Food Research International 53 (2013) 449–455

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Interactions of black and green tea polyphenols with whole milk

Jianhui Ye [a,b], Fangyuan Fan [a], Xinqing Xu [b], Yuerong Liang [a,*]

[a] Zhejiang University Tea Research Institute, Hangzhou 310058, China
[b] CSIRO Animal, Food and Health Sciences, 671 Sneydes Road, Werribee, VIC 3030, Australia

ARTICLE INFO

Article history:
Received 19 February 2013
Accepted 26 May 2013

Keywords:
Tea polyphenols
Caseins
FTIR
Fluorescence quenching
ORAC assay

ABSTRACT

Interactions of black tea polyphenols (BTP) and green tea polyphenols (GTP) with whole milk were comparatively studied. Upon the ultracentrifugation of BTP– and GTP–milk systems (40% v/v milk), most BTP and GTP partitioned into milk protein fractions: whey proteins and casein micelles, with 35.1% and 73.0% of total catechins bound to the casein micelles accordingly. The affinities of catechins for casein micelles were differentiated by the structures of catechins in the GTP–milk system but the BTP–milk system, being enhanced by a gallate group in catechins and the *cis*-form and weakened by a pyrogallol group. Fourier transforms infrared spectroscopy (FTIR) analysis showed that TP binding altered the secondary structures of milk proteins by reducing inter β-sheet, random coil and the large loop and increasing α-helix, intra β-sheet and turn structures, and more intense hydrophobic interaction was observed in the BTP–milk system. UV–vis spectra indicated no obvious impacts on TP molecules at a low concentration of milk proteins. With the increasing addition of tea infusion, BTP exhibited a similar fluorescence quenching ability to GTP, but the variance in the ORAC values of BTP–milk system was different from that of the GTP–milk system, suggesting that fluorescence quenching may not fully represent the interactions between polyphenols and proteins.

1. Introduction

Tea is a worldwide beverage having various health benefits and physiological functionalities, such as antioxidative (Benzie & Szeto, 1999), anticarcinogenic and antimutagenic effects (Dufresne & Farnworth, 2000; Gupta, Saha, & Giri, 2002), due to the principal bioactive components — tea polyphenols (TP). Tea catechins are the major components of TP, which consist of (−)-epigallocatechin gallate (EGCg), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), (−)-epicatechin (EC), and their epimerization isomers (+)-gallocatechin gallate (GCg), (+)-gallocatechin (GC), (+)-catechin gallate (Cg), and (+)-catechin (C) (Goto, Yoshida, Kiso, & Nagashima, 1996). Black tea (fully fermented) and green tea (non-fermented) are two major tea varieties with different chemical components named black tea polyphenols (BTP) and green tea polyphenols (GTP). Consumption of black tea with milk is a regular practice in daily life, and the application of tea or tea extract to dairy product is becoming popular thanks to the antibacterial effect and bioactivities of TP (Ferruzzi & Green, 2006). Studies have been extensively carried out concerning the impact of the addition of milk or milk proteins on the antioxidant potentials of TP (Arts et al., 2002; Dubeau, Samson, & Tajmir-Riahi, 2010; Langley-Evans, 2000b; Lorenz et al., 2007; Serafini, Ghiselli, & FerroLuzzi, 1996), and three types of results were achieved: non-masking effect of adding milk or milk proteins (Kyle, Morrice, McNeill, & Duthie, 2007; Leenen, Roodenburg, Tijburg, & Wiseman, 2000; Richelle, Tavazzi, & Offord, 2001), differentiated masking effect on the antioxidant potentials of black tea and green tea (Arts et al., 2002; Dubeau et al., 2010), and total inhibition on both (Serafini et al., 1996). Understanding the interactions of BTP and GTP with milk is important to clarify the controversies over milk impacts on the antioxidant activities of black tea and green tea.

Whole milk contains 85.5–88.7% water, 2.3–4.4% protein and 2.4–5.5% fat. Caseins and whey proteins are the major proteins in milk, which account for 80% and 20% of milk proteins. Caseins exist in the form of casein micelles with hydrophilic surface layer and hydrophobic interior (Sahu, Kasoju, & Bora, 2008). β-Lactoglobulin is the major component of whey proteins. Due to the rich contents in proteins and lipids, milk or milk isolates have been used as carriers for bioactive compounds via ligand binding in order to improve the sensation, water solubility or bioavailability of bioactives (Bohin, Vincken, van der Hijden, & Gruppen, 2012; Livney, 2010; Staszewski et al., 2012). The interactions between individual catechins (e.g. EGCg, EGC and ECg) and pure proteins (e.g. β-casein, α-casein and β-lactoglobulin) or milk proteins occurred with the formation of catechin–protein complexes, and EGCg had stronger affinities for the pure proteins or milk proteins compared with C, EC and EGC (Bohin et al., 2012; Hasni et al., 2011; Kanakis et al., 2011; Xiao et al., 2011). However, the behaviors of tea catechins might be different in the compound milk system reconstituted by BTP or GTP rather than individual catechins, and the relevant information is scarce.

* Corresponding author. Tel./fax: +86 571 88982704.
E-mail address: yrliang@zju.edu.cn (Y. Liang).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.05.033

Fluorescence spectroscopy has been widely used to interpret the interactions between polyphenols and proteins (Bourassa, Kanakis, Tarantilis, Pollissiou, & Tajmir-Riahi, 2010; Hemar, Gerbeaud, Oliver, & Augustin, 2011), and the oxygen radical absorbance capacity (ORAC) assay is a common method for measuring the antioxidant capacities of polyphenols and milk (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002; Zulueta, Esteve, & Frigola, 2009; Zulueta, Maurizi, Frigola, Esteve, Coli and Burini, 2009). Normally, the interaction between polyphenol and protein is assumed as the main reason for protein impacts on the antioxidant activities of polyphenols, but the correlation between them is still equivocal. In this study, the affinities of tea catechins for different components of milk in both the BTP– and GTP–milk systems were investigated by using ultracentrifugation separation. Fourier transforms infrared spectroscopy (FTIR) and UV–vis spectroscopic methods were used to investigate the impacts of TP–protein interactions on the secondary structures of milk proteins and the molecules of TP. Protein fluorescence quenching study was carried out to interpret the interactions between TP and milk proteins, and the correlations between the fluorescence quenching ratio% and the variances in the ORAC values of TP–milk systems were analyzed.

2. Materials and methods

2.1. Materials and chemicals

Green tea and black tea were supplied by the CinoTea CO., Ltd. (Hangzhou, China). Ultra—high temperature processed (UHT) full cream milk (3.2 g protein, 3.3 g fat, 120 mg calcium per 100 mL) was purchased from the local supermarket. Phosphate buffer (PB), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), sodium carbonate, catechins (C), Folin–Ciocalteu reagent, HPLC standards (EGCg, EGC, ECg, EC, GCg, GC, Cg and C) as well as other chemical reagents of HPLC grade were purchased from Sigma-Aldrich (Sydney, Australia).

2.2. Preparation of tea infusion stocks

Green tea and black tea were ground by Breville CG2B grinder (Breville Group Ltd., Australia) and sifted through 0.45 mm sifter respectively. The ground tea samples (10.0 g) were brewed with 300 mL boiling water for 3 min. After filtering through the silicone treated filter paper (Whatman International Ltd., Maidstone, England), the tea infusions were centrifuged at 10,000 *g* and 20 °C for 45 min. The obtained supernatants were kept at 4 °C, namely black tea stock (pH 5.18) and green tea stock (pH 5.82).

2.3. Partition of BTP– and GTP–milk systems

Milk tea is a common object in the study of milk impacts on antioxidant potentials of tea in vivo and in vitro, thus a milk tea formula with the addition of 40% v/v milk was applied to investigate the distributions of tea catechins in the BTP– and GTP–milk systems according to Sharma, Kumar, and Rao (2008). 3 mL tea infusion stock was mixed with 2 mL whole milk (pH 6.63) or 2 mL water (control), and the mixture was kept at room temperature 20 °C for 1 h. The obtained BTP– and GTP–milk systems (pH 6.50 and 6.60) as well as the tea infusion controls were ultracentrifuged (Beckman Coulter Optima L-90K ultracentrifuge) at 367,290 g and 20 °C for 1 h, with no obvious effect on the components of ultracentrifuged fractions over the pH range of 6.5–6.7 at 20 °C (Anema & Klostermeyer, 1997). Three fractions were obtained from the TP–milk system: the cream layer (mainly milk fat globule, the top layer), the skimmed serum (mainly whey proteins, the middle layer) and the casein micelle pellet (mainly casein micelles, the bottom layer) (Garcia-Risco, Ramos, & Lopez-Fandino, 2002). The cream layer and the skimmed serum were collected respectively, and the skimmed serum was made up to 5 mL using water, same volume as before ultracentrifugation.

TP was extracted from the cream layer and the adjusted skimmed serum with ethanol. 200 μL sample was mixed with 800 μL ethanol to achieve a final concentration of 80% v/v ethanol so as to denature proteins. After centrifugation at 10,000 *g* and 20 °C for 15 min, the protein precipitate was re-extracted twice with fresh 80% ethanol, and the supernatants from the same sample were combined and made up to 5 mL for HPLC analysis and total phenolics analysis.

HPLC analysis of individual catechins: injection volume 10 μL, VYDAC C_{18} monomeric 238EV52, column temperature 28 °C, mobile phase A = acetonitrile + 0.08% trifluoroacetic acid, mobile phase B = water + 0.1% trifluoroacetic acid, linear gradient elution: from 2% (v) A/98% (v) B to 24% (v) A/76% (v) B during early 45 min and then 2% (v) A/98% (v) B till 50 min, flow rate 200 μL min^{-1}. The eluate was monitored by Surveyor PDA Detector at 280 nm. Catechins were identified and quantified by comparing the retention time and the peak area with the external standards with known concentrations.

Total phenolics analysis by Folin–Ciocalteu assay (Komes, Horzic, Belscak, Ganic, & Vulic, 2010): 100 μL sample and 80 μL 6-fold diluted Folin–Ciocalteu's phenol reagent were loaded onto a 96-well microplate and stood for 3 min. Then sodium carbonate solution (7.5% w/v, 120 μL) was added, mixed well and kept in dark for 2 h. The absorbance of the mixture was measured by microplate reader at 765 nm. The concentration of TP was expressed as mg (+)-catechins (C) L^{-1}, using the linear calibration curve of C (0.005–0.05 mg mL^{-1}).

2.4. FTIR spectroscopic measurements

In order to diminish the interference of milk fat, the whole milk was skimmed by centrifugation at 3000 *g* for 20 min (Corredig & Dalgleish, 1996). BTP– and GTP–milk samples with the addition of 40% v/v milk were reconstituted as above, using the skimmed milk. The skimmed milk, the BTP– and GTP–milk mixture samples and the tea infusions were freeze dried (Christ Alpha 1–2 LD plus Freeze Drye, German) for FTIR analysis. KBr pellet was prepared by admixing 2–3 mg of obtained sample with 300 mg of spectroscopy-grade KBr and pressing the mixture into a 13-mm disk at 4 t pressure with a die press. The spectra of pellets were recorded by a Nicolet AVATAR 370 FTIR Spectrometer (Thermo Fisher, Massachusetts, USA) from 400 to 4000 cm^{-1}.

The secondary structures of proteins were analyzed according to the reported method (Bourassa et al., 2010). Fourier self-deconvolution and secondary derivative were applied to the range of 1700–1660 cm^{-1} assigned to the amide I band in protein FTIR spectrum. The FTIR spectra were smoothed, and their baselines were corrected automatically using Thermo Scientific OMNIC software. By means of the second derivative in the spectral region of 1700–1600 cm^{-1}, the major peaks for protein secondary structure were resolved. The above spectral region was deconvoluted by the curve-fitting method with the Levenberg–Marquadt algorithm and the corresponding peaks were adjusted and the area measured with the Gaussian function: α-helix (1652 ± 2 cm^{-1}), intermolecular β-sheet (1624–1610 cm^{-1}), intramolecular β-sheet (1645–1625 cm^{-1}), turn (1685–1660 cm^{-1}), random coil (1648–1641 cm^{-1}), and β-antiparallel (1690–1700 cm^{-1}). The bands located at 1658 ± 2 cm^{-1} were assigned to the large loop rather than α-helix or β-turns (Curley, Kumosinski, Unruh, & Farrell, 1998; Farrell, Wickham, Unruh, Qi, & Hoagland, 2001). The area of all the component bands assigned to a given conformation was summed up and divided by the total area. Peak analysis was performed by the Origin Pro 8.5.1 software.

2.5. UV–vis spectroscopy analysis

UV–vis spectroscopy was carried out on a Shimadzu UV-1700 spectrophotometer to investigate the UV–vis spectral profiles of TP