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Extrusion treatment for improved physicochemical and antioxidant properties of high-molecular weight polysaccharides isolated from coarse tea

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ABSTRACT

Extrusion processing was applied in pretreatment of coarse green tea and tea polysaccharide (TPS) was obtained, the physicochemical, thermal and antioxidant properties of which were comparatively studied by gas chromatography (GC), gel permeation chromatography (GPC), differential scanning calorimetry (DSC), scanning electron microscopy (SEM). The antioxidant activity of TPS was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Results showed that extrusion treatment could improve the yield of TPS from 1.26% to 6.14%. The treatment resulted in the changes of TPS on the monosaccharide composition, molecular weight, thermal properties and the morphological properties. In the scavenging activity against DPPH free radicals, the TPS from extruded tea showed better activity than the untreated one. Extrusion treatment of tea could change the physicochemical properties of TPS and improve the yield and the antioxidant property of TPS, which might be helpful for the tea polysaccharide related products in the food industry.

1. Introduction

Tea, an infusion prepared with the leaves of *Camellia sinensis*, is the most widely consumed beverage in the world besides water (Cabrera, Artacho, & Giménez, 2006). Tea is also one of the first set of medicinal herbs documented in ancient Chinese medicinal literature. The consumption of green tea is especially popular in Asian countries, and its association with human health benefits has resulted in the application of green tea extracts as common botanical ingredients in dietary supplements, nutraceuticals, and functional foods (El-Hanfy, Shawky, & Ramadan, 2011). Coarse green tea was traditionally used to cure diabetics in East Asia, especially in China and Japan. Polysaccharide was considered as one of the effective antidiabetic components in coarse green tea (Zhou et al., 2007).Great progresses had been made on the studies of composition, **p**hysicochemical properties, and bioactivities of tea polysaccharide in recent years (Chen, Qu, Fu, Dong and Zhang, 2009, Chen, Ye, Cheng, Jiang and Wu, 2009; Chen, Zhang, Qu, & Xie, 2007; Wang et al., 2009).

Polysaccharides and their conjugates from varieties of sources such as animals, plant cell walls, and fungal cells, possess marked immunological properties, such as antitumor, antiviral, and anti-infective effects, antioxidant, antimutagenic, and hematopoitic activities, etc. (Srivastava & Kulshreshtha, 1989). The polysaccharide and its conjugates from green tea have also been reported to possess immunological, anti-radiation, anti-blood coagulation, anti-HIV, and hypoglycemic activities (Nie & Xie, 2011; Wang, Wang, Li and Zhao, 2009, Wang, Wei and Jin, 2009). Polysaccharide played an important role in the development of functional food as well as the research of medicine as one of the bioactive macromolecules. So it is essential to study the physicochemical, thermal and rheological properties of polysaccharide.

Extrusion is a mechanical process exposing material to high temperature, shear force and pressure over a short period of time. It is a technology applied in the food and pharmaceutical industry for affecting product microstructure, product chemistry or the macroscopic shape of products (Singh & Smith, 1997). Extrusion processing was widely used for the production and modification or improvement of quality of various products especially in starch, lipids and protein products (Abbas, Khalil, & Hussin, 2010; Camire, 1991; Zhang, Bai, & Zhang, 2011). Extrusion of starchy material conventionally resulted in gelatinization of starch, denaturation of protein and formation of complexes between starch, lipids and proteins (Wolf, 2010). This combination of extreme processing conditions promoted both cell disruption and protein denaturation that seemed to favor aqueous extraction of oil and proteolytic attack (Jung & Mahfuz, 2009). Studies on starch and protein showed that moderate heat treatment such as extrusion, which employed high temperatures over short times, promoted partial protein denaturation and starch gelatinization (Wolf, 2010). The thermal process altered the distribution pattern of hydrophobic and hydrophilic patches on the protein surfaces, consequently affecting their interfacial properties (Camire, 2002). Furthermore, starch based products were often extruded to break down the starch granule to render it digestible and to produce a shaped product. Encapsulation

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of flavors, nutrients and drugs were other frequent applications of extrusion processing.

Extrusion processes are applied to polysaccharides for specific purposes such as physical modification or chemical modification (reactive extrusion), manufacture of confectionary gels and encapsulation of flavors or drugs. Non-starch polysaccharides and confectionary gels had been extruded in recent studies (Bueno, Pereira, Menegassi, Areas, & Castro, 2009; Vauchel, Kaas, Arhaliass, Baron, & Legrand, 2008; Zhang et al., 2011). Tea polysaccharide was applied in food products such as beverage and additives (Nie & Xie, 2011; Guo, Liang, & Du, 2011) and the effect of tea polysaccharide on gelatinization and retrogradation of wheat starch was studied in Zhou's study (Zhou, Wang, Zhang, Du, & Zhou, 2008). However, the effect of extrusion process on the physicochemical properties of tea polysaccharides was unknown till now.

The objectives of the present study were to investigate the effects of extrusion processing on the physicochemical and thermal properties of polysaccharide from coarse green tea, and the antioxidant properties of tea polysaccharides after extrusion treatment were also determined.

2. Materials and methods

2.1. Extrusion treatment of coarse tea

The coarse green tea was produced in Huang Mountain, Anhui province. Tea samples were extruded with a DS56-X twin-screw co-rotating, self-wiping extruder (Jinan Saixin Machinery Co., Jinan, China) with length/diameter ratio of 25, screw speed up to 600 rpm. The barrel temperature profile was maintained at 100, 130, and 160 °C, respectively. Feed moisture content of the tea samples in the extruder was adjusted to 4%, 8%, or 12%. Processed tea samples were made in triplicate for each treatment. The samples were stored at −20 °C prior to analysis.

2.2. Extraction of tea polysaccharide

Extraction of tea polysaccharide was performed according to our previous studies (Chen, Zhang, Qu, & Xie, 2008). Briefly, extruded green tea of each sample (50 g) as well as that of the untreated sample were mixed with 500 mL of 80% (v/v) ethanol and shaken at 30 °C for 24 h to remove most of the polyphenols and monosaccharide. After the mixture was filtered, the residues were dried in air and then extracted with hot water (80 °C) three times (1:20, w/v). The tea extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at 4 °C for 24 h, and then centrifuged (3000 × *g*, 10 min). The precipitate was vacuum freeze-dried, and the crude tea polysaccharide (TPS) from extruded and untreated coarse tea samples was obtained.

2.3. Purification of tea polysaccharide

Tea polysaccharide samples were purified by decoloring and deproteining procedures as follows. Crude tea polysaccharide conjugates (TPS) were dissolved in water and deprotein by Sevag method (Staub, 1965), in which the polysaccharide solution was mixed with chloroform and n-butanol in the proportion of 25:5:1. After three times of deprotein process, TPS was precipitated by 95% (v/v) ethanol at 4 °C for 24 h and decolored by ethyl alcohol. The yields of TPS were determined and the content of total sugar in TPS was determined by the sulfuric acid-phenol method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with glucose as standard. The standard calibration curve was obtained as follows: $A = 4.3667\ C + 0.0879$, $R^2 = 0.9998$.

2.4. Compositional analysis of monosaccharide

Uronic acid content was determined by the carbazole-sulfuric acid method using galacturonic acid as standard (Bitter & Muir, 1962). Gas chromatography (GC) was used for identification and quantification of neutral monosaccharides in tea polysaccharide according to our previous studies (Chen et al., 2008). GC was performed on a Shimadzu GC-6A instrument (Japan) with a WCOT column containing OV1701 (30 m × 0.32 mm × 0.5 μm). First, the tea polysaccharide (10 mg) was hydrolyzed with 4 mL of 2 M TFA at 120 °C for 2 h, and then hydrolyzed products were dried at reduced pressure. The hydrolysates were then converted into alditol acetates according to conventional procedures. The GC operation was performed using the following conditions: H_2: 30 mL/min; air: 300 mL/min; N_2: 20 mL/min; injection temperature: 250 °C; detector temperature: 250 °C; column temperature programmed from 150 to 180 °C at 20 °C/min, then increasing to 240 °C at 2 °C/min and holding for 5 min at 240 °C. Monosaccharides including rhamnose, arabinose, mannose, glucose and galactose (Sigma-Aldrich, Shanghai, China) were used as the standards.

2.5. Molecular weight determination

The molecular weight of polysaccharides was determined using gel permeation chromatography on Sephadex G-150 column (60 cm × 2.5 cm, i.d.) according to Fu, Chen, Dong, Zhang, and Zhang (2010). The column was eluted by 0.02 M PBS at a flow rate of 8.0 mL/h. Fractions were collected for every 4 mL. The total carbohydrate of each fraction was determined and the molecular weight of polysaccharides was obtained from the regression line of the standard molecular weight versus fraction number plot. The calibration curve was made with dextran standards of different molecular weights (Pharmacia, Uppsala, Sweden). The dextran standards were Dextran Blue (Mw > 2,000,000), T-500 (Mw 450,000), T-70 (Mw 68,500), T-40 (Mw 44,400), and T-10 (Mw 10,400). The calibration curve was constructed by plotting logMw vs Kav of dextran standards ($\log Mw = 5.335 - 1.619\ Kav$) with a correlation coefficient of 0.9970.

2.6. Morphological properties

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-30). The polysaccharide samples from extruded and untreated tea were placed on a specimen holder with the help of double-sided adhesive tapes and coated with gold powder (Yang, Zhao, Shi, Yang, & Jiang, 2008). Each sample was observed with 200 and 1000 fold magnification at an accelerating potential of 20 kV during micrography.

2.7. Thermal properties of TPS

Thermal characteristics of isolated TPS were studied by using a differential scanning calorimeter-DSC204, HP (NETZSCH, Germany) equipped with a thermal analysis station. TPS (5 mg, dry weight) was loaded into a 40 rmul capacity aluminum pan (Mettler, ME-27331) and distilled water was added with the help of a Hamilton micro syringe to achieve a polysaccharide-water suspension containing 70% water. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The DSC analyzer was calibrated using indium and an empty aluminum pan was used as reference. Sample pans were heated at a rate of 15 °C/min from 20 to 150 °C. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH_{gel}) were calculated automatically (Wang, Yu, Liu, & Chen, 2008).

2.8. Scavenging activity against DPPH free radicals

DPPH scavenging activities of TPS from extruded and untreated tea were detected according to our previous studies (Chen, Qu, et al., 2009). One hundred microliters of various concentration of the tea polysaccharide was mixed with 2900 μL DPPH solution (120 μM) in ethanol and incubated in darkness at 37 °C for 30 min. The absorbance was recorded at 517 nm. Percentage (%) inhibition of TPS on free radical