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Comparative characterisation of green tea and black tea cream: Physicochemical and phytochemical nature

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ABSTRACT

Tea cream is prevalent in various types of tea, yet a comparison of the mechanism of creaming in different teas remains uncertain. Here, we compared physicochemical characteristics, phytochemical composition, and simulated digestive profiles of green tea and black tea cream, looking to exploit their concentration and structure based mechanisms and *in vitro* bioaccessibility. Green tea cream particles were roughly one order of magnitude larger than those of black tea in size. Moreover, creaming concentrations of catechins, proteins and methylxanthines of green tea were dramatically higher than black tea. As major creaming components, gallated catechins, theaflavins, thearubigins, theabrownines, proteins and methylxanthines also exhibited high creaming affinities. Green tea cream particles, which were completely destroyed by simulated digestion, had few impacts on digestive recoveries of catechins and methylxanthines. In comparison, black tea cream particles were more stable under mimicking digestion, and clarification remarkably decreased the *in vitro* bioaccessibility of catechins and methylxanthines.

1. Introduction

Tea (*Camellia sinensis*), a distinguished source of natural bioactive compounds (tea polyphenols, methylxanthines, etc.), has evolved to be one of the most consumed non-alcoholic beverages worldwide. Despite their prevalence, most of the clear ready-to-drink (RTD) tea beverages suffer from tea cream, which is a noticeable precipitate that forms spontaneously in a hot and strong tea infusion on cooling (Dickinson, 1994). Tea cream was first recognised in black tea and many studies were devoted to profiling its chemical composition (Nagalakshmi & Seshadri, 1983; Powell et al., 1992; Seshadri & Dhanaraj, 1988), physicochemical properties (Penders, Jones, Needham, & Pelan, 1998; Penders, Scollard, Needham, Pelan, & Davies, 1998) and molecular interactions based mechanisms (Charlton et al., 2000; Jöbstl, Fairclough, Davies, & Williamson, 2005). Tea cream is not limited to fermented tea, but it is also a common property of unfermented tea (images of creaming black tea and green tea are available in Supplementary Data). Accordingly, much research over the past decade was performed to investigate the characteristic features of green tea cream (Kim & Talcott, 2012; Lin et al., 2014; Xu et al., 2014; Yin, Xu, Yuan, Luo, & Qian, 2009) and its underlying mechanism (Hayashi, Ujihara, & Kohata, 2004; Sato, Kinoshita, Tsutsumi, Yamamoto, & Ishizu, 2012). However, apart from evidence that fermentation of tea leaves significantly affected tea cream formation (Liang, Lu, & Zhang, 2002), a comparison between the mechanism of creaming in green tea and black tea cream remains to be elucidated. Thus, we performed comparative characterisations for green tea and black tea cream from physicochemical and phytochemical perspectives by means of dynamic light scattering (DLS), laser doppler velocimetry (LDV), high performance liquid chromatography (HPLC) and ultraviolet–visible (UV–vis) spectrometry, aiming to explore the distinction in the size, light scattering, zeta potentials, and chemical composition of creaming particles between green tea and black tea. To account for these differences, concentration-creaming amount and structure-creaming tendency relationships between tea infusions and tea cream were exploited. For the former mechanism, creaming concentrations were correlated with original concentrations of major chemical components in tea infusions extracted with a different tea-water ratio. For the latter mechanism, creaming affinities of various tea components were compared by calculating their distribution coefficients between precipitates and supernatants and the impacts of structural details involved in tea components molecules on their creaming behaviours were discussed. These findings expanded our basic

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knowledge of the comparative characteristics for green tea and black tea cream and provided a new insight into the concentration and structure based mechanisms of tea cream.

Though physical filtration was a prevalent, even dominant approach for the clarification of RTD tea beverages, previous research proved that tea cream removal dramatically attenuated the *in vitro* anti-oxidant (Kim & Talcott, 2012) and anti-inflammatory (Lin et al., 2014) potentials of tea leaves. Conversely, retaining tea cream in RTD beverages would probably be challenged by the bioaccessibility of bioactive components contained in tea precipitates. Several questions raised by this assumption remain unanswered. For instance, can tea cream particles be dissolved by the gastrointestinal digestion? More fundamentally, does the clarification affect the digestive recoveries of major bioactive components in these tea cream particles? Is there any difference in the *in vitro* digestive stability between green tea and black tea in presence or absence of tea cream? To address these questions, *in vitro* gastrointestinal digestion of tea infusions and supernatants from green tea and black tea were conducted. Changes of average size, total intensities and catechins and methylxanthines concentrations of tea colloidal particles in tea infusions and supernatants that occurred after *in vitro* digestion were evaluated. These results revealed the significant impact of tea cream on the *in vitro* bioaccessibility of tea components and advanced our understanding of simulated digestion of tea beverages.

2. Materials and methods

2.1. Materials and chemicals

An apical bud and shoots with two adjoining leaves of Yunnan Daye tea (*C. sinensis*) were freshly plucked in spring at Xiangwo tea plantation (Xinxing County, Guangdong Province, PR China). Fresh tea leaves for green tea were first roasted at 260–300 °C for fixation followed by a 30 min-rolling. For black tea, fresh tea leaves pre-withered for 8–10 h were rolled for 50–60 min and were fermented at 25 °C with a relative humidity of 95% for 3–4 h. Finally, rolled tea leaves for green tea and fermented leaves for black tea were dried at 110–120 °C for 10–15 min and re-dried at 85–95 °C after a 40 min-cooling period, respectively.

The following chemicals were used: epigallocatechin gallate (EGCG, ⩾95%), gallocatechin gallate (GCG, ⩾98%), epicatechin gallate (ECG, ⩾98%), catechin gallate (CG, ⩾98%), epigallocatechin (EGC, ⩾98%), gallocatechin (GC, ⩾98%), epicatechin (EC, ⩾98%), -(−)catechin (C, ⩾98%), gallic acid (GA, 97.5–102.5% by titration), theophylline (⩾99.0%), theobromine (⩾99.0%), pepsin from porcine gastric mucosa (⩾400 units/mg protein), lipase from porcine pancreas (Type II, 100–400 units/mg protein), pancreatin from porcine pancreas (reagent grade) and bile salts (approximately 50% sodium cholate and 50% sodium deoxycholate) were purchased from Sigma–Aldrich Trading Co., Ltd. (Shanghai, PR China); Caffeine (99.9%) was purchased from Sangon Biotech Co., Ltd. (Shanghai, PR China). Milli-Q water (18.2 MΩ cm) was produced by Milli-Q Integral 3 from Merck-Millipore (Molsheim, France). Methanol (HPLC grade) was purchased from Honeywell Burdick & Jackson (Ulsan, Korea). Formic acid (HPLC grade, 96%) was purchased from Tedia Company Inc. (Fairfield, USA).

2.2. Tea extraction and tea cream preparation

Nine grams of tea leaves were brewed with boiled water at 10% w/v in a boiling water bath for 30 min with three agitations (10 min-interval). Tea slurry was subsequently filtrated with quantitative filter paper (Whatman-Xinhua Filter Paper Co., Ltd., Hangzhou, PR China) and the filtrate was then diluted with Milli-Q water to 100 mL. Aliquot tea infusions were refrigerated at 4 °C for 12 h for tea cream development. Cloudy infusions were centrifuged at 4 °C at 10,000*g* for 30 min (Centrifuge 5804R, Eppendorf A G, Hamburg, Germany) to separate tea cream and supernatants. After a 10-fold dilution, tea infusions and supernatants, as well as tea cream dissolved in Milli-Q water, were immediately tested for total solid amount and tea polyphenols, theaflavins (TFs), thearubigins (TRs), theabrownines (TBs), proteins, carbohydrates and amino acids concentrations. Aliquot parts of these solutions acidified with 2% acetic acid (2:1, v/v) were stored at −40 °C until HPLC analysis of catechins and methylxanthines.

Original tea infusions and supernatants without dilution were used to determine particle size and light scattering intensities by DLS, and used for zeta potential determination by LDV.

For correlation of tea cream formation with the chemical composition of tea infusions, green tea and black tea of different weights (1.6, 2.0, 2.5, 3.2, 4.0, 5.0, and 8.0 g) were extracted with 80 mL boiled Milli-Q water for 30 min in a boiling water bath to obtain a series of tea infusions with different total solid amount (green tea: 6.8, 8.1, 9.7, 12.2, 14.3, 17.4, and 23.6 mg mL^{-1}; black tea: 5.7, 6.9, 8.3, 10.2, 12.2, 14.8, and 19.4 mg mL^{-1}). Total solid amount and tea polyphenols, TFs, TRs, TBs, proteins, methylxanthines, carbohydrates and amino acids concentrations in tea infusions and precipitates were detected.

2.3. In vitro digestion

In vitro digestion of tea infusions and supernatants in green tea and black tea were performed according to Green, Murphy, Schulz, Watkins, and Ferruzzi (2007) with a slight modification as follow: 4 mL of tea solution diluted with 2 mL saline (0.9%) was mixed with 0.6 mL gastric liquid (40 mg mL^{-1} porcine pepsin in 0.1 M HCl) in an amber vial. The pH of mixtures was adjusted to 2.0 ± 0.1 with 1.0 M HCl. The headspace of the vial was then filled with N_2 for 5 min to replace the oxygen. After a shaking incubation at 37 °C (150 rpm, 1 h), the pH of the gastric digesta was adjusted to 5.3 ± 0.1 with $NaHCO_3$ (0.1 M) and NaOH (1.0 M). Subsequently, 1.8 mL intestinal solution (1 mg mL^{-1} porcine lipase, 2 mg mL^{-1} pancreatin and 12 mg mL^{-1} bile salts in 0.1 M $NaHCO_3$) was added. Final pH of the mixtures was adjusted to 7.2 ± 0.1 with 1.0 M NaOH and these mixtures were diluted to 10 mL with saline (0.9%). After the headspace of the vial was filled with N_2 for 5 min, the mixtures were incubated at 37 °C (150 rpm) for 2 h to mimic intestinal digestion. The impacts of gastrointestinal digestion on size distribution and intensities of colloidal particles in tea infusions and supernatants with or without *in vitro* digestion were determined by DLS. Moreover, the concentrations of catechins and methylxanthines in tea infusions and supernatants before and after simulated digestion were measured via HPLC and their digestive recoveries were compared to evaluate the influence of clarification.

2.4. DLS and LDV measurements

DLS analysis was performed to test light scattering intensities (derived count rates) and hydrodynamic diameter (D_H) distribution of colloidal particles in a disposable sizing cuvette (DTS 0012) on Zetasizer Nano ZS 90 with Malvern DTS 6.20 software (Malvern Instruments Ltd., UK) at 25 °C. Zeta potentials of colloidal particles were analysed by LDV in a disposable folded capillary cell (DTS 1070) at 25 °C.

2.5. Analysis of chemical components

As described in our recent study (Lin et al., 2014), total solid amount was gravimetrically determined and total polyphenols, soluble proteins, carbohydrates and free amino acids were