Data S1 Materials and methods

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Chemicals

Phenol, potassium sodium tartrate, 3,5-dinitrosalicylic acid, anhydrous sodium sulphite, coomassie brilliant blue G250, absolute ethyl alcohol, 80% phosphoric acid, bovine serum albumin (BSA), anthrone, concentrated sulfuric acid, dextrose anhydrous, carbazole, galacturonic acid, gallic acid, folin-ciocalteu's phenol reagent, sodium carbonate, acarbose, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), standard sample of monosaccharide (L-rhamnose, D-mannose, D-glucose, D-galactose, L-arabinose, D-xylose, galacturonic acid) and all the catechin standards were purchased from Sigma Chemical Co. (MO, USA). Methanol and acetonitrile of HPLC grade were purchased from Tianjin Shield Co. (Tianjin, China). All other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

Raw materials

Tea (*Camellia sinensis* L.) fruit was collected from Panban tea garden (Zhejiang, China) in December, 2012. The fruit was washed in distilled water three times and then the peel was separated. After oven-dried at 40 °C for 24 h, the separated peel was then ground to pass through a 20 mesh screen by pulverizer (XB-02, Xiaobao Machinery Co., Zhejiang, China) and stored at -20 °C until needed.

Experimental design

The ground fruit peel of tea was treated with ethanol beforehand, followed by water extraction. Central composite design was applied to determine the optimum condition of water extraction of total polysaccharide from ground seed peel.

The optimum condition of hot water extraction of total polysaccharide from samples was studied by using response surface methology. In this experiment, three factors X_1 (extraction temperature, °C), X_2 (extraction time, min) and X_3 (liquid:solid ratio, ml/g) were studied, with three variation levels each, at X_1 (50 °C, 70 °C, 90 °C), X_2 (30 min, 60 min, 90 min) and X_3 (15:1, 20:1, 25:1), according to previous experiments. The complete design of 20 experimental points including five replications of the center points is shown in Table 1. Effects of extraction temperature, extraction time and ratio of water to material on yield were studied. The experimental procedure was designed referring to central composite design and the predictive polynomial quadratic equation model and interactions among three factors were developed by Design-Expert (Version 8.0, Stat-Ease Inc., Minneapolis, MN, USA) analysis. The significance of all terms in the polynomial was judged statistically by computing the F-value at a probability (p) of 0.001, 0.01 or 0.05. Experimental data was fitted to Design-Expert, and tri-dimensional response surfaces and contour plots were also generated. These response surfaces and contour plots could visualize the relationship between the response and experimental levels of each factor and deduce parameters interactions (Triveni *et al.*, 2001). The behavior of the surface was investigated for the response function (Y) using the regression equation (Lee *et al.*, 2006). Responses were monitored and results were compared with model predictions. The optimum condition was verified by conducting experiments under these conditions.

Table 1 Experimental design and response values

Run	Coded variable levels			Observed (*Y*)
	X_1 [a]	X_2 [b]	X_3 [c]	
1	-1(50)	-1(30)	-1(15:1)	3.32
2	1(90)	-1(30)	-1(15:1)	4.09
3	-1(50)	1(90)	-1(15:1)	3.87
4	1(90)	1(90)	-1(15:1)	4.53
5	-1(50)	-1(30)	1(25:1)	3.46
6	1(90)	-1(30)	1(25:1)	4.31
7	-1(50)	1(90)	1(25:1)	3.82
8	1(90)	1(90)	1(25:1)	4.63
9	-1(50)	0(60)	0(20:1)	3.62
10	1(90)	0(60)	0(20:1)	4.44
11	0(70)	-1(30)	0(20:1)	4.07
12	0(70)	1(90)	0(20:1)	4.99
13	0(70)	0(60)	-1(15:1)	4.73
14	0(70)	0(60)	1(25:1)	4.85
15	0(70)	0(60)	0(20:1)	4.67
16	0(70)	0(60)	0(20:1)	4.82
17	0(70)	0(60)	0(20:1)	4.84
18	0(70)	0(60)	0(20:1)	4.65
19	0(70)	0(60)	0(20:1)	4.14
20	0(70)	0(60)	0(20:1)	4.74

[a] Extraction temperature (°C);
[b] Extraction time (min);
[c] Liquid/solid ratio (v/w).

Extraction of polysaccharides and fractionation

The ground fruit peel of tea was subjected to extraction with 10 vol. (V/W) of 80 % ethanol for 24 h to remove a portion of polyphenols and monosaccharide. After filtering, the residues were air-dried and then extraction of polysaccharide from tea fruit peel was performed twice under 70 °C (1:20, V/W, 1.5 h). Prior to being treated by four-fold vol of 95% ethanol for 24 h, the extract was concentrated to 10 % with a vacuum evaporator at 4 °C. The precipitate was collected by centrifugation (6000 rpm, 10 min), then dissolved in hot water (70 °C), deproteinized by Sevag method (Wang *et al.*, 2007), centrifuged, dialyzed against distilled water for 48 h with dialysis tubing (molecular weight cut-off, 7000 Da) to remove low-molecular weight substance. The dialysate was then concentrated under vacuum and precipitated, freeze dried to give crude polysaccharides, which was kept in the dark at 4 °C before use.

Two g crude polysaccharide was used for each treatment, dissolved with 60 % ethanol, stirred, stood, and centrifuged (8000 r/min, 10 min). The supernate was first treated under reduced pressure in a rotary evaporator to remove the ethanol, and then lyophilized to get TFPP-60. The precipitate was collected and dissolved in 40 % ethanol, and underwent the same treatment to get TFPP-40. TFPP-20 was obtained with the same process with the only exception that ethanol ratio was reduced to 20%. Similarity, TFPP-0 was collected after same treatment with distilled water as a dissolving medium.

The content of polysaccharides in extract was determined by phenol-sulphuric acid method (Dubois *et al.*, 1956). The yield (%, w/w) of total polysaccharide was calculated as the total polysaccharide weight of extraction divided by the weight of dried sample.

Composition analysis

Neutral sugar content was measured by anthrone-sulfuric acid method (Morris, 1948) using D-glucose as a standard reference. Uronic acid content was determined by carbazole-sulfuric acid method using galacturonic acid as standard (Bitter and Muir, 1962). Protein was analyzed by the method of Bradford (1976) using bovine serum albumin as standard (Bradford, 1976).

Monosaccharide composition analysis

Hydrolysis of the tea fruit polysaccharides

Two mg of polysaccharides were hydrolyzed with 2 ml of 3 M TFA at 100 °C for 8 h. After cooled, the supernate was collected by centrifugation (1000 r/min, 5 min), then concentrated under vacuum and precipitated, freeze dried, dissolved in 1 ml distilled water.

Preparation of standard solution

Stock standard solutions (2.0 mM) were prepared by dissolving each standard monosaccharide in a mixture of water solution containing 10 % methanol. Working standard solutions were further obtained by appropriate dilution of the stock standard solutions with deionized water. The sample solutions were filtered through a 0.22 μm syringe filter and were degassed using an ultrasonic bath for 2 min prior to use. All the solutions prepared were stored in the dark at 4 °C until being used.

Derivatization procedure

1-phenyl-3-methyl-5-pyrazolone (PMP) derivatization of monosaccharides was carried out as described previously with proper modification (Zhang *et al.*, 2003). Briefly, 10 standard monosaccharides or the hydrolyzed samples of the tea fruit polysaccharides were dissolved in 0.3 M aqueous NaOH (50 μl) and a 0.5 M methanol solution (50 μl) of PMP was added to each. Since PMP was neutralized with NaOH, the resultant solution was almost neutral. Each mixture

was allowed to react for 60 min at 70 °C, then cooled to room temperature and neutralized with 50 µl of 0.3 M HCl. The resulting solution was extracted with chloroform (1 ml) and the process was repeated three times, then the aqueous layer was filtered through a 0.45 µm membrane.

HPLC equipment and conditions

The analysis of the monosaccharides was carried out on a Shimadzu LC-2010A HPLC system equipped with a quaternary gradient pump unit, an UV-Vis detector (190-700 nm), an autosampler (0.1-100 µl) and the column oven (273-333 K) was controlled by Shimadzu Class-VP 6.1 chromatography workstation. The analytical column used was a RP-C_{18} column (4.6 mm *i.d.* × 250 mm, 5 µm, Venusil, USA). The wavelength for UV detection was 250 nm. Elution was carried out at a flow rate of 0.8 ml/min at 35 °C. The mobile phase A consisted of acetonitrile and the mobile phase B was 0.045 % KH_2PO_4-0.05 % triethylamine buffer (pH 7.0) using a gradient elution of 20-29-30-35 % B by a linear decrease from 0-15-30-35 min. The injection volume was 20 µl.

FTIR spectroscopy

The organic functional groups of the four polysaccharides were identified using an FTIR spectrophotometer (FTIR-8400S, Shimadzu co., Japan) within 4000-400 cm^{-1} via the KBr pressed-disc method.

Antioxidant capacity

ABTS•+ scavenging capacity

ABTS•+ assay was carried out according to the method introduced by Cai *et al*. (2004). The ABTS•+ solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate and then incubated in the dark at room temperature for 12 h. The resulting solution was diluted with water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. ABTS•+ solution (3 ml) was added to 0.1 ml of the test sample with various concentrations (156.25 to 10000 µg/ml) and mixed vigorously. The absorbance was measured at 734 nm after the sampling standing for 6 min with BHT as a positive control. The ABTS•+ scavenging effect was calculated as follows:

$$\text{ABTS+• scavenging effect (\%)} = (1 - A_{samp} / A_{cont}) \times 100$$

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control (BHT), respectively. EC_{50} values (mg/ml), the effective amount of the sample needed to scavenge ABTS•+ by 50 %, were determined from the plotted graphs of scavenging activity against the concentration of the extracts.

Ferric-reducing antioxidant power

The FRAP assay was performed according to a modified method of Benzie and Strain (1999). Briefly, the working FRAP reagent was prepared by mixing 25 vol of 300 mM acetate buffer (pH 3.6) with 2.5 vol TPTZ (10 mM) in HCl (40 mM) and 2.5 vol of 20 mM $FeCl_3$. Newly prepared FRAP reagent was warmed up to 37 °C, and a reagent-alone blank reading was taken at 593 nm. Subsequently, 0.6 ml test samples was added to the FRAP reagent (4.5 ml). A second reading at 593 nm was taken after 8 min. The initial blank reading was subtracted from that with the sample to determine the FRAP value of the sample. A standard curve was prepared using a mixture of 0.5 ml TPTZ (10 mM), 5 ml acetate buffer (300 mM, pH 3.6) and 0.5 ml $FeSO_4 \cdot 7H_2O$ solution with concentrations varying from 25 µM to 1500 µM. BHT was used as the positive control. The reducing ability of the extracts was expressed as the equivalent to that of 1 µM $FeSO_4 \cdot 7H_2O$.

α-Glucosidase inhibitory activity

The α-Glucosidase inhibitory activity of the tea fruit peel extracts was determined according to the method described by Apostolidis and Lee (2010) with a slight modification. A mixture of 50 µl of sample and 100µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution(1 U/ml) was incubated in 96 well plates at 25 °C for 10 min. After preincubation, 50 µl of 5 mM *p*NPG solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 min. Before and after incubation, absorbance was recorded at 405 nm by microplate reader (SpectraMax M5, Molecular Devices, CA, USA). Acarbose was used as the positive control. The α-glucosidase inhibitory activity was expressed as inhibition percentage and was calculated as follows:

Inhibition (%) = (1 − A_{samp} / A_{cont}) × 100,

where A_{samp} and A_{cont} were defined as absorbance of the sample and the control (acarbose), respectively.

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