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Composition and bioactivity of tea flower polysaccharides obtained by different methods

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ABSTRACT

The effects of different extraction methods on the composition and bioactivity of tea flower polysaccharides (TFPS) were investigated. Traditional water extraction (TWE), microwave-assisted water extraction (MAE), and ultrasound-assisted water extraction (UAE) were compared to extract TFPS. TWE was found to be the optimal method with highest yield of TFPS and highest neutral and acid saccharides contents in TFPS. TFPS obtained by TWE mainly consisted of two kinds of polysaccharides with the molecular weight of 31 kDa and 5000 Da. TFPS obtained by MAE and UAE generally had very low inhibitory effects on α-glucosidase. TFPS (2 mg/mL) obtained by TWE exhibited a strong inhibitory effect on α-glucosidase with the inhibitory rate of 83.3%. TFPS obtained by TWE had stronger proliferation effect on lymphocyte at the concentration of 3.0 μg/mL than that of tea leave polysaccharides (TPS).

1. Introduction

Green tea, made up from leaf of *Camellia sinensis*, is the second most consumed beverage in the world (Krafczyk & Glomb, 2008; Sultana et al., 2008; Tsubaki, Iida, Sakamoto, & Azuma, 2008; Xiao et al., 2008). Compared with leaves, tea flowers have similar chemical components and contain less caffeine but comparable amounts of total catechins (Yung, Sang, & Jen, 2003). Tea flowers contained many nutrition compounds, such as protein, sugar, sucrose, vitamin, amino acid, tea polyphenols and caffeine (Yang, Xu, Jie, He, & Tu, 2007). From this point, tea flowers are also of important application value as leaves. For a long time, however, there are very few studies about the tea flowers.

Tea flower polysaccharides (TFPS) are the main effective components in tea flowers, accounting for a comparative large proportion. Recently, the tea leaves polysaccharide (TPS) has attracted great interest among researchers (Chen et al., 2003; Lee et al., 2006; Monobe, Ema, Kato, & Maeda-Yamamoto, 2008; Zhou et al., 2007). The studies and application of TFPS are also becoming valuable. The increasing interest in plant bio-active components is accompanied by a need to expand the application of plant-extraction protocols. The extraction methods of polysaccharides commonly included traditional water extraction (TWE), and methods assisted by ultrasonic wave and microwave to improve the extraction efficacy (Hou & Chen, 2008; Hou, Zhang, Xiong, Li, & Yang, 2008; Liang, 2008; Wang, Zhou, & Wen, 2006). As TFPS, however, are a sort of composite polysaccharides with complicated structures which are possible to be altered or lose its activities during extractions, the traditional water extraction (TWE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) of TFPS were investigated in this paper. And the bioactivity of TFPS was also discussed.

2. Materials and methods

2.1. Materials

Tea flowers was obtained commercially from Hebei province of China. *m*-Hydroxyl biphenyl was purchased from Fluka Co. (MO, USA) and α-glucosidase was purchased from Sigma Co. (MO, USA). *p*-Nitrophenol-α-D-glucopyranose was purchased from Xibao Co. (Shanghai, China). Reduced glutathione, 1640 cell culture medium, Coomassie brilliant blue G-250 and bovine serum albumin were provided from Sinopharm Chemical Reagent Co. (Shanghai, China). All other reagents and solvents were of analytical reagent grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using newly double-distilled water.

2.2. Analytical methods of components in tea flowers

Total polysaccharides were determined by the phenol–sulfuric acid method (Tatsuga et al., 2005). The tea polyphenols content

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was determined by the ferrous tartrate method (Ozkan, Sagdic, Baydar, & Karamahmutoglu, 2004). The soluble protein (SP) was determined by the Coomassie brilliant blue G-250 method (Murphey, Spayd, & Powers, 1989). The total flavonoids concentration was measured using a colorimetric assay based on the quantification of yellow color produced by the interaction of flavonoids with $AlCl_3$ reagent (Pourmorad, Hosseinimehr, & Shahabimajd, 2006).

2.3. Traditional water extraction (TWE)

The dry flowers, cut into small pieces (50 g), were extracted with 400 mL distilled water in water bath at a controlled temperature for 2 h. After filtered, the flowers were extracted again with 300 mL distilled water in water bath at the same temperature for another 1 h. The extraction temperatures were 20, 40, 60, 80, and 100 °C, respectively. Then the extracts were centrifuged to remove the contaminants. The supernatant was concentrated via rotary evaporation method and precipitated with 95% alcohol. Then the precipitation was dissolved with water and dialyzed to remove the small molecules. The dialyzed solution was freeze-dried to yield polysaccharides powder.

2.4. Microwave-assisted water extraction (MAE)

The dry ground flowers (50 g) and 400 mL distilled water were transferred into Erlenmeyer flasks (1000 mL), which were then placed into a microwave-assisted extraction equipment. Extractions were carried out under a controlled microwave power for 5 min. After extraction time was completed, the flasks were allowed to cool to room temperature and the extract was filtered. Then the flowers were extracted again with 300 mL distilled water with the same microwave power for another 5 min. The microwave powers were set at 127.5, 300, 495, 637.5, and 750 W, respectively. The microwave power was adjusted according to the experimental trial. The separation and further treatment of the filtrates were the same as described for the water extraction.

2.5. Ultrasound-assisted water extraction (UAE)

The dry flowers (50 g), cut into small pieces, and 400 mL distilled water were put into Erlenmeyer flasks (1000 mL), which were then placed into an ultrasonic cleaning bath and extracted with a controlled ultrasonic power for 5 min. After filtered, the flowers were extracted again with 300 mL distilled water with the same ultrasonic power for another 5 min. The extraction was conducted by applying various accumulated powers, 100, 150, 200, 250, and 300 W, respectively. The extraction was carried out at 25 °C. The separation and further treatment of the filtrates were the same as described for the water extraction.

2.6. Determination of molecular weight of tea flower polysaccharides

The molecular weight of TFPS was determined by HPGPC method (Hao, Dai, Chen, Zhu, & Ying, 2007). Samples extracted by different methods were dissolved in 0.1 mol/L $NaNO_3$ to a concentration of 10 mg/mL. After centrifuged the foregoing solutions at 10,000 r/min for 10 min, 20 μL of the supernatant was injected for HPGPC analysis.

The chromatographic separation was performed on an Ultrahydrogel™ Linear column (7.8 × 300 mm, 10 μm). The mobile phase consisted of 0.1 mol/L $NaNO_3$ and the flow rate was 0.9 mL/min. The column compartment was maintained at 45 °C. The molecular weight of the tea flower polysaccharide was calculated by constructing a calibration curve.

2.7. Determination of α-glycosidase inhibitory activity

The α-glucosidase inhibitory activity of tea flower polysaccharide was determined according to the chromogenic method described by Tremblay et al. with slight modifications (Chapdelaine, Tremblay, & Dube, 1978). The substrate solution *p*-nitrophenyl α-D-glucopyranoside (pNPG) was prepared wither 0.1 M Na-phosphate buffer (pH 6.8). The reaction mixture was described as follows: 0.1 mol/L Na-phosphate buffer (pH 6.8), 2 mL; 5 mg/mL TFPS solution, 20 μL; 1 mg/mL reduced glutathione, 50 μL; 1 U/μL α-glucosidase, 20 μL. The mixed solution was incubated at 37.5 °C for 10 min. The enzymatic reaction was initiated by adding saturated pNPG and the reaction mixture was incubated for another 30 min at 37.5 °C. The catalytic reaction was terminated by addition of 10 mL of 0.1 M Na_2CO_3 solution. The reaction system without polysaccharides was used as blank test and the system without α-glucosidase was used background test. The Na-phosphate buffer (pH 6.8) was used as zero-setting solution for determination of the absorbance at the wavelength of 400 nm. The inhibitory rate of sample on α-glucosidase was calculated by the following formula:

$$\text{Inhibition percentage } (\%) = [A_{\text{blank}} - (A_{\text{sample}} - A_{\text{background}})] \times 100/A_{\text{blank}}.$$

2.8. Determination of immunological activity in vitro

The spleen was removed under aseptic condition, chopped and washed through screen mesh (200 meshes) with sterile normal saline and centrifuged at 2000 r/min for 5 min for three times. The precipitation of spleen cells were suspended with 1 mL of complete culture medium and seeded at 2×10^6 cell/mL per well into 96 well plates. Some wells of the cell culture were added with tea flower polysaccharides. The cells were incubated at 37 °C for 72 h in a humidified atmosphere of 5% CO_2 in air. Ten microliters of 5 mg/mL MTT was added into the cell culture per well at the 68th h, incubated continuously for the rest 4 h. After incubation for 72 h, 100 μL of 10% SDS was added in per well and mixed thoroughly to dissolve the dark blue crystals. The plate was kept overnight at room temperature. On the next day, the plate was read with an ELISA reader, using test wavelength of 570 nm and a reference wavelength of 630 nm.

3. Results and discussion

3.1. Nutritional components of tea flower

The main nutrition components in tea flowers and tea leaves were determined and shown in Table 1. As shown in Table 1, the tea flowers contained similar nutrients as tea leaves. However, the total saccharides and flavonoids in flowers were much higher than that of leaves. TPS and TPP including flavonoids play a key role in bio-activities of tea (Anesini, Ferraro, & Filip, 2008; Kang et al., 2008; Kato et al., 2008; Xiao et al., 2008). The tea flowers the same as leaves can be used as medical and health food.

Table 1
Nutritional components in tea flowers and leaves.

Part of tea	Flavonoids (%)	Saccharides (%)	Polyphenol (%)	Protein (%)	Fat (%)
Flowers	11.67	62.84	10.66	3.64	3.43
Leaves	7.91	22.80	19.62	1.80	–

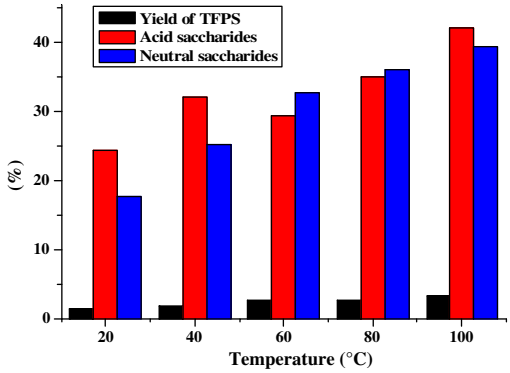

Fig. 1. Effect of the temperature of TWE on the composition and yield of TFPS.

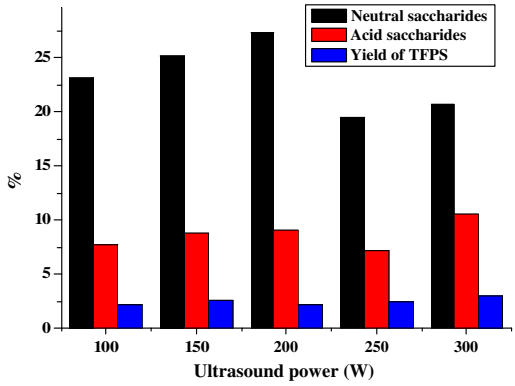

Fig. 3. Effect of the ultrasound power of UAE on the composition and yield of TFPS.

3.2. Effects of TWE, MAE, UAE on the composition and yield of TFPS

Fig. 1 showed the effect of the temperature of water extraction on the composition and yield of TFPS. As shown in Fig. 1, the yield of TFPS increased with the increasing extraction temperature. From 20 to 100 °C, the yield of TFPS increased to twice. The extraction temperature also affected the composition of TFPS. With the improved temperature, the neutral saccharides in TFPS increased up to 39.5%. The acid saccharides in TFPS, however, changed irregularly related to the temperature. When the temperature is 100 °C, the yield of TFPS is highest. But it is energy-costing and difficult to control the water in 100 °C. Here, 90 °C was selected as the optimal extraction temperature for TWE.

Fig. 2 showed the microwave power of MAE on the composition and yield of TFPS. As shown in Fig. 2, the yield of TFPS irregularly changed with the increasing microwave power. However the variation of TFPS yield was not extreme. With the increasing microwave power, the neutral saccharides in TFPS increased up to 37.0%. The acid saccharides in TFPS, however, at first increased and then decreased related to the improving microwave power.

Fig. 3 showed the ultrasound power of UAE on the composition and yield of TFPS. As shown in Fig. 3, the yield of TFPS changed slightly with the increasing ultrasound power (2.14–2.95%). However, with the increasing ultrasound power, the neutral saccharides and acid saccharides changed irregularly related to increasing ultrasound power.

Extraction of natural products by different methods may yield different chemical components (Bengtsson, Namutebi, Alminger, & Svanberg, 2008; Cao, Xiao, & Xu, 2007; Cubas, Lobo, & González, 2008; Kalia, Sharma, Singh, & Singh, 2008; ma et al., 2008; Sharma et al., 2008). From Figs. 1–3, it was found that the composition and yield of TFPS were significantly affected by extraction methods and parameters. Compared with TWE, the UAE dramatically weakened the neutral and acid saccharides content in TFPS. UAE uses high frequency sound to disrupt or detach the target components from the matrix. Ultrasonic wave has powerful mechanical force and may degrade polysaccharides into smaller pieces during the extraction process, which caused the loss of polysaccharides. Therefore, UAE was not suitable to extract the polysaccharides from tea flowers, even if with high yield of TFPS.

While compared with TWE, MAE almost had no neutral polysaccharides. However, with the augment of microwave power, the acid polysaccharides content increased first, and then decreased rapidly. Considered that the yield of TEPS by MAE was almost the same as that of TWE, the condition of MAE can be improved through further experiment. MAE offers a rapid delivery of energy to the solvent and microwave radiation can be focused directly onto the sample, thus the heating is more efficient, the cell walls of the plants was disrupted mainly due to the mechanical effects of microwave radiation. In this study, MAE was presented as an "environmentally friendly" extraction method suitable for extraction of TEPS (Sharma et al., 2008). But because the solubility of TEPS obtained by MAE was low, the further work should focus on the extraction parameters to improve the solubility of TEPS.

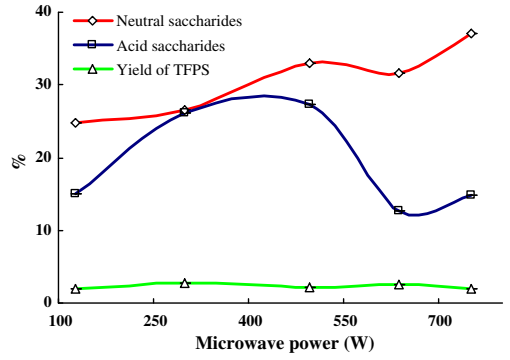

Fig. 2. Effect of the microwave power of MAE on the composition and yield of TFPS.

3.3. Effect of different extraction technologies on molecular weight of TFPS

Different extraction technologies could affect the molecular weight distribution of TFPS comparing TWE, MAE, UAE by HPGPC. The components of TFPS by TWE (90 °C) mainly consisted of two kinds of polysaccharides with the peak molecular weight of 31 kDa and 4400 Da. Comparing the peak molecular weight of TFPS obtained by UAE with the ultrasonic power of 200 and 100 W, it could be concluded that the peak molecular weight of TFPS decreased with the increasing ultrasonic power. Here, we confirmed above hypothesis that ultrasonic wave had powerful mechanical force, which could cut polysaccharides into small pieces during the long extraction process.

Compared the peak molecular weight of TFPS obtained by MAE with the microwave power of 750 and 550 W, it was found that along with the augment of microwave power, the peak molecular weight of TFPS increased. Probably, the microwave radiation enhanced the release, pervasion and solubility of the materials inside of the cell, and accelerated the compounds effectively dissolving into the solvent, which may result in the TFPS with higher molecular weight. The peak molecular weight of TFPS obtained by MAE with the microwave power of 750 W is higher that of TFPS obtained by TWE (90 °C).

3.4. Inhibitory effect of TFPS on α-glucosidase

The α-glucosidase inhibitors are currently focused on for diabetic treatment as oral hypoglycemic agents for its high affinity to α-glucosidase. The α-glucosidase inhibitors act on the brush border of intestinal mucosa to inhibit the post-meal blood glucose level from rising and decrease fasting blood glucose to some extent by delaying the carbohydrates digestion and absorbance at intestine (Quan, Yin, Jin, & Shen, 2003). The α-glucosidase inhibitors are mostly evaluated by determination of α-glucosidase inhibitory activity using pNPG as the reaction substrate.

TFPS obtained by MAE, UAE, and TWE (90 °C) were tested for their inhibitory effect on α-glucosidase. TFPS obtained by MAE and UAE generally had very low inhibitory effects on α-glucosidase. It might be due to the poor solubility of the samples extracted by these two methods. TFPS obtained by TWE (90 °C), however, had an obvious inhibitory effect on α-glucosidase. Fig. 4 showed the dose–response curve of the inhibitory effect of TFPS obtained by TWE (90 °C) on α-glucosidase. It was seen that the inhibitory effect of TFPS on α-glucosidase increased rapidly with the addition of TFPS. The TFPS (2 mg/mL) exhibited a strongly inhibitory effect on α-glucosidase with the inhibitory rate of 83.3%.

3.5. Immunological activity of TFPS

Cell culture method was adopted to determine the effect of TFPS on mice splenic lymphocyte *in vitro*. The lymphocytes were seeded into 96 well plates divided into three experimental groups of blank control group, TFPS group and TPS group. Each experimental group was added with the sample solution at gradient final concentrations of 0.03, 0.3, 3, 30, 300 μg/mL and repeated for four wells. The results are represented in the form of "mean number ± standard deviation". Tea flower polysaccharide samples extracted by microwave-assisted method, ultrasound-assisted method and water extracting method (excepting for 90 °C water extraction) were found to no proliferation effect on the mice splenic lymphocyte.

Fig. 5 showed the proliferation effect of TEPS and TPS on the mice splenic lymphocyte. TPS with low concentrations (0.03 and 0.3 μg/mL) had higher proliferation effect on lymphocyte than that of TFPS. However, the results also indicated that the TFPS had stronger proliferation effect on lymphocyte at the concentration of 3.0 μg/mL ($P < 0.01$) than that of TPS. The pharmacological effect of TPS as immunomodulator has broadly been accepted by many researchers. Fig. 5 showed that TFPS had the ability to directly promote the mice splenic lymphocyte as TPS.

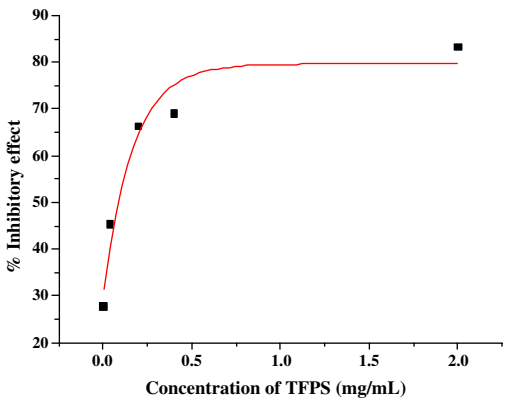

Fig. 4. The dose–response curve of the inhibitory effect of TFPS obtained by TWE (obtained by TWE with 90 °C) on α-glucosidase.

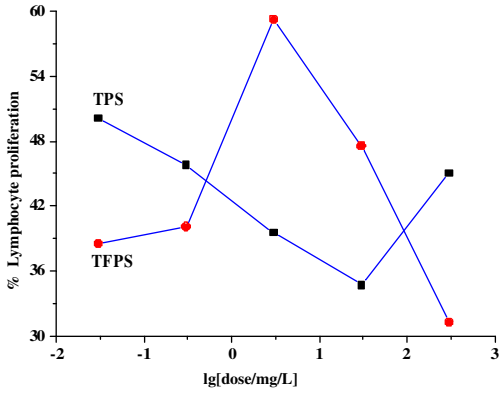

Fig. 5. The proliferation effects of TEPS (obtained by TWE with 90 °C) and TPS on the mice splenic lymphocyte.

4. Conclusion

Regarded the components, molecular weight and bioactivity of tea flower polysaccharides, the traditional water extraction was the ideal way to extract polysaccharides from tea flowers. The tea flower polysaccharides obtained by traditional water extraction mainly consisted of two kinds of polysaccharides. The peak molecular weight of tea flower polysaccharides obtained by ultrasound-assisted water extraction was smaller than that of traditional water extraction. The tea flower polysaccharides extracted by microwave-assisted or ultrasound-assisted water extraction had poor inhibitory effects on α-glucosidase. The inhibitory effect of tea flower polysaccharides obtained by traditional water extraction on α-glucosidase increased rapidly with increasing dose. Tea flower polysaccharides (2 mg/mL) obtained by traditional water extraction exhibited a strong inhibitory effect on α-glucosidase with the inhibitory rate of 83.3%. These results indicated that the tea flower polysaccharides extracted by water had stronger proliferation effect on lymphocyte at the concentration of 3.0 μg/mL ($P < 0.01$) than that of tea leave polysaccharides.

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