Food Chemistry 172 (2015) 831–835

Contents lists available at ScienceDirect

Food Chemistry

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

The major factors influencing the formation of sediments in reconstituted green tea infusion

Yong-Quan Xu [a,b,1], Xiong-Fei Hu [b,1], Ping Tang [c], Yong-Wen Jiang [a], Hai-Bo Yuan [a], Qi-Zhen Du [b,*], Jun-Feng Yin [a,*]

[a] Tea Research Institute, Chinese Academy of Agricultural Sciences; Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, 9 South Meiling Road, Hangzhou 310008, China
[b] Institute of Food Chemistry, Zhejiang Gongshang University, 149 Jiaogong Road, Hangzhou, Zhejiang 310012, China
[c] Hangzhou Vocational and Technical College, 68 Xueyuan Road, Hangzhou 310018, China

ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form 1 September 2014
Accepted 25 September 2014
Available online 5 October 2014

Keywords:
Reversible sediment
Irreversible sediment
Caffeine
Polyphenols
Ca^{2+}

ABSTRACT

The effects of Ca^{2+}, caffeine and polyphenols on the formation of reversible tea sediments (RTS) and irreversible tea sediments (IRS) in green tea infusion were studied. Adding Ca^{2+} (2 mmol/l) was found to increase the formation of RTS by 8% and IRS by 92%, while adding chelating ions of Na_2EDTA significantly decreased the amount of RTS by 14.6%, but not the amount of IRS. Under acid conditions, Ca^{2+} combined with oxalic ions to form indissoluble oxalate that is the principal constituent of IRS, despite the existence of the chelating ions. Decaffeination largely inhibited the formation of RTS (73%) and IRS (60%), even in the presence of Ca^{2+}. The amount of sediment could be reduced by removing polyphenols using polyvinyl-polypyrrolidone. The results suggest that sediment formation in green tea infusions can be inhibited by lowering the concentration of Ca^{2+}, caffeine or polyphenols.

1. Introduction

Tea cream emerges from a hot aqueous green tea infusion as it cools, and sediments appear as the tea cream settles. Tea sediment not only has an unattractive appearance but also influences the flavour and colour of the tea infusion (Liang & Xu, 2001; Penders, Jones, Needham, & Pelan, 1998). Tea sediment consists of reversible tea sediment (RTS) primarily and irreversible tea sediment (IRS) (Xu et al., 2014). The main chemical composition of RTS is very different from that of IRS, especially the mineral composition (Xu, Chen, Wang, et al., 2012). RTS mainly comprises polyphenols, total sugar, caffeine, flavones and proteins, and IRS consists of oxalates of Ca, Mg, Ga and Mn (Xu et al., 2014).

The constituents of tea sediment include polyphenols (thearubigins, theaflavins and catechins), caffeine, protein, free amino acids (theanine, aspartic acid and glutamic acid), carbohydrate (pectin and polysaccharides), organic acids and minerals (Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}) (Chao & Chiang, 1999a; Jöbstl, Fairclough, Davies, & Williamson, 2005; Yin, Xu, Yuan, Luo, & Qian, 2009). The formation of tea sediment is influenced by various parameters, including the trace chemicals in the water used for its extraction (Couzinet-Mossion et al., 2010; Xu et al., 2013), extraction temperature (Liang & Xu, 2003), pH (Liang & Xu, 2001), the components of the tea leaves (Xu, Chen, Shen, & Yin, 2011) and the concentration of solids (Xu, Chen, Yuan, Tang, & Yin, 2012). Polyphenols and caffeine are the principal components of tea sediment (Chao & Chiang, 1999b; Yin et al., 2009). Protein is a primary component of black tea cream (Wu & Bird, 2010), oolong tea cream (Chao & Chiang, 1999a) and green tea cream (Kim & Talcott, 2012). However, in green tea infusions, the concentration of soluble protein is too low to influence the formation of tea sediment, with Ca^{2+} playing an important role in the formation of tea cream and sediments (Jöbstl et al., 2005). It is still unclear how polyphenols, caffeine and Ca^{2+} influence the formation of reversible and irreversible tea sediment in green tea infusions.

In this study, we aimed to investigate the action of polyphenols, caffeine and Ca^{2+} on the formation of RTS and IRS in green tea infusions by selectively removing caffeine, polyphenols or Ca^{2+} (chelation). The results contribute to clarifying how to resolve the sediment problem in green tea infusions.

* Corresponding authors. Tel.: +86 571 88081935; fax: +86 571 88823509 (Q.-Z. Du). Tel.: +86 571 86650031; fax: +86 571 86650056 (J.-F. Yin).
E-mail addresses: qizhendu@126.com (Q.-Z. Du), yqx33@126.com, yinjf@mail.tricaas.com (J.-F. Yin).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.foodchem.2014.09.143
0308-8146/

2. Materials and methods

2.1. Materials

Instant green tea powder, with a polyphenol content of 55.4% (w/w) and caffeine 4.8% (w/w), was provided by the Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou, China. The powder was prepared by extracting green tea leaves with pure water at 85 °C for 25 min, filtering the extraction solution, concentrating the filtrate using a reverse osmosis membrane to 18 Brix and spray-drying. The reagents used, $CHCl_3$, polyvinyl-polypyrrolidone (PVPP), $CaCl_2$, NaOH, Na_2EDTA and $H_2C_2O_4$, were obtained from Sigma–Aldrich China (Shanghai, China).

2.2. Effect of Ca^{2+} on the formation of green tea sediment

The effect of Ca^{2+} on the formation of green tea sediment was investigated using an infusion reconstituted from instant green tea powder because an infusion using direct extraction from tea leaves would have been less stable. The reconstituted infusion was prepared by dissolving the tea powder in hot water (40 °C) at a proportion of 2.5% (w/w). Na_2EDTA and/or Ca^{2+} ($CaCl_2$) were added to the 2.5% reconstituted green tea infusion with stirring at 40 °C over 5 min to a concentration of 2 mmol/l. The tea infusions with different treatments (Control, Ca^{2+}, Na_2EDTA, Ca^{2+} + Na_2EDTA) were then sterilized at 90 °C for 5 min and stored at 4 °C for 14 days. For analysis, the tea sediment was separated from the supernatant by centrifugation (8000*g*, 15 min, 4 °C).

2.3. Effect of caffeine on the formation of green tea sediment

The decaffeinated tea infusion was prepared from the reconstituted green tea infusion by a double extraction with $CHCl_3$ at a proportion 1:1 (v/v) at 25 °C for 1 h. Ca^{2+} (2 mmol/l, $CaCl_2$) was added to the decaffeinated tea infusion to investigate its effect on the formation of tea sediments.

2.4. Effect of polyphenols on the formation of green tea sediment

The reconstituted green tea infusion (2.5%, w/w) was treated with polyvinylpolypyrrolidone (PVPP) (0.0%, 0.2% and 0.4%, w/v) at 40 °C to remove the polyphenols. Ca^{2+} (2 mmol/l, $CaCl_2$) was then added to this modified tea infusion to investigate its effect on the tea sediments.

2.5. Analysis of the mechanism of Ca^{2+} participation in the formation of tea sediment

Ca^{2+} (1 mmol/l) was added to a 1% green tea infusion with different combinations of NaOH, Na_2EDTA or $H_2C_2O_4$ included (Table 1). The mixtures were treated at room temperature for 30 min, then the turbidity of each mixture was determined by a turbidimeter (WZT-3A, Shanghai Jinjia Scientific Instrument Co. Ltd., Shanghai, China). The interactions between Ca^{2+} and EDTA and oxalic ions were analysed from the changes in turbidity.

Table 1
Addition of Ca^{2+} to green tea infusions under different conditions.

No.	Treatments	Turbidity (NTU)
1	Control (1% w/w, pH 6)	62.47 ± 5.72^{c}
2	NaOH (to pH 8)	0.74 ± 0.23^{a}
3	Ca^{2+} (1 mmol/l)	210.80 ± 17.41^{f}
4	NaOH (to pH 8) + Ca^{2+}	81.08 ± 6.18^{d}
5	Na_2EDTA (1 mmol/l)	45.20 ± 4.27^{b}
6	Na_2EDTA + Ca^{2+}	153.15 ± 10.06^{e}
7	Na_2EDTA + NaOH (to pH 8) + Ca^{2+}	0.68 ± 0.33^{g}
8	Na_2EDTA + Ca^{2+} + $H_2C_2O_4$ (1 mmol/l)	>600^{a}
9	Na_2EDTA + NaOH (to pH 8) + Ca^{2+} + $H_2C_2O_4$ (1 mmol/l)	0.55 ± 0.26^{a}

Data are means (±SD) of three replicates. $^{a-g}$Different letters indicate a significant difference ($p < 0.05$).

2.6. Separation and preparation of IRS and RTS

The sediments were obtained by centrifugation (8000*g*, 15 min, 4 °C) then dispersed in distilled water at 60 °C. After 20 min, the dissolved portion was categorised as RTS, and the insoluble part as IRS, which was separated by centrifugation at 8000*g* at 25 °C for 15 min. After drying at 80 °C for 48 h, the IRS and RTS were weighed (Nagalakshmi, Ramaswamy, Natarajan, & Seshadri, 1984).

2.7. Analysis of major constituents of the tea infusion

2.7.1. Protein and flavones

The protein concentrations were determined using the Enhanced BCA Protein Assay Kit (Beyotime Biotechnology, Haimen, China). The flavones components were determined by spectrophotometry with 1% $AlCl_3$ at 420 nm (Yin et al., 2009). Absorbance (A_1) at 420 nm of the reaction solution (containing 0.5 ml sample and 9.5 ml 1% $AlCl_3$ solution) was determined in a 1 cm photometer cuvette using a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan). Absorbance (A_2) at 420 nm of a control reaction solution (distilled water replacing the tea sample) was similarly determined. The content of flavones was calculated by the equation: flavones (mg ml^{-1}) = $(A_1 - A_2) \times 0.32$.

2.7.2. Polyphenols and total sugar

The polyphenol contents were determined by the spectrophotometric method using $FeSO_4$, 3.5×10^{-3} M potassium sodium tartrate and buffer described by Liang, Lu, Zhang, Wu, and Wu (2003). The absorbance (E_1) at 540 nm of the reaction solution was determined in a 1 cm photometer cuvette as before. Absorbance (E_2) at 540 nm of a control reaction solution (containing 5 ml distilled water, 5 ml dye solution and 15 ml buffer) was again determined as above. The content of tea polyphenols was calculated by the equation: polyphenols (mg ml^{-1}) = $(E_1 - E_2) \times 3.9133$.

The total sugar content was determined by the anthrone-sulphuric acid reaction, using glucose as a standard (Chen, Zhang, Qu, & Xie, 2008). Two ml of tea infusion was reacted with 8 ml anthrone reagent (2 g anthrone dissolved in 1000 ml analytically pure sulphuric acid) at 100 °C for 10 min. After being cooled rapidly for 10 min, the absorbance (A_{620}) of the solution was determined using a Shimadzu UV-2550 spectrophotometer. The total sugar content was calculated by the equation: total sugar (mg ml^{-1}) = $0.1296 \times A_{620} + 0.0065$.

2.7.3. Caffeine and catechins

The analysis of tea catechins and caffeine was carried out using HPLC (El-Shahawi, Hamza, Bahaffi, Al-Sibaai, & Abduljabbar, 2012). The tea infusion was filtered through a 0.2 μm Millipore filter before injection (LC-2010A, Shimadzu). The HPLC injection volume was 5 μl into a Diamonsil™ C18 column (250 × 4.6 mm I.D., 5 μm), and the column temperature was 40 °C. The mobile phase was a mixture of A, acetonitrile/acetic acid/water (6:1:193) and B, acetonitrile/acetic acid/water (60:1:139). The mobile phase was changed linearly from 100% A to 100% B over the first 45 min and then held at 100% B up to 60 min. The mobile phase flow rate was 1 ml min^{-1}. Detection used an SPD ultraviolet detector (Shimadzu) at 280 nm.