Extraction and preparation of high-aroma and low-caffeine instant green teas by the novel column chromatographic extraction method with gradient elution

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Abstract The lack of aroma and natural taste is a critical problem in production and consumption of instant green teas. A method to prepare instant green teas high in-natural-aroma and low-caffeine by the novel column chromatographic extraction with gradient elution is reported. This method simultaneously extracted aroma (or volatile) and non-aroma compounds from green tea. Green tea was loaded into columns with 2.0-fold of petroleum ether (PE): ethanol (8:2). After standing for 3 h until the aroma compounds dissolved, the column was sequentially eluted with 3.0-fold 40% ethanol and 3.5-fold water. The eluant was collected together and automatically separated into PE and ethanol aqueous phases. The aroma extracts was obtained by vacuum-evaporation of PE phase at 45 °C. The ethanol aqueous phase was vacuum-concentrated to aqueous and partially or fully decaffeinated with 4% or 9% charcoal at 70 °C. A regular instant green tea with epigallocatechin-3-gallate: caffeine of 3.5:1 and a low-caffeine instant green

tea (less than 1% caffeine) with excellent aroma and taste were prepared, by combining the aroma and non-aroma extracts at a 1:10 ratio. This work provides a practical approach to solve the low-aroma and low-taste problems in the production of high quality instant green teas.

Keywords Instant green tea · Column chromatographic extraction · Decaffeination

Introduction

Instant tea has become a fast growing drinking product worldwide, as it's fully soluble, convenient to use and flexible to adjust the taste and components, comparing to the traditional leaf tea products. Green tea contains high amount of bioactive compounds such as polyphenolics, caffeine, theanine and soluble polysaccharides. There is a large amount of green tea materials that can be used to make instant green tea (Wang et al. 2012). However, instant teas often lack of natural tea aroma and taste, which are the critical problems in the production and consumption.

The aroma compounds contribute greatly to the tea odor and taste and are the important sensory factors in the quality evaluation of tea and instant tea (Kraujalytė et al. 2016). Most of the aroma compounds are low-polar volatile substances which were not effectively extracted by the traditional extraction methods using hot water or ethanol aqueous solution, or lost during the concentration of tea extracts at high temperatures (Zhu et al. 2008; Huo et al. 2014; Katsuno et al. 2014). Some efforts have been made to extract or recover the tea aroma compounds in the production of instant tea, such as vacuum-distillation, simultaneous distillation and extraction, resin adsorption,

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supercritical extraction, microwave assisted extraction and freeze drying (Lv et al. 2012; Ye et al. 2014). These methods are ineffective or uneconomic for industrial production of instant green teas.

Instant green tea is often made from summer and autumn tea leaves which account for 60% of annual tea output. As the high temperature and high intensity of illumination, the tea plant synthesizes more polyphenolics and caffeine in summer and autumn, compared to the spring tea. Polyphenolics and caffeine contents are also quite different from tea cultivars and production places (Ye 2012). The high content of caffeine makes the tea taste bitter. Many reports indicate that caffeine or high amount caffeine have side effects on certain groups of people such as children and pregnant women. Though it is available to breed low caffeine tea varieties through biotechnological methods (Yadav and Ahuja 2007), limited resource of low-caffeine tea is available. Decaffeination is important in the production of instant tea (Vuong et al. 2013). Many decaffeination methods for tea leaves or instant tea have been reported, including hot water treatment of tea leaves (Liang et al. 2007; Vuong et al. 2013), solvent fractionation (Dong et al. 2011), supercritical extraction (Park et al. 2007; Kim et al. 2008), adsorption by a variety of adsorbents (Ye et al. 2007; Liu et al. 2012), membrane separation (Kumar et al. 2012) and biological treatments with microbes or enzymes (Fan et al. 2011). These methods have their own characteristics and weaknesses in removal of caffeine from tea extracts.

We have reported the highly efficient column chromatographic extraction (CCE) of green tea (Wang et al. 2012). However, the aroma compounds in the green tea were not effectively extracted and the tea extracts lack the unique aroma and taste of natural green tea. The objective of this work was to simultaneously extracting low-polar aroma, high-polar and water soluble compounds from green tea using the CCE method with gradient elution and prepare high-aroma and low-caffeine instant green teas after quantitative removal of caffeine with charcoal (Ye et al. 2007).

Materials and methods

Green tea and chemical reagents

Leaf green tea was obtained from the Tea Research Center of Guangdong Academy of Agricultural Sciences. The tea material was dried at 65 °C for 4 h in an electric oven, ground and sieved. Green powder with the particle size between 20 and 80 mesh was used for the experiments.

Standard compounds of epigallocatechin-3-gallate (EGCG), caffeine and theanine were purchased from

Sigma (St. Louis, MO, USA). HPLC-grade solvents were purchased from Burdick & Jackson Inc. (Muskegon, MI, USA). Charcoal was purchased from Guangzhou Chemical Factory (Guangzhou, China). Two organic solvents of petroleum ether (PE) and food grade ethanol (95%) used in this study are permitted for food processing by the US Food and Drug Administration. Other chemical reagents are analytical grade and were purchased from local suppliers.

Analysis of compounds

Total amounts of polyphenolics, EGCG, caffeine, soluble polysaccharides and theanine in the tea material were extracted according to the National Standard Method of the China National Accreditation Service for Conformity, which were used as content controls of 100% (Wang et al. 2012). Polyphenolic content was determined by the ferrous tartrate colorimetric method (GB/T8313–2002). Polysaccharides were determined by the phenol sulphate colorimetric method. EGCG, caffeine and theanine were determined by HPLC in a Shimadzu SPD-20A HPLC system (Shimadzu, Japan) with YMC-packed ODS column (250 mm × 4.6 mm, 5 µm). EGCG and caffeine were analyzed with methanol: water: acetic acid (25:73:2, v/v/v) as the mobile phase and UV detection at 280 nm; theanine was analyzed with a mobile phase of 0.05% trifluoroacetic acid aqueous solution and UV detection at 203 nm. The sample peaks were identified by their retention times and co-injection tests with their corresponding standard compounds, and the quantitative analysis of each compound was performed using the peak area based on the corresponding standard curves.

The low-polar aroma substances were analyzed by visual observation on thin layer chromatographic (TLC) plates. TLC analysis was conducted on silica gel G TLC plates (Merck Co., Germany). The developing solvents were PE: ethyl acetate (8.5:1.5, v/v) for low-polar components, and isobutanol: H₂O: acetic acid (4:5:1, v/v/v) for high-polar components. The developed TLC plates were stained in iodine vapor.

Column chromatographic extraction of compounds in green tea

Non-gradient CCE with single solvent to extract the low-polar, high-polar or water soluble compounds, and the determination of their optimal solvents, minimum volume (MV) of the solvent for the material to be fully absorbed and dissolving time were conducted as described before (Wang et al. 2012).

Gradient column chromatographic extraction of all soluble compounds from green tea

The gradient CCE for simultaneous extraction of all soluble compounds from green tea was performed based on our previous report with modifications (Han et al. 2014). The tea powder (5 g, unless for scale up experiments with 2.0 kg) was loaded into a column with one MV (2.0-fold of the tea material, v/w) of PE:ethanol 8:2 at a column diameter: height of 1:5 through the wet column preparation method. After standing for 3 h at room temperature, the column was sequentially eluted with one MV (3.0-fold of the tea material, v/w) of ethanol: water 4:6 and one MV of water (3.5-fold of the tea material, v/w), at a flow rate of one MV/h.

All elutes were collected together, mixed thoroughly and automatically separated into an ether phase and an ethanol aqueous phase. The ether phase containing low-polar aroma compounds was vacuum-evaporated to dry at 45 °C to obtain the aroma extracts, and the PE solvent was recovered for reuse. The ethanol aqueous phase containing high-polar and water soluble compounds (the non-aroma extracts) was vacuum-evaporated at 70 °C to aqueous solution for the next decaffeination or for spray-drying to make non-aroma extracts, and the ethanol were recovered for reuse.

Decaffeination of green tea extracts

Charcoal was pretreated sequentially with 1 mol/L HCl, 1 mol/L NaOH and 95% ethanol for 4 h or more and washed extensively with water after each pretreatment. The pretreated charcoal was dried in an oven. For decaffeination, pre-treated charcoal was added to the aqueous solution of the tea extracts and absorbed for 60 min with 50 rpm shaking in a water bath at 70 °C. The solution was separated from charcoal by centrifugation 3000×*g* for 5 min (small scale experiments) or by filtration through double-layer filter paper (scale-up experiments). The contents of EGCG, caffeine and theanine in the solution were analyzed by HPLC.

Preparation of standard and low-caffeine instant green teas

The aroma extracts was dissolved with tenfold (v/w) of 80% ethanol and centrifuged at 5000×*g* for 10 min at room temperature to remove insoluble substances. The aroma solution was added into the decaffeinated aqueous solutions at aroma extracts: non-aroma extracts 1:10. Instant tea powders were made by spray-drying of the mixture at inlet air temperature 190 °C, spraying flow at 500 ml/h and air flow at 600 L/h.

The qualities or the aroma and taste of the instant tea infusions with different levels of decaffeination and different ratios of aroma: non-aroma were evaluated by sensory ratings of 20 graduate and undergraduate students in our lab (10 males and 10 females), based on the National Tea Sensory Evaluation Method GB/T 23776-2009 and comparing to the tea infusion prepared with leaf tea product and boiled hot water.

All experiments in this study were repeated at least three times. The results presented are the averages of three independent repeats ± standard errors (SE).

Results and discussion

Optimal solvents and conditions for extraction of low-polar, high-polar and water soluble compounds

Using the traditional maceration extraction method, the optimal solvents and conditions for the extraction of low-polar, high-polar and water soluble groups of compounds were determined. As shown in Fig. 1a, in a series solvents of PE:ethanol from 10:0 (I), 8:2 (II), 6:4 (III) and 4:6 (IV), solvent II was selected as the optimal solvent to extract low-polar compounds which were not extracted by ethanol: water 4:6 (VII), based on visual observation on TLC plates. The MV of solvent II was determined to be 1.8-fold (2.0-fold was used in this study, v/w) over the tea material and the optimal time for solvent II to dissolve low-polar compounds was determined to be 3 h.

Similar to our previous reported (Wang et al. 2012), the high-polar group containing polyphenolics and caffeine was effectively extracted by a series of ethanol aqueous solutions, ethanol: water of 6:4 (V), 5:5 (VI), 4:6 (VII) and 3:7 (VIII, Fig. 1b). Solvent VII (ethanol: water 4:6) was selected as the optimal solution to extract high-polar compounds from green tea, based on HPLC analysis of EGCG and caffeine in the extracts. The MV for solvent VII was determined to be 2.9-fold (3.0-fold was used in this study, v/w), and the optimal time for solvent VII to dissolve high-polar compounds was 1 h. Water was used to extract soluble polysaccharides and other water-soluble compounds, with a MV of 3.5-fold and the optimal dissolving time of 1 h (Fig. 1c).

Extraction of low-polar, high-polar and water soluble compounds in green tea with their corresponding optimal solvents

The low-polar, high-polar or water soluble groups of compounds in green tea were independently extracted with their corresponding optimal solvent and conditions.

Fig. 1 Determination of optimal solvents and conditions for the extraction of low-polar, high-polar and water soluble compounds in green tea (**a–c**) and the extraction results by single solvent CCE (**d–f**) and gradient CCE (**g, h**) **a** Determination of optimal solvent and conditions for the extraction of low-polar compounds; *I, II, III* and *IV* represent petroleum ether:ethanol of 10:0, 8:2, 6:4 and 4:6, respectively. *VII* is the control of high-polar compounds extracted with ethanol: water 4:6. *II* was selected as the optimal solvent with a MV of 1.8 and a dissolving time of 3 h). **b** Determination of optimal solvent and conditions for the extraction of high-polar compounds; *V, VI, VII* and *VIII* represent ethanol: water of 6:4, 5:5, 4:6 and 3:7, respectively. *IX* is EGCG standard. *VII* was selected as the optimal solvent with a MV of 2.9 and a dissolving time of 1 h. **c** Determination of optimal conditions for water extraction of soluble polysaccharides, with a MV of 3.5 and a dissolving time of 1 h. **d** TLC analysis of CCE extracts (three repeats) of low-polar compounds with petroleum ether:ethanol of 8:2 (single solvent). *1, 2* and *3* represent the 1st, 2nd and 3rd MV of elution. **e** CCE extracts of EGCG and caffeine with ethanol: water of 4:6 (single solvent). **f** CCE extracts of soluble polysaccharides with water (single solvent). **g, h**, low-polar compounds (**g**) and EGCG, caffeine and soluble polysaccharides (**h**) in the CCE extracts by gradient elution with three solvents

Figure 1d shows the extraction of low-polar compounds with solvent II. The low-polar substances were extracted completely with 1 MV of solvent II and were not visible in the 2nd and 3rd MV of extracts on TLC plates.

In the high-polar compounds, EGCG and caffeine were extracted with ethanol: water 4:6 for 88.2 and 92.3% in the 1st MV, 9.5 and 6.2% in the 2nd MV, and 2.3 and 0.5% in the third MV of extracts, respectively (Fig. 1e). In the

water soluble compounds, soluble polysaccharides were extracted by 90.2, 8.3 and 1.5% in the 1st, 2nd and 3rd MV of extracts, respectively. The results indicated that the three groups of tea components were effectively extracted with one MV of their corresponding optimal solvent.

Simultaneous extraction of low-polar, high-polar and water soluble compounds by the CCE with gradient elution

Based on the results above, green tea was extracted by the CCE method with gradient elution. Green tea powder was loaded with one MV of PE:ethanol (8:2) in a column. The column was eluted with one MV of ethanol: water (4:6) and then with water. One MV each of the three solvent extracts were collected and mixed thoroughly. The mixed extraction solution was automatically separated into an ether phase (aroma extracts) containing low-polar compounds, and an ethanol aqueous phase (non-aroma extracts) containing high-polar and water soluble compounds. The ether phase was vacuum-evaporated to dry at 45 °C and the aroma extracts were obtained. The ethanol aqueous phase was vacuum-concentrated to aqueous solution at 70 °C, and was used for decaffeination or for spray-drying to make the non-aroma extracts.

Based on the TLC analysis, the low-polar compounds were effectively extracted and the yield of aroma extracts was $2.98 \pm 0.11\%$ over the tea material (Fig. 1g). Quantitative analyses of the non-aroma extracts by HPLC indicated that EGCG, caffeine and soluble polysaccharides were extracted by 93.8, 95.6 and 92.3%, respectively. The yield of non-aroma extracts were $38.4 \pm 0.3\%$ over the tea material (Fig. 1h).

In the gradient CCE, compounds in each polar group were not only extracted by their own solvent, but also cross-extracted partially by the front or the later solvents. As shown in Fig. 1a, the low-polar solvent can extract part of the high-polar compounds (original point of sample on the TLC plate), the high-polar solvent can extract part of the low-polar and the water soluble compounds, and the water can also extract high-polar compounds left in the tea material (Wang et al. 2012). Therefore, the advantage of the gradient CCE is just the extraction of all low-polar aromas and other compounds. It used less solvents and obtained higher extraction efficiencies, comparing to the original CCE method with single solvent (Wang et al. 2012).

Decaffeination and preparation of high-aroma instant green teas

Charcoal is an effective absorbent to remove caffeine in tea extracts with limited lose of other compounds (Ye et al.

2007). The effects of charcoal amount, temperature and absorption time on the absorption of EGCG, caffeine and theanine were investigated. As shown in Fig. 2a, at 30, 50 and 70 °C, the absorption rate by charcoal (5%) increased for caffeine, but slightly decreased for EGCG and theanine as the temperature increased, indicating that a relatively high temperature is in favor of caffeine removal with less lose of EGCG and theanine. The charcoal amount showed good dose–response relationship with caffeine removed. However, EGCG and theanine showed similar proportional loses as charcoal amount increase (Fig. 2b). The optimal decaffeination time were determined to be 60 min (Fig. 2c, d). Based on the results, the decaffeination conditions were determined to be with charcoal at 70 °C for 60 min in aqueous solution Fig. 3.

A series of decaffeinated green tea solutions by different amount of charcoal were prepared. Sensory evaluation indicated that the treatments with 4 and 9% charcoal tasted better than the others. Analysis of the components indicated that the tea extraction solution (aqueous solution without decaffeination) contained caffeine 6.7, polyphenolics 44.5, EGCG 13.8 and theanine 1.4 mg/mL, with an EGCG: caffeine ratio of 2.1. The solution treated by 4% charcoal contained caffeine 3.4, polyphenolics 38.1, EGCG 12.4 and theanine 1.3 mg/mL with an EGCG: caffeine ratio of 3.5, in which 49.3% caffeine was removed, while polyphenolics, EGCG mg and theanine lost 14, 10.1 and 7.1%, respectively. The treatment by 9% charcoal contained caffeine 0.1, polyphenolics 22.7, EGCG 8.68 and theanine 1.1 mg/mL, with 98.8% caffeine removed and 49.0, 37.1 and 21.4% lost for polyphenolics, EGCG and theanine, respectively.

The dried aroma extracts had strong green tea fragrance, while the non-aroma extracts had very low or no green tea fragrance. Instant green teas were prepared with different ratios of aroma to non-aroma extracts at 1:5, 1:10, 1:20, 1:50, and 1:100 on dried extracts bases (w/w). Sensory evaluation indicated that the instant green tea made with 1:10 ratio of aroma to non-aroma extracts had the best fragrance and taste, same as or better than the natural leaf green tea. The green tea fragrance was too strong at 1:5 ratio, but light at 1:20, and too light or not sensible at 1:50 and 1:100 ratios of aroma: non-aroma extracts. Based on the results, two all-natural instant green teas, a standard and a low-caffeine instant tea, were successfully prepared using the aroma extracts and quantitatively decaffeinated non-aroma extracts at the ratio of 1:10. The standard instant green was treated by 4% charcoal or with the EGCG: caffeine ratio between 3.0 and 3.5, and the low-caffeine instant green tea was treated with 9% charcoal or with less than 1% caffeine in dried powder.

Fig. 2 Effects of temperature (a), charcoal amount (b) and times (c for 5% charcoal and d for 9% charcoal) on the decaffeination of tea extraction solution with charcoal.

a Adsorption of EGCG, caffeine and theanine by 5% charcoal at different temperatures.

b Adsorption of EGCG, caffeine and theanine by different amount of charcoal in aqueous solution of green tea extracts; the columns in the figure of each compound represent 1, 2, 3, 4, 5, 6, 7, 8, 9, 10% charcoal, respectively from left to right. **c**, **d** Adsorption of caffeine, EGCG and theanine by 4% (c) and 9% (d) charcoal at 70 °C for different times

Fig. 3 Diagram of extraction of all soluble compounds in green tea by the gradient CCE technology and preparation of all-natural and high aroma instant green teas

Conclusion

This work developed a new CCE method with gradient elution to simultaneously extract all soluble compounds in green tea, including low-polar aroma compounds and high-polar and water soluble non-aroma compounds. More than 92% of all soluble compounds in green tea were extracted with one MV each of the low-polar solvent (PE:ethanol 8:1), high-polar solvent (ethanol: water 4:6) and water. The new method can extract simultaneously all aroma and non-aroma compounds in green tea with much less solvents compared to the original CCE method with single solvent (Wang et al. 2012). The extraction mixture was automatically separated into PE and ethanol aqueous phases, which

let the PE phase containing green tea aromas be able to separately vacuum-evaporated and dried at a low temperature of 45 °C. The ethanol aqueous phase containing non-aroma compounds was vacuum-evaporated at 70 °C and then quantitatively decaffeinated with different amount of charcoal. A standard instant green tea with a EGCG: caffeine 3.0–3.5:1 and a low-caffeine instant green tea (caffeine ≤ 1%) were successfully prepared by combining the aroma and non-aroma extracts at the ratio of 1:10. Both instant green teas are all-natural and had the best green tea aroma and taste. This work solved the problems of low-aroma and low-taste in instant green teas. All solvents used in the whole procedure were recovered for reuse without environmental contamination.

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