Effects of Soluble Tea Polysaccharides on Hyperglycemia in Alloxan-Diabetic Mice

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The effects of tea water extracts (TWE), crude tea polysaccharides (CTP), and a tea polysaccharide fraction (TPF) were tested on hyperglycemic diabetic mice. Results indicated that TWE, CTP, and TPF could significantly decrease fasting blood glucose (FBG) and glucosylated serum protein (GSP) in alloxan-induced diabetic mice compared to the control group. In vitro antioxidant activities of TWE, CTP, and TPF for scavenging hydroxyl radicals and superoxide radicals decreased with the degree of purification and were lowest for TPF. High-performance gel permeation chromatography (HPGPC) and component analysis revealed the molecular mass distribution and constituents of TWE, CTP, and TPF, indicating that a 100−120 kDa fraction contained the hypoglycemic activity. This fraction was essentially composed of polysaccharides (~90%) with substantial amounts of arabinogalactan proteins. The second-derivative IR spectra of TWE, CTP, and TPF with peak intensity around 1075 and 1045 cm^{-1}, which characterize galactopyranose in the backbone and arabinofuranose units in side branches, respectively, further substantiated the importance of the arabinogalactan proteins. Taken together, the results indicate that a soluble tea polysaccharide is the major hypoglycemic factor in tea and that this polysaccharide may be developed to a potential natural hypoglycemic functional ingredient.

KEYWORDS: Tea water extracts; tea polysaccharide; arabinogalactan proteins; hypoglycemic effect; alloxan-diabetic mice; antioxidant activities

INTRODUCTION

Diabetes, a disorder of metabolism, is increasingly affecting more and more people in the world. Currently nearly 180 million people worldwide are estimated to suffer from diabetes, and the number is predicted to reach 325 million by the year 2025 (*1*). Diabetes is associated with long-term complications that affect eye, heart, blood vessel, and kidney function. It has been established that non-starch polysaccharides are likely to be associated with less risk of diabetes (*2*). Reports indicate that some polysaccharides could prevent and treat a variety of ailments such as hyperglycemia, diabetes, hypertension, hepatitis, cancers, and AIDS (*3*). Many of these polysaccharides have been reported to have effects on hyperglycemia (*4−7*).

It is estimated that more than 200 species including many common plants, such as pumpkin, wheat, celery, wax gourd, lotus root, and bitter melon, exhibit hypoglycemic properties (*8*). Unfortunately, focus is on the mechanism of these plants,

and it is ignored that finding the active components may lead to the development of drug or functional food ingredients.

One of the potential candidates with hypoglycemic properties is tea polysaccharide (TPS), which is extracted from tea leaves. TPS components and activities have been studied in the past few years (*9−11*). One of the difficulties is that there are many active elements in tea such as polyphenols, pigments, theine, theanine, vitamins, and minerals besides TPS (*12−15*), and these active elements usually combine with TPS and often occur in products of TPS. This brings uncertainty in pharmacology and the investigation of the mode of action of TPS.

Up to now, whether the hypoglycemic effect of TPS is induced by other active elements present in TPS or by TPS itself or whether it is a synergistic effect is not well understood. Little information exists on the comparison and the influence of different purities of TPS on hyperglycemia in diabetic mice.

The main purpose of the presented work was to study the effect of tea water extracts (TWE), crude tea polysaccharides (CTP), and a tea polysaccharide fraction (TPF) on fasting blood glucose (FBG), glucosylated serum protein (GSP) level, and maleic dialdehyde (MDA) in alloxan-induced diabetic mice. The molecular mass distribution and major components of these three substances were compared. This may help to elucidate the

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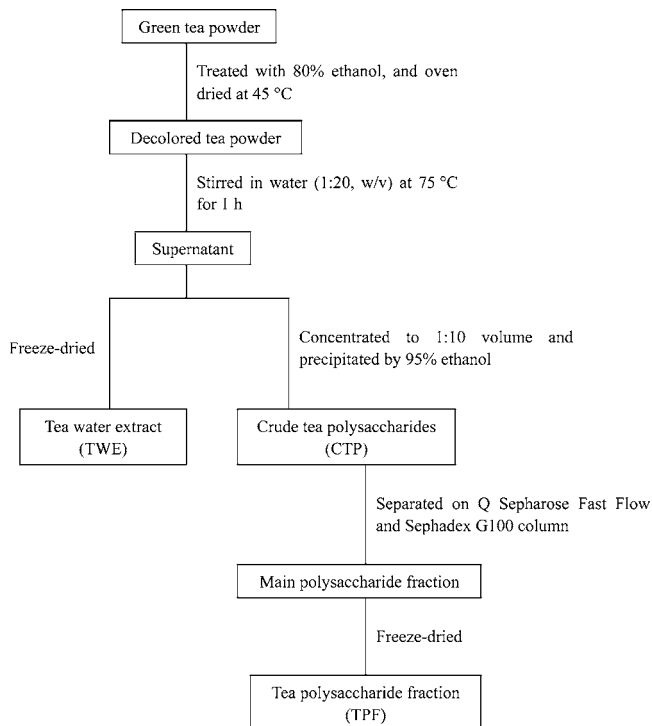[#] Nestlé R&D Center Shanghai Ltd.

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Figure 1. Isolation procedure of TWE, CTP, and TPF of green tea.

function of TPS and promote further research and development of functional food with TPS.

MATERIALS AND METHODS

Chemicals. Q Sepharose Fast Flow and Sephadex G100 were purchased from Amersham Pharmacia Biotech (China) Ltd. Alloxan was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analysis grade and from China Chemicals Co. Ltd. The water used was bidistilled water.

Preparation of TWE, CTP, and TPF (Figure 1). Green tea (produced at Laoshan, Qingdao, China) was pulverized into a powder that was treated with 80% ethanol to remove most of the pigment and oven-dried at 45 °C. The decolored tea powder was stirred in water (1:20, w/v) at 75 °C. After 1 h, the extracts were filtered, and the filtrate was centrifuged at 4000 rpm for 10 min. The obtained supernatant (TWE) was freeze-dried.

The TWE prepared as above was concentrated to 1:10 volume in a rotary evaporator under reduced pressure and precipitated by 95% ethanol (1:4, v/v). The precipitate was collected by centrifugation at 4000 rpm for 10 min and desiccated under vacuum to yield CTP as a solid.

Further purification of the CTP was performed through ion exchange chromatography on a Q Sepharose Fast Flow (20 mm × 60 cm) (*16*). Two peaks were eluted by a NaCl gradient at pH 8.0. The main fraction was collected and subsequently precipitated, desiccated under vacuum, and further purified on a Sephadex G100 column (16 mm × 60 cm). The main polysaccharide fraction was collected and freeze-dried to yield TPF as a powder.

Experimental Animals. Male Kunming mice weighing 24−28 g were purchased from Qingdao Medicament Inspection Institution, China. They were housed in environmentally controlled conditions with a 12 h light/dark cycle at a temperature of 22−24 °C and fed with standard laboratory diet (*17*) and tap water.

Alloxan-Induced Hyperglycemia in Mice and FBG Measurement. Hyperglycemia was induced by the tail vein injection of alloxan at a dose of 55 mg/kg, dissolved in normal saline solution. A standard fasting protocol developed by the American National Institute of Health was adopted in testing the FBG of the experimental animals. Short fasting from 7 a.m. to 1 p.m., with blood drawn at 1 p.m., was implemented. FBG of the mice was tested by the One-Touch Ultrasoft monitor
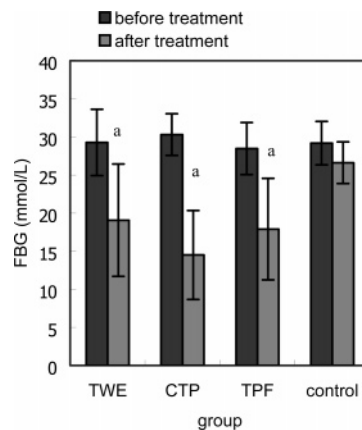

Figure 2. Comparison of blood glucose concentration in diabetic mice before and after treatment with TWE, CTP, and TPS ($n = 8$, mean ± SD; a, $P < 0.01$).
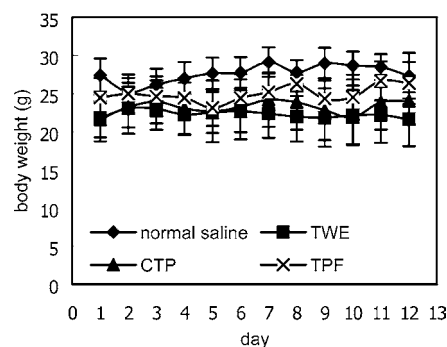

Figure 3. Body weight fluctuation of diabetic mice during treatment with TWE, CTP, and TPF, and the control group ($n = 8$, mean ± SD).

(Lifescan Co.) the third day after alloxan injection. Mice having FBG values >15 mmol/L, and considered to be hyperglycemic, were selected for the test on diabetic mice and randomized into groups of mice according to FBG.

Administration of TWE, CTP, and TPF to the Diabetic Mice. The diabetic mice were randomly divided into four groups of eight mice each and subsequently administered by oral infusion TWE, CTP, and TPF dissolved in normal saline at doses of 60, 60, and 20 mg/100 g/day, respectively. The control group of diabetic mice received normal saline. After 12 days of consecutive administration, FBG of the diabetic mice was measured as described before.

Assay of GSP and MDA. GSP and MDA levels were determined by using commercial kits from Jancheng Institute of Biotechnology (Nanjing, China). The GSP level was determined by the nitroblue tetrazolium (NBT) colorimetric test (*18*), based on the ability of the ketoamine group of glycated proteins to reduce tetrazolium salt under alkaline conditions. Determination of MDA was by the thiobarbituric acid (TBA) colorimetric test at 532 nm. Absorbance was determined using an UV spectrophotometer.

Body Weight of the Animal. The body weight of experimental mice was recorded at 10 a.m. every day until they were sacrificed.

In Vitro Antioxidant Activities Assay. The capability of scavenging superoxide radicals and scavenging hydroxyl radicals was analyzed on the hypoxanthine/xanthine oxidase generating system and the Fenton reaction system, respectively, using the commercial kits from the Jancheng Institute of Biotechnology (Nanjing, China). Absorption of the reaction mixtures was monitored spectrophotometrically at 550 nm.

High-Performance Gel Permeation Chromatography and Component Analysis. The purity and the molecular mass of TPS were determined by high-performance liquid chromatograghy (HPLC) using a TSK3000 column (7.8 mm × 30.0 cm) that was eluted isocratically with Milli-Q filtered and degassed water at 35 °C with a flow rate of 0.5 mL/min and ultraviolet absorption and refractive index detectors. Standard dextran of T-10, T-40, T-70, T-110, and T-500 and blue

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Table 1. Effect of TWE, CTP, and TPF on FBG in Diabetic Mice ($n = 8$, Mean $\pm$ SD)

treatment group	dose (mg/100 g/day)	value of FBG[a] (mmol/L)
normal saline	90	26.61 $\pm$ 2.74
TWE	60	19.06 $\pm$ 7.37 a
CTP	60	14.50 $\pm$ 5.83 b
TPF	20	17.90 $\pm$ 6.66 a

[a] Significance was determined by Dunnett's *t* test: a, $P < 0.05$; b, $P < 0.01$.

Table 2. Effect of TWE, CTP, and TPF on GSP Concentration in Diabetic Mice ($n = 8$, Mean $\pm$ SD)

treatment group	dose (mg/100 g/day)	value of GSP[a] (mmol/L)
normal saline	90	4.68 $\pm$ 0.64
TWE	60	4.00 $\pm$ 0.31 a
CTP	60	3.61 $\pm$ 0.34 b
TPF	20	3.69 $\pm$ 0.49 b

[a] Significance was determined by Dunnett's *t* test: a, $P < 0.05$; b, $P < 0.01$.

Table 3. Influence of TWE, CTP, and TPF on Serum MDA in Diabetic Mice ($n = 8$, Mean $\pm$ SD)

treatment group	dose (mg/100 g/day)	value of MDA[a] (nmol/L)
normal saline	90	15.82 $\pm$ 2.36
TWE	60	12.86 $\pm$ 2.90 a
CTP	60	12.76 $\pm$ 1.99 a
TPF	20	13.35 $\pm$ 1.58

[a] Significance was determined by Dunnett's *t* test: a, $P < 0.01$.

Table 4. IC_{50} Values of TWE, CTP, and TPF for Scavenging Hydroxyl and Superoxide Radicals ($n = 3$, Mean $\pm$ SD)

sample	IC_{50} $^{\bullet}$OH (μg mL^{-1})	IC_{50} $^{\bullet}O_2^-$ (μg mL^{-1})
TWE	5 $\pm$ 0.5	40 $\pm$ 4.6
CTP	15 $\pm$ 3.1	200 $\pm$ 26.5
TPF	36 $\pm$ 5.2	400 $\pm$ 43.5

dextran (Pharmacia) were used for calibration of the molecular size. The polysaccharide content was determined according to the Dubois method (*19*) and protein according to the Bradford method (*20*). Total polyphenol content was determined by the method using ferrous tartrate (*21*). TPF (25 mg) was dissolved in 6 mol/L HCl and hydrolyzed in sealed vacuated tubes at 110 °C for 20 h, and the respective products of hydrolysis were assayed in a Hitachi 835-50 amino acid analyzer. The monosaccharide analysis of TPF was conducted according to Endwin's method (*22*) on an Agilent GC 6890 using a DB225 capillary column (30 m $\times$ 0.32 mm $\times$ 25 μm), with a gas velocity of 1 mL/min, FID detector, and a column temperature of 220 °C. The uronic acid content in TPF was determined according to the method of Blumenkrantz (*23*).

Fourier Transform Infrared Spectroscopy. The samples of TWE, CTP, and TPF were each pulverized with KBr. The infrared spectra were collected on a NEXUE 470 spectrometer (Nicolet) equipped with a DTGS detector. They were recorded from the accumulation of 32 scans in the 1200−800 cm^{-1} range with a resolution of 4 cm^{-1}. The second-derivative IR spectra were obtained using OMNIC E.S.P. V6 software.

Statistical Analysis. SPSS 11.0 programs were used in the statistical analysis. All results were expressed as mean $\pm$ SD. Data were analyzed by one-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) between means were determined using Dunnett's *t* test (two-sided). A paired-sample *T* test was used when blood glucose concentration in diabetic mice before and after treatment was compared. *P* values of less than 0.05 or 0.01 were considered to be significant.

RESULTS

Hypoglycemic Effect of TWE, CTP, and TPF in Diabetic Mice. FBG of TWE, CTP, and TPF treatment groups were significantly lower than those of the control [19.06 $\pm$ 7.37 mmol/L (TWE) and 17.90 $\pm$ 6.66 mmol/L (TPF) versus 26.61 $\pm$ 2.74 mmol/L of control group, $P < 0.05$] and especially the CTP group (14.50 $\pm$ 5.83 mmol/L, $P < 0.01$) (**Table 1**). FBG values of TWE, CTP, and TPF groups after treatment were significantly lower than those before treatment, whereas the values of the control showed no significant difference from the initial values (**Figure 2**).

Effect of TWE, CTP, and TPF on GSP in Diabetic Mice. Persistently high levels of blood glucose in combination with serum proteins will result in high GSP levels. GSP has been used as a marker to reflect the blood glucose concentration during a short period (1−2 weeks) (*24*). In this study, the average levels of GSP in the TWE, CTP, and TPF groups were found to be 4.00 $\pm$ 0.31, 3.61 $\pm$ 0.34, and 3.69 $\pm$ 0.49 mmol/L, respectively. They were significantly lower than the 4.68 $\pm$ 0.64 mmol/L of the control ($P < 0.05$) (**Table 2**). The reductions were 15, 23, and 21% in the TWE, CTP, and TPF groups, respectively.

Influence of TWE, CTP, and TPF on Serum MDA in Diabetic Mice. MDA is a final product of lipid peroxidation and a well-established parameter to determine the increase of free radicals. The levels of MDA in normal saline (control), TWE, CTP, and TPF group were 15.82 $\pm$ 2.36, 12.86 $\pm$ 2.90,

12.76 $\pm$ 1.99, and 13.35 $\pm$ 1.58 nmol/mL, respectively (**Table 3**). Only the TWE and CTP treatments gave a significant difference compared to the control ($P < 0.01$).

Fluctuation of Body Weight of Diabetic Mice. Statistical analysis of the data (**Figure 3**) indicated that there was no significant difference among the body weights of all groups at the end and at the beginning of the experiment. That means the body weights of diabetic mice were not greatly affected by the TWE, CTP, and TPF treatments.

In Vitro Antioxidant Activities of TWE, CTP, and TPF. The IC_{50} values for hydroxyl radicals ($^{\bullet}$OH) and superoxide radicals ($^{\bullet}O_2^-$) of TWE, CTP, and TPF were decreased in turn (**Table 4**). This indicated that their potentials for scavenging hydroxyl and superoxide radicals decreased with the degree of the purification of the tea polysaccharide.

Molecular Mass Distribution and Major Components of TWE, CTP, and TPF. The elution profiles of TWE, CTP, and TPF after HPGPC are shown in **Figure 4**. It can be seen that the molecular mass of substances in TWE ranged from about 120 kDa to very low values. The proportion of the 100−120 kDa polysaccharide fraction increases as the purity of tea polysaccharide increases. By comparing the major components in TWE, CTP, and TPF, the proportion of polysaccharides was found to increase from 34.5 $\pm$ 1.93 and 62.57 $\pm$ 2.54% to 90.26 $\pm$ 5.25%, respectively. Contrarily, the proportion of tea polyphenols and protein decreased (**Table 5**) and no polyphenols were detected in TPF.

Monosaccharide, Uronic Acid, and Amino Acid Analysis of TPF. The molar ratio of monosaccharides in TPF is as follows: L-Rha/L-Fuc/L-Ara/D-Xyl/D-Man/D-Gal/D-Glc = 1:1.01:18.86:2.47:5.73:18.54:1.01. The result suggested that TPF was mainly constituted of arabinose and galactose and of the less abundant rhamnose, fucose, xylose, mannose, and glucose. The uronic acid content was about 10 $\pm$ 0.16% (w/w), and TPF
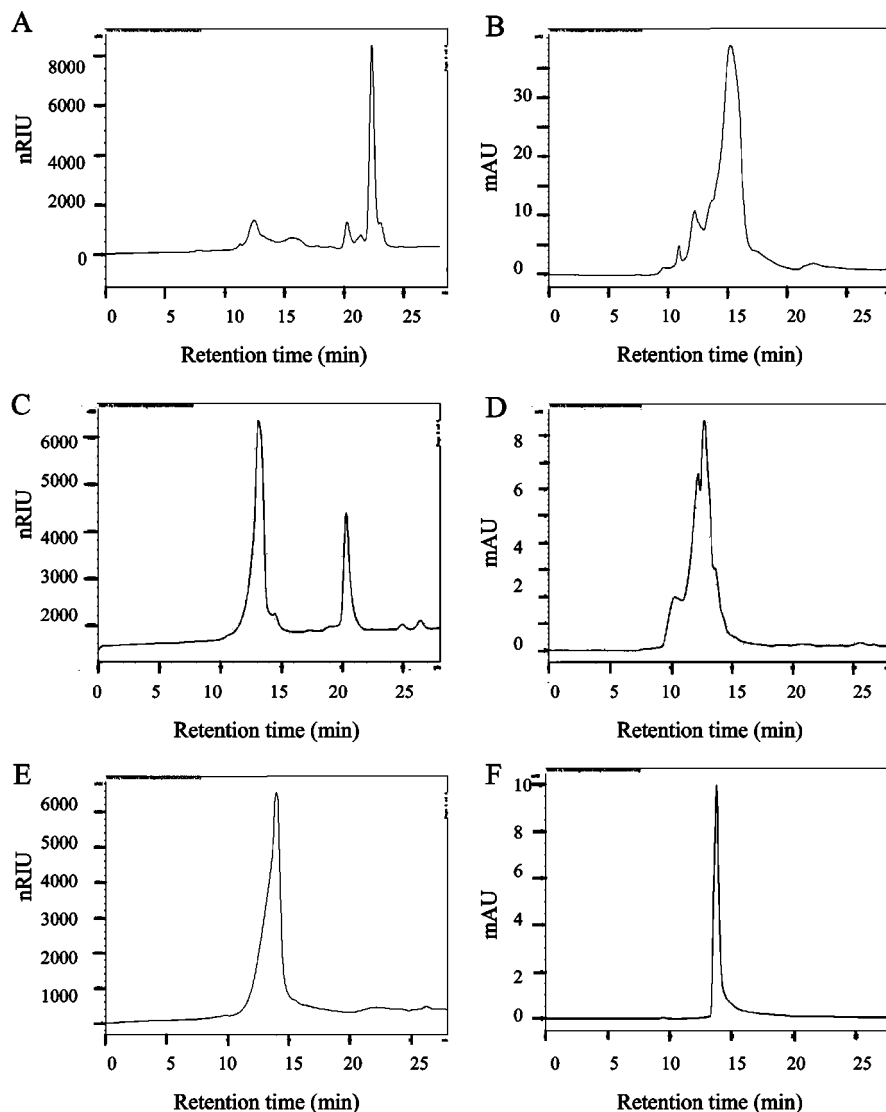
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Figure 4. HPGPC chromatogram of TWE, CTP, TPF: (**A**) TWE (RID signal); (**B**) TWE (UV signal at 280 nm); (**C**) CTP (RID signal); (**D**) CTP (UV signal at 280 nm); (**E**) TPF (RID signal); (**F**) TPF (UV signal at 280 nm).

Table 5. Comparison of Major Components in TWE, CTP, and TPF (%, w/w) ($n = 3$, Mean ± SD)

component	TWE	CTP	TPF
protein	16.3 ± 0.58	8.3 ± 0.79	3.5 ± 0.34
polyphenols	9.2 ± 1.50	4.6 ± 0.17	ND[a]
polysaccharides	34.5 ± 1.93	62.57 ± 2.54	90.26 ± 5.25

[a] Not detected.

contained 17 kinds of amino acids (data not shown) with the proportions of Glu, Gly, and Ala being relatively higher than the other amino acids.

Second-Derivative IR Spectra Characteristics and Similarity of TWE, CTP, and TPF between 1200 and 800 cm^{-1}. The 1200−800 cm^{-1} spectra region in which each particular polysaccharide has a specific band maximum is used in polysaccharide investigations (*25*). The peak around 1097 cm^{-1} in the spectra of TWE is not found in the spectra of CTP and TPF. The peak around 1006 cm^{-1} in the spectra of CTP and TPF does not appear in the spectra of TWE. The intensity of peaks around 1149 and 887 cm^{-1} decreases gradually as the purity of TPS increases. On the other hand, the intensity of the peaks around 1075 and 1045 cm^{-1} (**Figure 5**), which characterize galactopyranose in the backbone and arabinofuranose units

in side branches, respectively (*26*), increases gradually as the purity of TPS increases.

DISCUSSION

Green tea, a popular beverage in Asia, was recently reported to have an effect on blood glucose levels and serum proteomic patterns in diabetic mice and to promote glucose metabolism in healthy humans (*27*). However, it was not clear what were the main hypoglycemic factors. The presented study compared the hypoglycemic effects of TWE, CTP, and TPF at the same time in mice. Interestingly, all of the fractions examined (**Tables 1** and **2**; **Figure 2**) mitigated the hyperglycemia in alloxan-induced diabetic mice. The FBG values and GSP levels of all treatment groups were significantly lower than those of the control. Whereas the purity and content of polysaccharides (100−120 kDa) increased in TWE, CTP, and TPF, the content of other potentially active constituents decreased (**Figure 4**; **Table 5**). This suggests that the TPS is one of the major hypoglycemic factors in tea. In fact, the monosaccharide composition of TPF is different from the former reports on heteropolysaccharides from green tea (*10*, *11*). The presence of arabinose and galactose as main monosaccharides in a molar proportion of 1:1, a protein content of <10%, a higher
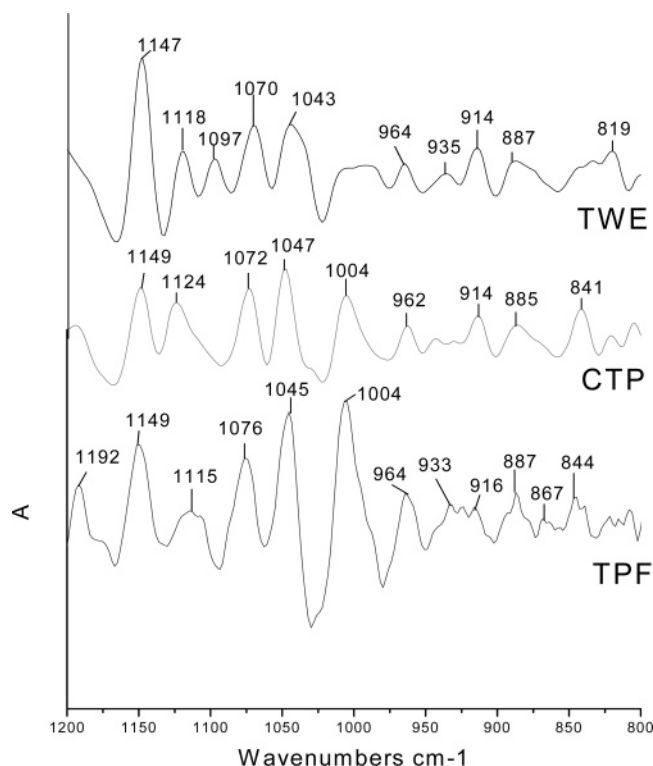
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Figure 5. Second-derivative spectra characters of the 1200–800 cm^{-1} region for TWE, CTP, and TPF. Second-derivative data are multiplied by −1.

proportion of glycine and alanine in TPF, and the second-derivative IR spectra of TPF all indicate that arabinogalactan proteins (AGPs) are the main active components of TPS. AGPs are water-soluble macromolecules that widely exist in plants, playing an important role in vegetative, reproductive, and cellular growth and development as well as programmed cell death. Reports also indicate that AGPs are potential immunological regulators for human health (*28*, *29*).

Recently, AGPs extracted from wolfberry (*Lycium barbarum*) were identified and revealed to have a hypoglycemic effect on alloxan-induced diabetic rabbits (*4*, *30*). However, the mode of action of these AGPs on hyperglycemia animals remains unknown. How does TPF act on the diabetic animals at the molecular level? Does TPF affect the function of pancreas cells or liver cells? What about the advanced structure of TPF? Questions such as these need to be elucidated by further research and may contribute to the understanding of its hypoglycemic effect.

Not surprisingly, the in vivo and in vitro antioxidant activities of TWE and CTP were found to be superior to those of TPF. This can be explained by the higher proportion of tea polyphenols, tea pigments, vitamins, and other antioxidant substances in the cruder fractions. Therefore, in view of an application, CTP may be a better compromise as it combines the antioxidant and hypoglycemic activities better than TWE and TPF. Nevertheless, the natural and hypoglycemic activity of TPS makes it a potential functional food ingredient that deserves further testing regarding its structural identity and its benefits on humans.

ABBREVIATIONS USED

TWE, tea water extracts; CTP, crude tea polysaccharides; TPF, tea polysaccharide fraction; TPS, tea polysaccharide; FBG, fast blood glucose; GSP, glucosylated serum protein; MDA, maleic dialdehyde; HPGPC, high-performance gel permeation chromatography; AGP, arabinogalactan proteins.

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