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Short Communication

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Composition and Biological Activity of Tea Polysaccharides Obtained by Water Extraction and Enzymatic Extraction

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***SUMMARY*. The composition and biological activities of tea polysaccharides (TPS) obtained by traditional water extraction, boiling water extraction and enzymatic extraction were investigated. Boiling water extraction (100 °C for 2 h) was found to be the optimal method with higher yield of TPS (1.91%) consisting of higher contents of neutral saccharides (57.82%) and acid saccharides (26.95%) with lowest protein content (3.06%). TPS obtained by boiling water extraction exhibited a strong inhibitory effect on α-glucosidase with the inhibitory rate of 86.67%. The inhibitory effect of TPS on α-glucosidase increased with increasing neutral polysaccharides content in TPS. TPS obtained by boiling water extraction (50 μg/mL) had very strong proliferation effect on lymphocyte.**

INTRODUCTION

Green tea *(Camellia sinensis)* is the second most consumed beverage in the world and has caused great interest among researchers [1-5]. Tea polysaccharides (TPS) are shown to have a variety of bioactivities, such as immunostimulating activity [6], hypoglycemic effect [7,8], and anti-bacterial activity [9].

Enzyme-assisted aqueous extraction has often been used to eliminate starch, pectin, protein, and other interfering substances. Protease, amylase, cellulase and pectinase are most frequently used for enzyme-assisted aqueous extraction [10-13]. Those fat-soluble and water-insoluble components can be transferred to water-soluble glycosides through the digestion of partially hydrolyzed starches using glucose hydrolase or glycoside transferase. This paper focused on the composition and biological activities of tea polysaccharides (TPS) obtained by water extraction and enzymatic extraction.

MATERIALS AND METHODS

Materials and reagents

Tea leaves were obtained commercially from Hebei province of China. Tea hydrolase was provided from Novozymes Co. (Beijing, China). MTT (3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide) and α-glucosidase were 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.

Enzymatic extraction (EE)

Dry ground tea leaves (80 g) and 800 mL distilled water were put into a beaker (2000 mL) and then 0.64 g of tea hydrolase was added. Extraction was carried out at 50 °C for 1 h. After filtered, the tea leaves were extracted again with 640 mL of distilled water with the same temperature for another 1 h. Then the combined ex-

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tracts were centrifuged to remove the contaminants. The supernatant was concentrated via rotary evaporation method and precipitated with 95% alcohol. The precipitate was dissolved with water and dialyzed to remove small molecules. The dialyzed solution was freeze-dried to yield polysaccharides powder.

Traditional Water Extraction (TWE)

Dry ground tea leaves (80 g) and 800 mL distilled water were put into a beaker (2000 mL). Extraction was performed at 55 °C for 2 h. After filtered, the tea leaves were extracted again with 640 mL distilled water at 55 °C for another 2 h. The further treatment of the filtrates was the same as described for the enzymatic extraction.

Boiling Water Extraction (BWE)

Dry ground tea leaves (80 g) and 800 mL distilled water were put into Beaker (2000 mL). Extraction was performed at 100 °C for 2 h. After filtered, the tea leaves were extracted again with 640 mL distilled water at 100 °C for another 2 h. The further treatment of the filtrates was the same as described for the enzymatic extraction.

Analytical methods of components in tea leaves

Polysaccharide yield was determined by the A/B ratio: A, the quality of tea polysaccharide powder obtained (g) and B, the quality of raw tea leaves (g). The neutral polysaccharide was determined by the phenol-sulfuric acid method [14,15]. The acid polysaccharide was determined by Inter-hydroxy biphenyl colorimetry.

Determination of α-glycosidase inhibitory activity

The α-glucosidase inhibitory activity of tea polysaccharide was determined according to the chromogenic method described by Tremblay *et al.* with slight modifications [16]. The substrate solution p-nitrophenyl α-D-glucopyranoside (pNPG) was prepared with 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 TPS solution, 20 µL; 1 mg/mL reduced glutathione, 50 µl; 1U/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 as 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 equation [**1**].

$$\text{Inhibition percentage (\%)} = [A_{blank}-(A_{sample}-A_{background}) \times 100/A_{blank} \quad [\mathbf{1}]$$

Determination of immunological activity in vitro

The Kunming mice (4 to 6 six weeks old, weighing 18~22 g) were obtained from experimental animal central of Nantong Medical College. All procedures were performed according to the Institute Ethical Committee for Experimental Use of Animals. The spleens of mice were removed under aseptic condition, chopped and washed through screen mesh (200 mesh) with saline and centrifuged at 2000 r/min for 5 min for 3 times. The precipitates 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. The cells were incubated with TPS 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 hour, incubated continuously for the rest 4 h. After incubation for 72 h, 100 µL of 10% SDS (sodium dodecylsulphate) 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.

RESULTS AND DISCUSSION

Comparison of different extraction methods on the composition and yield of TPS

Extraction of natural products by different methods may yield different chemical components [17-20]. Figure 1 showed the composition of TPS obtained by EE, TWE and BWE. The neutral polysaccharides contents of TPS obtained by BWE, EE, and TWE were 57.82%, 54.27%, and 39.00%, respectively. For BWE, high temperature can denature protein. Thus it improved the purity of TPS. Tea leaves hydrolase can effectively degrade cell wall and the polysaccharides dissolved out. Additionally, to a certain extent,

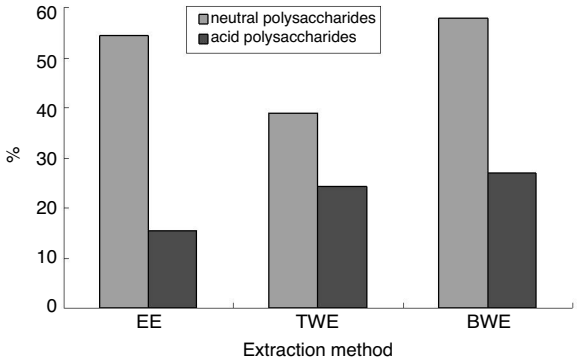

Figure 1. Composition of TPS obtained by EE, TWE and BWE.

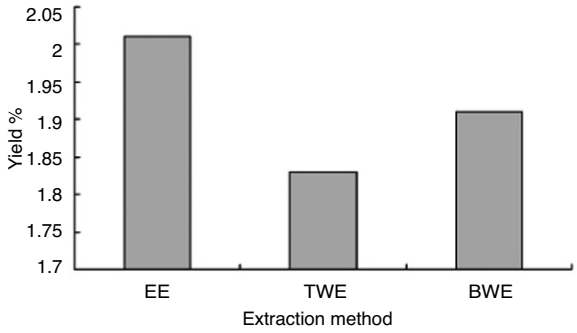

Figure 2. Yield of TPS obtained by EE, TWE and BWE.

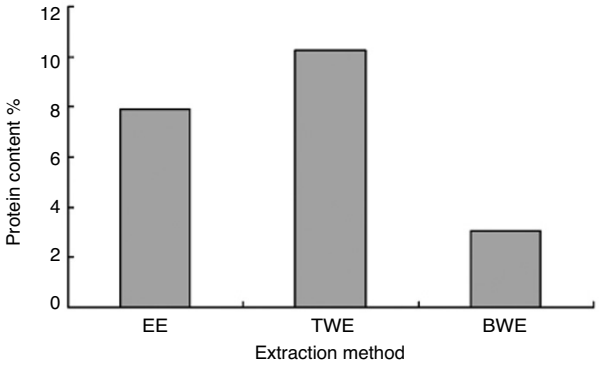

Figure 3. Protein contents in TPS obtained by EE, TWE and BWE.

the enzyme made TPS sugar chains degrade, and then the fracture of sugar chain that combined with protein, polyphenols and other non-polysaccharide material had been removed together, so then increased the purity of TPS. Substances such as protein and pectin, which existed in TPS by TWE, were difficult to remove, and therefore neutral polysaccharides content was low.

The acid polysaccharides contents of TPS obtained by BWE, EE, and TWE were 26.95%, 15.41%, and 24.27%, respectively. This result indicated that acid polysaccharide was resistant to high temperature. The other hand warm water extraction was no effective made acid polysaccharides to dissolve out. Enzymes may disrupt the structure of acid polysaccharides. Enzymatic extraction was able to lower content of acid polysaccharides of TPS. Figure 2 showed the yields of TPS obtained by EE, TWE and BWE. The yields of TPS obtained by BWE, EE, and TWE were 1.91%, 2.01%, and 1.83%, respectively. Enzyme and higher temperature can improve the yield of TPS. Figure 3 showed the protein contents in TPS obtained by EE, TWE and BWE. The protein contents in TPS obtained by BWE, EE, and TWE were 3.06%, 7.91%, and 10.25%, respectively.

The inhibition rate of α-glucosidase by TPS

Diabetes is characterized as high concentration of blood sugar, which can cause serious complications, such as the kidneys, eyes and cardiovascular were being destructed and function failure [21]. So the treatment of diabetes mainly focuses on reducing fluctuations in blood sugar and complications [22]. The α-glucosidase inhibitors are currently used for diabetic treatment as oral hypoglycemic agents for its high affinity to α-glucosidase [23]. The α-glucosidase inhibitors inhibit degradation of disaccharides to monosaccharide [24,25]. The α-glucosidase inhibitors are mostly evaluated by determination of α-glucosidase inhibitory activity using pNPG as the reaction substrate.

Table 1 showed the inhibitory effect of TPS obtained by EE, TWE and BWE on α-glucosidase. The inhibitory percentage of different TPS were determined as: BWE > EE > TWE. As shown in Figure 4, the inhibitory effect of TPS on α-glucosidase increased with increasing neutral polysaccharides content in TPS. However, there is no relationship between the inhibitory effect on α-glucosidase and the content of acid polysaccharides inTPS.

Sample	Inhibitory rate (%)
Acarbose (control)	99.50%
TPS-EE	73.35%
TPS-TWE	53.85%
TPS-BWE	86.67%

Table 1. Inhibitory effect of TPS on α-glucosidase.

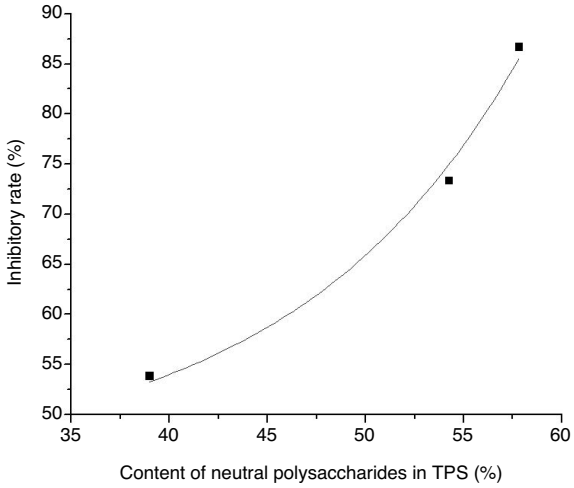

Figure 4. Relationship of neutral polysaccharides content and inhibitory effect on α-glucosidase of TPS.

Immunological activity of TPS

Cell culture method was adopted to determine the effect of TPS on mice splenic lymphocyte *in vitro*. The lymphocytes were seeded into 96 well plates divided into three experimental groups of negative control group, positive control group and TPS group. Each experimental group was added with the sample solution at gradient final concentrations of 0.005, 0.05, 0.5, 5,50, 500 μg/mL and repeated for four wells.

Table 2 showed the proliferation effect of TPS on mice splenic lymphocyte. These results indicated that the proliferation effect of TPS obtained by EE on mice splenic lymphocyte was lower than that of the negative control and positive control groups. The proliferation effect of TPS obtained by TWE was higher than that of the positive control group at the concentration of 5.0, 0.5, and 0.05 μg/mL. The proliferation effects of TPS obtained by BWE were higher than that of positive control group at the concentration of 50, 5, and 0.5 μg/mL.

The proliferation effect of TPS obtained by BWE (50 μg/mL) has very strong proliferation effect on lymphocyte. Many researchers had broadly accepted the pharmacological effect of TPS as an immunomodulator. The TPS had the capability to inhibit the cancer cells proliferation mainly by activating immune cells and enhancing the immunity of the body. Table 2 showed that TPS had the ability to directly promote the mice splenic lymphocyte.

CONCLUSION

The present experiment used boiling water extraction, traditional water extraction, and enzymatic extraction to extract polysaccharides from tea leaves. The results show that enzymatic extraction was propitious to improve the yield of polysaccharides. The sugar by TPS obtained by boiling water extraction consisting of neutral polysaccharides and acid polysaccharides had strong inhibition effect on α-glucosidase. Experimental results about immunological activity in vitro showed that TPS obtained by boiling water extraction had stronger proliferation effect on lymphocyte at the concentration of 50 μg/mL.

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REFERENCES

1. Pongsuwan, W., T. Bamba, K. Harada, T. Yonetani, A. Kobayashi & E. Fukusaki (2008) *J. Agric. Food Chem.* **56**: 10705-8.
2. Jayabalan, R., S. Marimuthu, P. Thangaraj, M. Sathishkumar, A.R. Binupriya, K. Swaminathan & S.E. Yun (2008) *J. Agric. Food Chem.* **56**: 9064-71.

Concentration (μg/mL)	TPS-EE	TPS-TWE	TPS-BWE	Positive control	Negative control
500	0.4308	0.4019	0.4063	0.8011	0.6615
50	0.4731	0.5996	0.7122	0.6966	0.6615
5	0.4919	0.6875	0.6965	0.5809	0.6615
0.5	0.5208	0.7033	0.6766	0.6468	0.6615
0.05	0.5326	0.6777	0.5859	0.6365	0.6615
0.005	0.5025	0.5341	0.6610	0.6603	0.6615

Table 2. Effect of TPS on Lymphocyte Proliferation *in Vitro*.

3. Munoz-Munoz, J.L., F. García-Molina, M. Molina-Alarcón, J. Tudela, F. García-Cánovas & J.N. Rodríguez-López (2008) *J. Agric. Food Chem.* **56**: 9215-24.
4. Krafczyk, N. & M.A. Glomb (2008) *J. Agric. Food Chem.* **56**: 3368-76.
5. Xiao, J., X. Chen, L. Zhang, S.G. Talbot, G.C. Li, & M. Xu (2008) *J. Agric. Food Chem.* **56**: 910-5.
6. Monobe, M., K. Ema, F. Kato & M. Maeda-Yamamoto (2008) *J. Agric. Food Chem.* **56**: 1423-7.
7. Zhou, X., D. Wang, P. Sun, P. Bucheli, L. Li, Y. Hou & J. Wang (2007) *J. Agric. Food Chem.* **55**: 5523-8.
8. Chen, J.G., Y. Wang, S. Mei, W.Q. Lai, Y. Fu & X. Hu (2003) *Acta Nutrimenta Sin.* **25**: 253-5.
9. Lee, J.H., J.S. Shim, J.S. Lee, J.K. Kim, I.S. Yang, M.S. Chung & K.H. Kim (2006) *J. Agric. Food Chem.* **54**: 8717-23.
10. Iwanaga, D., D.A. Gray, I.D. Fisk, E.A. Decker, J. Weiss & D.J. McClements (2007) *J. Agric. Food Chem.* **55**: 8711-6.
11. Servili, M., A. Taticchi, S. Esposto, S. Urbani, R. Selvaggini & G.F. Montedoro (2007) *J. Agric. Food Chem.* 55: 7028-35.
12. Ugolini, L., G. de Nicola, & S. Palmieri (2008) *J. Agric. Food Chem.* **56**: 1595-600.
13. Kapchie, V.N., D. Wei, C. Hauck & P.A. Murphy (2008) *J. Agric. Food Chem.* **56**: 1766-71.
14. Wuxi institute of light industry (1983) *Food Analysis*. Beijing: Light Industry Press.
15. Zhang, W.J. (1999) *Investigative Technology of Biochemistry on Glycoconjugate*. Second Edition. Hangzhou: Zhejiang University Press.
16. Chapdelaine, P., R.R. Tremblay & J.Y. Dube (1978) *Clin. Chem.* **24**: 208-11.
17. Kalia, K., K. Sharma, H.P. Singh & B. Singh (2008) *J. Agric. Food Chem.* **56**: 10129-34.
18. Bengtsson, A., A. Namutebi, M.L. Alminger & U. Svanberg (2008) *J. Agric. Food Chem.* **21**: 134-43.
19. Sharma, U.K., K. Sharma, N. Sharma, A. Sharma, H.P. Singh & A.K. Sinha (2008) *J. Agric. Food Chem.* **56**: 374-9.
20. Cao, H., J.B. Xiao & M. Xu (2007) *J. Food Compos. Anal.* **20**: 45-51.
21. Alarcon-Aguilar, F.J., M. Jimenez-Estrada & R. Reyes-Chilpa (2000) *J. Ethnopharm.* **69**: 207-15.
22. Kim, Y.M. & M.H. Wang (2005) *Nutrition* **21**: 756-61.
23. Bischof, H. (1994) *Eur. J. Clin. Investm.* **24** (**S3**): 3-10.
24. Viehayanrat, A., S. Ploybutr & M. Tunlakit (2002) *Diabetes Res. Clin. Pract.* **55**: 99-103.
25. Yoshikawa, M., T. Morikawa, H. Matsuda, G. Tanage & O. Muraoka (2002) *Bioorg. Med. Chem.* **10**: 1547-54.