renneted milk and tea-milk samples were pipetted to several test tubes in a waterbath maintained at 30 °C. The rennet reaction was stopped at specific time intervals by addition of 3% perchloric acid with subsequent vortexing. After overnight refrigerated storage, the supernatant was collected after centrifugation at 4500*g* for 15 min, filtered through 0.45 μm filter, and analysed by reversed phase HPLC (López-Fandiño, Olanoal, San Joséa, & Ramos,