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Cream formation and main chemical components of green tea infusions processed from different parts of new shoots

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ABSTRACT

The formation of green tea cream and its chemical components were investigated. The green tea infusions were processed from different parts of new shoots: the bud and the first leaf, the second leaf and its implicative stem, the third leaf and its implicative stem, and the fourth leaf and its implicative stem, which were named as first part, second part, third part and fourth part, respectively. The results showed that the formation of tea cream and its settlement slowed down gradually from the first part to the fourth part, and that the amount of tea cream also decreased accordingly. The amount of tea cream was influenced remarkably by the chemical components in the green tea infusion. The main components of green tea cream were polyphenols (29.86 –78.66%), total sugar (14.47–27.62%) and caffeine (2.35–10.43%). Catechins (12.8–42.5%) were the main components of polyphenols which participated in tea cream formation. The main components in the catechins were found to be (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin (EC) and (−)-epicatechin gallate (ECG). Seven minerals were found in the tea cream, including Ca^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Zn^{2+} and Ni^{2+}.

1. Introduction

Tea cream is formed whilst hot aqueous tea infusion cools down, which not only has an unattractive appearance but also damages both taste and colour of tea. The main constituents of black tea cream were thearubigins (TRs), theaflavina (TFs) and caffeine (Roberts, 1962, 1963; Smith, 1968). Collier, Mallows, and Thomas (1972), Rutter (1971) and Rutter and Stainsby (1975) concluded that the formation of tea cream was governed by various molecular types of interactions including polyphenol–caffeine complexation and polyphenol–polyphenol interactions. Polyphenol–caffeine complexation is influenced by a number of gallate and hydroxyl groups of the polyphenols. Protein (Nagalakshmi & Seshadri, 1983) and a lipid complex (Seshadri & Nagalakshmi, 1988) were identified in black tea cream. Tea cream also has been found in green tea infusion (Liang, Lu, & Zhang, 2002) and in semi-fermented tea infusion (Chao & Chiang, 1999a). However, most researches related to tea cream formation have focused on black tea. The primary components of semifermented tea cream were catechins (30%), caffeine (20%) and protein (16%), and EGCG and ECG were found to be the major catechins precipitated during creaming, constituting 19% and 5% of the tea cream, respectively (Chao & Chiang, 1999a).

Liang and Bee (1992) investigated the morphology of green tea cream particles by using optical and electron microscopes, and revealed that tea cream could be formed in the absence of thearubigins and theaflavins. Liang et al. (2002) also observed that caffeine, gallocatechine (GC) and EGCG were predominant in green tea cream, and that gallated catechins had stronger creaming ability than un-gallated catechins. However, little information on the chemical components except catechins and caffeine has been reported, and the influence of chemical components in green tea infusions when tea cream formed was not noticed.

The amount of tea cream formed in tea infusions is influenced by various parameters including chemical composition (Liang et al., 2002; Penders, Jones, Needham, & Pelan, 1998; Smith, 1989). There are different chemical compositions in different parts of new shoots. The contents of polyphenols, catechins, caffeine, free amino acids, protein, magnesium (Mg), zinc (Zn) and kalium (K) decrease gradually, whilst the contents of total sugar, flavones, starch, chlorophyll, calcium (Ca), manganese (Mn) and aluminium (Al) increase gradually, amongst one leaf and a bud, second leaf, third leaf and fourth leaf of the new shoots (Chen, 1982; Selvendran, Perera, & Selvendran, 1973; Wan, 2003). Consequently, green tea infusions processed from different parts of new shoots can demonstrate the effects of the chemical components in green tea infusion better when green tea cream formed. The objectives of this study were to investigate the formation of green tea cream,

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chemical compositions of the tea cream and the effects of the chemical components in the tea infusions processed from different parts of new shoots when tea cream formed.

2. Materials and methods

2.1. Tea leaves

Shoots with four leaves and a bud from tea plant (*Camellia sinensis* (L.) O. Kuntze cv. Zhenghe dabaicha) were harvested in August 2007 from tea garden of the Tea Research Institute of Chinese Academy of Agricultural Sciences. Every new tea shoot was separated into four parts, which were the bud and the first leaf, the second leaf and its implicative stem, the third leaf and its implicative stem, and the fourth leaf and its implicative stem. These parts of new shoots were named as first part, second part, third part and fourth part, respectively. After withering for 3 h, every part of the tea leaves was panned at 130 °C for 8 min, rolled for 5 min, first dried at 95 °C for 30 min and then dried at 80 °C to a final moisture content of 4%. The dry tea leaves were stored in a freezer at −20 °C for further use.

2.2. Preparation of the tea infusion

The ground tea leaves (20–60 mesh) were extracted with distilled water (1:20, w/w) at 70 °C for 10 min. The extract was filtered through 300 mesh screen and then quickly cooled down to 25 °C or lower with a glass condenser using water (15 °C) as a cooling medium. The cooled extract was then centrifuged at 10,000*g* at 10 °C for 15 min to obtain a clarified infusion.

2.3. Formation and separation of the tea cream

The clarified infusion was sterilized at 90 °C for 5 min, and every 45 ml was filled in a lathy transparent bottle. The bottles were sterilized beforehand. Tea infusion in the bottles was cooled down to 25 °C, and then stored at 10 °C for 20 days to observe tea cream formation. Tea cream was separated by centrifuging at 10,000*g* at 10°C for 15 min, and the supernatant was discarded. The precipitated tea cream was rinsed with 10 ml distilled water, and then dissolved in 5 ml of 50% (v/v) ethanol solution, and then diluted to 50 ml with distilled water.

2.4. Observation of tea cream formation

The observation team consisted of 10 members who were trained before. The infusions were observed by colour, clarity and the amount of tea cream at the bottom of the bottle during storage at 10 °C. The result was determined together by the team, and the observation indexes are described in Table 1.

2.5. Analysis of chemical constituents of tea infusion and tea cream

2.5.1. Analysis of total solids content and the amount of tea cream

The total solids contents of the tea infusions were determined by drying 20 ml of the infusion at 80 °C for 48 h. The amount of tea cream in tea infusions was determined by referring to the method described by Nagalakshmi, Ramaswamy, Natarajan, and Seshadri (1984). The tea liquor in the bottles was centrifuged at 10,000*g* at 10 °C for 15 min. The supernatant was discarded. The precipitated tea cream that was obtained was washed with two aliquots of 5 ml distilled water in a weighed glass dish having a diameter of 9 cm, and dried at 80 °C for 48 h.

Table 1
The observation index of phenomena for tea cream formation during storage at 10 °C.

Index	1	2	3	4
Colour	Green	Yellow-green	Green-yellow	Yellow
Clarity	Pellucid	Sub-pellucid	A little turbid	Turbid
Amount of tea cream	No deposit	A little deposit	Visible deposit	Much visible deposit

2.5.2. Analysis of free amino acids

The content of free amino acids in the tea infusions and tea cream was determined with a spectrophotometer (UV-2550, Shimadzu (Suzhou) Instruments Manufacturing, Co., Ltd., Suzhou, China) by ninhydrin colour reaction method (Zhong, 1989), which was performed at 540 nm with glutamic acids as the standard of free amino acids. The standard of the free amino acids graph is given as $C = (A_{570} + 0.0868) \times 1.51^{-1}$, $R^2 = 0.9996$. C was the concentration of the free amino acids, and A_{570} was the absorbance (A) at 570 nm.

2.5.3. Analysis of tea polyphenols

The content of tea polyphenols in the tea infusions and tea cream was determined by the spectrophotometric method with $FeSO_4$, 3.5×10^{-3} M potassium sodium tartrate and buffer described by Zhong (1989). Absorbance (E_1) of the reaction solution was determined at 540 nm in a 1 cm photometer cuvette with Shimadzu UV-2550 spectrophotometer. Absorbance (E_2) of the control reaction solution (containing 5 ml distilled water, 5 ml dyeing solution and 15 ml buffer) was determined at 540 nm as described earlier. The content of tea polyphenols was calculated using the following equation: TP (mg L^{-1}) = $(E_1 - E_2) \times 3.9133 \times 10^3$.

2.5.4. Analysis of protein, pectin and flavones

The protein content in the tea infusion and tea cream was determined by bicinchoninic acid (BCA) method (GENMED SCIENTIFICS INC., USA). The pectin content was determined by the procedure proposed by Blumenkrantz and Asoboe-Hansen (1973) using anhydrogalacturonic acid (AGA) as a standard. The flavones content in the tea infusions was determined at 420 nm by the spectrophotometric method with 1% $AlCl_3$ as described by Zhong (1989).

2.5.5. Analysis of total sugar and polysaccharides

The total sugar content was determined by the anthrone-sulphuric acid reaction, using glucose as a standard (Fu, Xie, Nie, Zhou, & Wang, 2001). Two milliliters of tea infusion was reacted with 8 ml anthrone reagent (2 g anthrone dissolves in 1000 ml analytically pure sulphuric acid) at 100 °C for 10 min, and then the absorbance (A_{620}) was determined with SHIMADZU UV-2550 spectrophotometer after being cooled rapidly for 10 min. The standard glucose graph is given as $C = 0.3589 * A_{620} - 0.0034$, $R^2 = 0.999$. C was the concentration of the glucose.

Preparation of polysaccharides: 5 ml of tea infusion was precipitated with 10 ml 100% (v/v) ethanol at 10°C for 24 h, and centrifuged (10 min, 5000*g*) to get the sediment (Chen, Zhang, & Xie, 2005). Polysaccharides were dissolved and fixed to 25 ml with distilled water, and then analysed by referring to total sugar method.

2.5.6. Analysis of tea catechins and caffeine

Analysis of tea catechins and caffeine was carried out by HPLC method (Liang et al., 2002). The tea infusion was filtered through a 0.2 μm Millipore filter before injection (Model Shimadzu LC-2010A, Shimadzu Corporation, Kyoto, Japan). The HPLC conditions were as follows: injection volume, 5 μl; column, 5 μm – Diamonsil™ C18 (4.6 mm × 250 mm); temperature, 40 °C; mobile phase A, acetonitrile/acetic acid/water (6:1:193); mobile phase B, acetonitrile/acetic acid/water (60:1:139); gradient, 100% mobile phase A to 100% mobile phase B by linear gradient during the early