



## Effect of saccharides on sediment formation in green tea concentrate



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### ABSTRACT

Reducing sediment in green tea concentrate utilized for tea production is an important process. In this study, the effect of saccharides on sediment formation in green tea concentrate was investigated. The results show that the amount of tea sediment significantly decreased (31.4%–86.4%) with the addition of fructose or sucrose and that the ratios of polyphenols and caffeine in the sediment sharply decreased (24.1%–49.7% and 2.4%–6.2%) while the proportion of total sugars markedly increased (20.9%–56.5%) in the sediment. Moreover, fructosyl was found to be a highly effective functional group for preventing sediment formation, on the basis of experimental results for a series of sugars with different numbers of fructosyl groups. This phenomenon was elucidated from the energies of interaction between typical sugars, polyphenols, and caffeine calculated by density functional theory method. Our results open new applications for tea concentrates.

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## 1. Introduction

Tea concentrate, which is used to prepare both mixed tea beverage and instant tea, is massively produced throughout the world. In China, green tea concentrate is one of the main tea-extract products, with about 15,000 tons consumed per year (Xu, Chen, Yuan, Tang, & Yin, 2012). Most of the green tea concentrate is used for the preparation of green tea, the bitterness of which is usually reduced by adding sugar processed from sugarcane or sugar beet (Valipour, 2012, 2015; Valipour et al., 2015). Tea concentrate is a colloidal dispersion that is unstable during storage. During low-

temperature storage, green tea concentrate yields a substantial amount of tea sediment, which mainly consists of polyphenols, sugars, caffeine, proteins, and minerals (Chao & Chiang, 1999; Xu et al., 2014; Xu, Chen, Shen, & Yin, 2011). Tea sediment not only has an unattractive appearance, but it also detracts from the taste and color of the tea infusion (Penders, Jones, Needham, Pelan, & Davies, 1998; Xu et al., 2014). Polyphenol–caffeine and polyphenol–polyphenol interactions are the major intermolecular phenomena leading to sediment formation (Rutter & Stainsby, 1975; Rutter, 1971).

In our previous study, we found a strong positive correlation between the amount of tea sediment and the solid concentration at 5–40° Brix, as well as improvement of the colloidal stability of the tea concentrate due to an increase in viscosity (Xu et al., 2012). The concentrations of Ca<sup>2+</sup>, caffeine, and polyphenols could

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significantly influence the amount of tea sediment; lowering their concentrations inhibited sediment formation in green tea infusions (Xu, Hu, Tang, Jiang, Yuan, Du, & Yin, 2015).

Adding carbohydrates is a useful method for preventing coalescence of aqueous colloids. Common saccharides, including glucose, sucrose, and maltose, can enhance the viscosity and colloidal stability of apple juice (Benítez, Genovese, & Lozano, 2009). Saccharides are effective at inhibiting tea sediment formation in black tea infusion (Liang & Bee, 1995). However, the action of saccharides in inhibiting sediment formation in tea infusion is still unclear. The effect of saccharide addition on sediment formation in green tea infusion is also unclear. Effective methods for preventing the formation of sediments in tea concentrates (20%–60%, w/v) are being sought since polyphenols and caffeine are the major components of tea concentrate. The objectives of this study were to investigate the effects of saccharides on sediment formation in green tea concentrate and to find an optimal way of inhibiting sediment formation.

## 2. Material and methods

### 2.1. Chemicals

Caffeine, (–)-epigallocatechin, (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate (EGCG), 1-kestose, nystose, 1<sup>F</sup>-fructofuranosylnystose, and gallic acid were bought from Sigma (Shanghai, China). Maltose, glucose, sucrose, and fructose were obtained from Henan Runcheng Chemical Products Co. Ltd. (Zhengzhou, China). Green tea instant powder was provided by Zhejiang Minghuang Natural Products Development Co. Ltd. (Hangzhou, China). Purified water (Hangzhou Wahaha Group Co. Ltd. Hangzhou, China) was used in all experiments. The pH of the water was  $6.81 \pm 0.05$ , and its electroconductibility was  $1.81 \pm 0.10 \mu\text{S}/\text{cm}$ .

### 2.2. Treatment of green tea concentrates using sugars

Green tea concentrate (30% w/w) was prepared by dissolving instant green tea powder with distilled water at 60 °C. Sugar (maltose, glucose, sucrose, or fructose) was added to the tea concentrate to a given concentration under magnetic stirring. Tea concentrate without added sugar was used as the control. After the concentrates were sterilized at 90 °C for 6 min, they were stored at 4 °C for 14 days for observations on the formation of tea sediment and colloidal stability.

### 2.3. Treatment of green tea concentrates using food hydrocolloids

Green tea concentrate (30% w/w) was prepared by dissolving instant green tea powder with distilled water (60 °C). Sodium carboxymethyl cellulose (CMC; 0%, 0.05%, 0.1%, 0.2%, and 0.4%), xanthan gum (XG, 0%, 0.02%, 0.06%, and 0.1%), and carrageenan (0%, 0.02%, 0.06%, and 0.1%) were added to the green tea concentrates (30% w/w) to different concentrations. A magnetic stirrer was used to ensure that the hydrocolloids were completely dispersed in the tea concentrate when hydrocolloids were added at 60 °C. The concentrates were sterilized at 90 °C for 6 min. The treated tea concentrates were then stored at 4 °C for 14 days for observations on tea sediment formation and colloidal stability.

### 2.4. Separation and determination of tea sediment

Tea sediment was separated by centrifuging at  $8000 \times g$  at 4 °C for 15 min. The amounts of sediment in concentrated tea were determined by weighing after drying at 80 °C for 48 h following the

method described by Nagalakshmi, Ramaswamy, Natarajan, and Seshadri (1984).

### 2.5. Analysis of chemical components in tea sediment

Tea sediment was separated and dissolved in hot distilled water (60 °C). The concentrations of chemical components were then determined as follows.

#### 2.5.1. Analysis of polyphenols and total sugar

The polyphenols in green tea concentrate were determined by spectrophotometric method using  $\text{FeSO}_4$ ,  $3.5 \times 10^{-3}$  M potassium sodium tartrate solution, and buffer, as described by Liang, Lu, Zhang, Wu, and Wu (2003). The absorbance of the reaction solution at 540 nm ( $E_1$ ) was measured using a 1-cm photometer cuvette and a Shimadzu UV-2550 spectrophotometer (Jiangsu, China). The absorbance of the control reaction solution (a mixture of 5 mL distilled water, 5 mL dyeing solution, and 15 mL buffer) at 540 nm ( $E_2$ ) was measured. The polyphenol content of tea was calculated through the following equation: Polyphenol content (mg/mL) =  $(E_1 - E_2) \times 3.9133$ .

The total sugar content was determined by the anthrone–sulfuric acid reaction using glucose as a standard. Reaction between 2 mL of tea infusion and 8 mL of anthrone reagent (2 g anthrone dissolves in 1000 mL of analytically pure sulfuric acid) was carried out at 100 °C for 10 min, and the absorbance ( $A_{620}$ ) was measured with a Shimadzu UV-2550 spectrophotometer after rapid cooling for 10 min. The total sugar content was calculated from the following equation: Total sugar content (mg/mL) =  $(A_{620} + 0.05)/7.715$ ,  $R^2 = 0.9907$  ( $p < 0.01$ ).

#### 2.5.2. Analysis of catechins and caffeine

Analysis of tea catechins and caffeine was carried out by HPLC (Xu et al., 2014) using a Shimadzu LC-2010A (Shimadzu Corporation, Kyoto, Japan). Tea infusion was filtered through a 0.2- $\mu\text{m}$  Millipore filter before injection into a DiamonsilTM C18 column ( $250 \times 4.6$  mm I.D. 5  $\mu\text{m}$ ). The HPLC injection volume was 5  $\mu\text{L}$ , and the column temperature was 40 °C. Gradient elution was performed with solvent A (acetonitrile/acetic acid/water (6:1:193) solution) and solvent B (acetonitrile/acetic acid/water (60:1:139) solution). The linear gradient was run from 100% solvent A to 100% solvent B in the first 45 min, and 100% solvent B was then maintained for another 15 min. The flow rate of the mobile phase was 1 mL/min, and the detection wavelength was 280 nm.

### 2.6. Analysis of viscosity

The viscosity of the tea concentrates was measured using a digital rheometer (DV-II + Pro; Brookfield Company, Stoughton, USA) with ULA spindle DV-II, with the torsion up to 80% before storage (Mao, Xu, & Que, 2007). The measurement temperature was 25 °C. The viscosity was calculated according to the formula,  $\eta$  (Pa·S) =  $A \times 0.125$ , where A is the reading on the digital rheometer.

### 2.7. Analysis of particles images

Images of the particles in the green tea concentrate were obtained by transmission electron microscopy (TEM; LEO Electron Microscopy Ltd. Cambridge, UK). The sample was prepared as follows: A drop of tea concentrate was mounted onto a carbon-coated copper grid. It was dried at room temperature and then examined without negative staining by TEM.

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