Analysis of cream formation in green tea concentrates with different solid concentrations

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Abstract The formation of tea cream in the green tea concentrates of different solid concentrations (5, 10, 20, 30, 40, 50 and 60°Brix) was investigated. The results showed a good positive correlation ($\gamma=0.98$, $p\leq 0.05$) between the amount of tea cream and the solid concentrations from 5 to 40°Brix, while the amount of tea cream in the tea concentrates of 50 and 60°Brix decreased acutely. Total sugar, caffeine and catechins were found to be the main chemical components of tea cream in the green tea concentrate. The large decrease of the amount of tea cream in the tea concentrates of 50 and 60°Brix may be induced by a sharp increase of the viscosity of the tea concentrates, which helped to improve the stability of tea concentrate. It may be indicated that the stability of green tea concentrate enhanced when the concentration higher than 50°Brix, which helped to restrain the formation of tea cream.

Keywords Cream formation · Green tea concentrate · Solid concentration · Chemical components · Viscosity

Introduction

Tea beverage is one of the most frequently consumed drinks in the world. Green tea is an unfermented tea, and contains more catechins than black tea or oolong tea. Green tea has been found to have many beneficial effects such as antioxidant, antimutagenic effect, antibacterial and antiviral activity (Cabrera et al. 2006). In China, there is about fifteen thousand tons of tea concentrate produced every year, which is used as additive or raw material, though transportation and storage of tea concentrate is intractable. Green tea concentrate is the main tea concentrate processed in China, because green tea accounts for more than 70% of the total tea yield. However, tea cream appears in tea concentrate, which not only has an unattractive appearance but also damages both taste and aroma quality of tea concentrate.

The formation of tea cream is governed by various parameters including extracting temperature (Liang and Xu 2003), pH (Liang and Xu 2001), chemical composition (Chao and Chiang 1999a) and solid concentration (Bee et al. 1987). Polyphenols, total sugar and caffeine were found to be the main components of green tea cream, and most catechins components and seven minerals were found in the tea cream (Yin et al. 2009).

Tea cream is also known as colloidal precipitate. In accordance with the classical theory of lyophobic colloids (Verwey and Overbeek 1948), the electrostatic repulsion between the charged droplet surfaces protects the droplets from coagulation. However, at high tea cream phase volumes, the phenomenon of phase inversion with a dilute phase dispersed into a continuous cream phase was also found by Bee et al. (1987). Penders et al. (1998) showed that in the concentrated region of the miscibility gap ($>2\%$ w/w), demixing (spinodal decomposition) was the predom-

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inant mechanism of cream formation, and the (narrow) metastable region of the miscibility gap was in general difficult to access even at low concentrations of tea and small temperature quench depths. The rheological behavior of a solid–liquid dispersion depends on the characteristics of the continuous phase (the solvent or serum), the dispersed phase (the particle), and the interactions between them. However, there was lack of study on tea cream formation and the stability of tea concentrate, especially green tea concentrate.

The objectives of this study were to analyze the formation of tea cream in green tea concentrates with different solid concentrations by investigating the amount of tea cream, the viscosity of tea concentrates and the main chemical components which participated in tea cream formation, and discuss the causation.

Materials and methods

Green tea concentrate

Green tea was bought from the Tea Market of South Zhejiang and used as tea material in the present paper. The green tea (20–60 mesh), separated into 7 shares randomly, was extracted with distilled water (1:15, w/w) at 75 °C for 15 min. The extract was filtered through 300 mesh screen and then quickly cooled down to 25 °C 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. The clarified infusion was concentrated with a centritherm (CT-1, FT Industrial Pty. Ltd., Australia) at 50 °C and 90 kPa, and seven samples of concentrated green tea infusion with different solid concentrations (5, 10, 20, 30, 40, 50 and 60°Brix) were prepared. The samples of tea concentrate were sterilized at 90 °C for 15 min, and every 45 ml were quickly infused in a transparent bottle. The bottles were sterilized beforehand. Green tea concentrates in the bottles, which were the original concentrate, were cooled down to 25 °C, and then stored at 4 °C for 30 days to observe tea cream formation. After storage, the tea cream was separated by centrifuging at 10,000 g at 4 °C for 15 min, and the supernatant were the clarified concentrate.

Analysis of the amount of tea cream

The amount of tea cream in the tea concentrates was determined according to the method described by Nagalakshmi et al. (1984). The tea concentrate in the bottles was centrifuged at 10,000 g at 4 °C for 15 min. The supernatant was discarded. The precipitated tea cream was obtained and the bottle 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.

Analysis of chemical constituents of tea concentrate

The contents of the chemical constituents in the tea cream were determined by calculating the contents in the original and clarified tea concentrates and then making a comparison. In other words, the contents of the chemical constituents which participated in tea cream formation were calculated by subtracting the contents of the clarified tea concentrates from those of the original tea concentrates. It should be calculated as follow: Participated content (mg ml^{-1}) = [original content (mg ml^{-1}) $\times V_1(\text{ml})$ – clarified content (mg ml^{-1}) $\times V_2(\text{ml})$]/ $V_1(\text{ml})$.

V_1 was the volume of the original concentrate, and V_2 was the volume of the clarified concentrate.

Analysis of total sugar The total sugar content was determined by the anthrone-sulphuric acid reaction, using glucose as a standard. Two ml of tea infusion were reacted with 8 ml anthrone reagent (2 g anthrone dissolves in 1,000 ml analytically pure sulphuric acid) at 100 °C for 10 min, and then the absorbance (A_{620}) was measured with a spectrophotometer (UV-2550, Shimadzu (Suzhou) Instruments Manufacturing, Co., Ltd., Suzhou, China) after being cooled down rapidly for 10 min. The standard glucose graph was given as $C = 0.3589 \times A_{620} - 0.0034$, $R^2 = 0.999$. C was the concentration of the glucose.

Analysis of free amino acids The content of free amino acids in the tea concentrates was determined with Shimadzu UV-2550 spectrophotometer by ninhydrin dyeing's method, which was performed at 570 nm with glutamic acids as the standard of the free amino acids. The standard of free amino acids graph was 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 at 570 nm.

Analysis of flavones The flavones content in the tea concentrates was determined at 420 nm by the spectrophotometric method with 1% AlCl_3 . A half ml of tea infusion were reacted with 9.5 ml 1% AlCl_3 solution at 25 °C for 10 min, and then the absorbance (A_{420}) was measured with Shimadzu UV-2550 spectrophotometer. The concentration of the flavones was calculated as follow: Flavones content ($\text{mg} \cdot \text{ml}^{-1}$) = $A_{420} \times 320 \times 1000^{-1}$.

Analysis of 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 μ l; column, 5 μ m—Diamonsil™ C18 (4.6 mm $\times$ 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 45 min and then 100% mobile phase B up to 60 min; flow rate, 1 ml min⁻¹; detector, Shimadzu SPD ultraviolet detector (Shimadzu Corporation, Kyoto, Japan) at 280 nm.

Analysis of minerals The minerals in the tea concentrates were determined by ICP-OES (IRIS/AP, Thermo Jarrell Ash Corp., America). Analytical conditions were as follows: Detector CID, low wavelength max integration time: 15 s; high wavelength max integration time: 5 s; nebulizer pressure: 193 kPa; pump speed: 100 rmin⁻¹; auxiliary gas: medium (1.0 Lmin⁻¹); RF power: 1,150 W.

Analysis of viscosity

The viscosity of the tea concentrates was measured using a digital rheometer (L-90, Shanghai Tongji Science & Technology Industrial Co., Ltd, Shanghai, China) with spindle SC4-18 at 45 rpm (Mao et al. 2007). The viscosity of the tea concentrates was measured at about 25 °C, and the viscosity was evaluated according to the formula: $\eta(\text{Pa} \cdot \text{s}) = A \times 0.125$. A was the reading of the digital rheometer.

Statistical analysis

Results are presented as mean value $\pm$ standard deviation (at least three replicates). Analysis of variance and significant differences amongst means and correlation analysis were done by one-way ANOVA using SPSS (Version 13.0, SPSS Inc., Chicago, USA).

Results and discussion

Tea cream formation in the green tea concentrates of different solid concentrations

From 5 to 40°Brix of the tea concentrates, with the increasing of solid concentration, the amount of tea cream increased (Table 1). Good positive correlation ($\gamma=0.98$, $p\leq0.05$) was found between the solid concentrations and the amount of tea cream in the tea concentrates from 5 to 40°Brix. Concentration is one of the parameters which determined the formation of tea cream, and it was also observed by Smith (1968) and Penders et al. (1998) that the amount of tea cream increased with the concentration to a certain extent.

Table 1 Amount of tea cream in the green tea concentrates of different solid concentrations

Solid concentration (°Brix)	Amount of tea cream(mg ml ⁻¹)
5	12.6 $\pm$ 0.26 ^a
10	21.4 $\pm$ 0.88 ^b
20	43.2 $\pm$ 4.45 ^c
30	66.5 $\pm$ 15.30 ^d
40	77.4 $\pm$ 14.67 ^d
50	28.2 $\pm$ 8.48 ^b
60	32.2 $\pm$ 9.23 ^{bc}

Data are means ($\pm$ SD) of 3 replicates

The same letters in a line are not significantly different ($P\geq0.05$)

However, the amount of tea cream decreased when the solid concentration achieved 50°Brix. Some irreversible deposit was found in the green tea concentrates from 30 to 60°Brix, which was also observed in green tea beverage by Niino et al. (2005).

Main chemical constituents participated in tea cream formation in the green tea concentrates of different solid concentrations

Total sugar and caffeine The content of total sugar and caffeine (Table 2) in the original and clarified green tea concentrates both increased with the solid concentration. And it could be found that total sugar and caffeine both participated in green tea cream formation, which was also observed as the main constituents of green tea cream in the former study (Yin et al. 2009). With the increasing of the amount of tea cream in the tea concentrates from 5 to 40°Brix, the contents of total sugar and caffeine which participated in tea cream formation increased. But in the tea concentrates of 50 and 60°Brix, similar to the amount of tea cream, the content of total sugar and caffeine which participated in tea cream formation were much lower than those in the concentrates of 30 and 40°Brix. However, good positive correlation was found between the amount of tea cream and the contents of total sugar ($\gamma=0.94$, $p\leq0.05$) and caffeine ($\gamma=0.96$, $p\leq0.05$) which participated in tea cream formation in the green tea concentrates from 5 to 60°Brix.

Total sugar participated in tea cream formation included pectin, starch and polysaccharides (Yin et al. 2009). Caffeine was also observed as a principal compound for inducing cream formation in semi-fermented tea, and creaming was found again after replenishing the decreamed tea infusion with caffeine (Chao and Chiang 1999b). The concentration of caffeine was found to affect the extent of tea cream formation (Rutter and Stainsby 1975). Complexation of caffeine and polyphenols was found the main form participating in tea cream formation,

Table 2 Contents of total sugar and caffeine participated in green tea cream formation

Solid concentration (°Brix)	Total sugar (mg ml ⁻¹)			Caffeine (mg ml ⁻¹)		
	Original	Clarified	Participated	Original	Clarified	Participated
5	20.2±0.38 ^a	16.8±3.58 ^a	3.5 ^a	4.0±0.84 ^a	1.7±0.31 ^a	2.4 ^a
10	40.7±2.15 ^b	26.1±0.73 ^b	15.1 ^b	6.3±1.60 ^a	2.4±0.93 ^a	4.0 ^b
20	91.7±11.95 ^c	56.4±9.20 ^c	37.6 ^c	11.2±1.48 ^b	5.3±0.13 ^b	6.2 ^c
30	126.3±13.16 ^d	90.6±8.41 ^d	42.1 ^d	20.0±2.23 ^c	9.6±1.50 ^c	11.1 ^d
40	172.3±10.06 ^e	134.3±12.48 ^e	48.7 ^e	27.3±1.98 ^d	18.7±1.37 ^d	10.1 ^d
50	224.1±22.33 ^f	203.9±20.56 ^f	26.4 ^f	34.2±1.29 ^e	31.6±0.34 ^e	3.6 ^b
60	250.3±8.12 ^f	239.9±18.02 ^f	17.6 ^b	41.4±1.44 ^f	36.5±1.44 ^f	6.0 ^c

Data are means (± SD) of 3 replicates

The same letters in a row are not significantly different ($P \geq 0.05$)

‘Participated’ were the contents of total sugar and caffeine which participated in tea cream formation, which were calculated as follow: Participated content (mg ml⁻¹) = [original content (mg ml⁻¹) × V_1 (ml) – clarified content (mg ml⁻¹) × V_2 (ml)] / V_1 (ml). V_1 was the volume of the original concentrate, and V_2 was the volume of the clarified concentrate

and the complex was found to form through the six-member rings of caffeine and methyl gallate eclipsed each other in a stacking arrangement with hydrophobic interaction, and simultaneous intermolecular hydrogen bonding stabilizes this arrangement (Couzinet-Mossion et al. 2010).

Free amino acids and flavones From Table 3, it could be found that free amino acids and flavones both participated in tea cream formation. Good positive correlation was also found between the amount of tea cream and the contents of flavones ($\gamma=0.91$, $p \leq 0.05$) which participated in tea cream formation in the green tea concentrates from 5 to 60°Brix. Tea flavonols, including myricetin, quercetin and kaempferol in green tea, make up 2–3% of the water-soluble solids of tea (Wang and Helliwell 2001). However,

flavonols are also regarded to be potent antioxidants due to the combination of a keto group conjugated to a double bond in the C ring, together with adjacent hydroxyl groups in the B ring. Flavonols was found to participate in tea cream formation in green tea infusion, which may be because of the hydroxyl groups that can be integrated with caffeine and protein.

The content of free amino acids participated in tea cream formation increased with the solid concentration of the tea concentrates. There was good positive correlation ($\gamma=0.99$, $p \leq 0.05$) between the contents of free amino acids which participated in tea cream formation and the solid concentration from 5 to 60°Brix, but not the amount of tea cream. Free amino acid was also found to participate in cream formation of Paochung tea (Chao and Chiang 1999a), while

Table 3 Contents of free amino acids and flavones participated in green tea cream formation

Solid concentration (°Brix)	Free amino acids (mg ml ⁻¹)			Flavones (mg ml ⁻¹)		
	Original	Clarified	Participated	Original	Clarified	Participated
5	3.0±0.13 ^a	2.8±0.10 ^a	0.3 ^a	1.6±0.26 ^a	0.91±0.21 ^a	0.7 ^a
10	4.9±0.04 ^b	4.4±0.04 ^b	0.6 ^b	2.3±0.21 ^b	1.4±0.31 ^a	1.0 ^b
20	8.8±0.12 ^c	8.2±0.03 ^c	1.0 ^c	4.1±0.52 ^c	2.7±0.44 ^b	1.5 ^c
30	14.2±0.29 ^d	13.4±0.05 ^d	1.7 ^d	6.8±0.76 ^d	4.5±0.63 ^c	2.7 ^d
40	20.1±0.70 ^e	18.9±0.11 ^e	2.7 ^e	9.6±0.64 ^e	7.7±0.41 ^d	2.5 ^d
50	25.9±0.04 ^f	23.9±0.59 ^f	2.7 ^e	12.0±0.23 ^f	11.9±0.05 ^e	0.3 ^c
60	31.4±0.80 ^g	28.8±0.54 ^g	3.5 ^f	15.2±0.36 ^g	14.2±0.32 ^f	1.4 ^c

Data are means (± SD) of 3 replicates

The same letters in a row are not significantly different ($P \geq 0.05$)

‘Participated’ were the contents of the free amino acids and flavones which participated in tea cream formation, which were calculated as follow: Participated content (mg ml⁻¹) = [original content (mg ml⁻¹) × V_1 (ml) – clarified content (mg ml⁻¹) × V_2 (ml)] / V_1 (ml). V_1 was the volume of the original concentrate, and V_2 was the volume of the clarified concentrate

Table 4 Contents of total catechins and gallated catechins participated in green tea cream formation

Solid concentration (°Brix)	Total catechins (mg ml ⁻¹)			Gallated catechins (mg ml ⁻¹)		
	Original	Clarified	Participated	Original	Clarified	Participated
5	16.0±0.56 ^a	10.5±0.53 ^a	5.7 ^a	9.2±0.25 ^a	4.7±0.41 ^a	4.6 ^a
10	26.6±1.19 ^b	14.8±0.78 ^b	12.1 ^b	15.0±0.53 ^b	6.1±0.34 ^a	9.0 ^b
20	47.7±3.57 ^c	30.1±1.99 ^c	18.8 ^c	26.0±1.86 ^c	13.3±0.65 ^b	13.3 ^c
30	80.8±0.67 ^d	50.0±2.40 ^d	34.4 ^d	45.4±0.32 ^d	23.2±1.17 ^c	23.9 ^d
40	111.2±6.94 ^e	84.1±4.58 ^e	33.8 ^d	62.5±3.18 ^e	43.6±2.23 ^d	22.4 ^d
50	134.7±1.41 ^f	127.6±3.11 ^f	10.9 ^b	75.7±1.09 ^f	72.8±0.48 ^e	5.1 ^a
60	175.6±10.57 ^g	157.2±3.02 ^g	23.1 ^e	98.0±4.76 ^g	85.3±1.46 ^f	15.3 ^c

Data are means (± SD) of 3 replicates

The same letters in a row are not significantly different ($P \geq 0.05$)

‘Participated’ were the contents of the total catechins and gallated catechins which participated in tea cream formation, which were calculated as follow: Participated content (mg ml⁻¹) = [original content (mg ml⁻¹) × V_1 (ml) – clarified content (mg ml⁻¹) × V_2 (ml)] / V_1 (ml). V_1 was the volume of the original concentrate, and V_2 was the volume of the clarified concentrate

Nagalashmi and Seshadri (1983) did not find a significant amount of theanine in the acid hydrolysate of black tea cream.

Catechins Catechins were the main components of polyphenols in green tea concentrates. It could be found that catechins components participated in tea cream formation (Table 4). Gallated catechins (48.93–79.77%), including (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate (ECG), (-)-catechin gallate (CG) and (-)-gallocatechin gallate (GCG), were the main components of the catechins which participated in tea cream formation. There were good positive correlations between the contents of total catechins ($\gamma=0.94$, $p \leq 0.05$) and gallated catechins ($\gamma=0.92$, $p \leq 0.05$) and the amount of tea cream in green tea concentrate from 5 to 60°Brix. Gallated catechins were prone to participate in tea cream formation, which was observed by Chao and Chiang (1999b). It was also thought that the gallated catechins which possessed a galloyl group and a hydroxylpheny B ring acted like a claw (Chao and Chiang 1999b). And gallated catechins having stronger creaming ability were considered to offer more hydroxyl groups for hydrogen bonding, which was thought to be an important force for aggregation of compounds involved in tea cream formation (McManus et al. 1985).

Viscosity of the tea concentrates

The viscosity of the clarified tea concentrates increased with the solid concentration (Fig. 1). From 5 to 40°Brix of the tea concentrates, there was a little increase in viscosity, but a rapid increase in viscosity appeared in the tea concentrates of 50 and 60°Brix, which was also found in cloudy apple juice (Genovese and Lozano 2000). This may

be the cause why the amounts of tea cream in the tea concentrates of 50 and 60°Brix were much lower than those in the tea concentrates of 30 and 40°Brix. Bee et al. (1987) reported that the diameter of the individual colloidal droplets and the phase volume of the disperse phase increased greatly with the solid concentrations of black tea infusion. Gong et al. (2006) observed that addition of konjac glucomannan helped to decrease tea cream and stabilize the suspended particles in tea infusion by forming many water-soluble complexes among the konjac glucomannan molecules and tea components and increasing the viscosity of the tea infusion. High viscosity may help to improve the stability of tea concentrate, which has been observed in apple juice concentrate (Benitez et al. 2009). However, there was difference between green tea concentrate and apple juice concentrate since the soluble solids in apple juice concentrate were mainly sugars, pectins and organic

Fig. 1 Viscosity of the clarified green tea concentrates

acids while tea concentrates mainly contained polyphenols, sugars, caffeine and amino acids.

Conclusion

The formation of tea cream in green tea concentrates of different solid concentrations was investigated. There was good positive correlation ($\gamma=0.98$, $p\leq 0.05$) between the amount of tea cream and the solid concentrations from 5 to 40°Brix, while the amount of tea cream in the tea concentrates of 50 and 60°Brix decreased acutely. Total sugar, catechins and caffeine were found to be the main components of the tea cream in the green tea concentrates. The large decrease of the amount of tea cream in the tea concentrates of 50 and 60°Brix may be induced by the sharp increase of the viscosity of the tea concentrates, which helped to improve the stability of tea concentrate. Tea cream formation in tea concentrate of high concentration was influenced by the stability of the colloidal dispersion, which increased with the viscosity in an extent. It may be indicated that the stability of green tea concentrate enhanced when the concentration higher than 50°Brix, which helped to restrain the formation of tea cream.

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