Food Research International 46 (2012) 158–166

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Fermentation process enhanced production and bioactivities of oolong tea polysaccharides

Yuefei Wang [a,*,1], Shuhong Shao [a,1], Ping Xu [a], Hao Chen [a], Shoei-Yn Lin-Shiau [b], Yea-Tzy Deng [c], Jen-Kun Lin [c,**]

[a] Department of Tea Science, Zhejiang University, Hangzhou 310058, PR China
[b] Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan
[c] Institute of Biochemistry, College of Medicine, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 23 September 2011
Accepted 29 November 2011

Keywords:
Tea polysaccharides
Oolong tea
Physicochemical characterization
Antioxidant activity
Alpha-glucosidase inhibitory effect

ABSTRACT

Oolong tea (OT) is unique for its flavor and healthy effects. We have reported that the polypheonols of OT possessed antioxidant anticancer, and hypolipidemic effects. In addition, tea polysaccharides (TPS) have long been recognized to have antioxidant, immunomodulatory and antidiabetic properties. Since there are three typical kinds of OTs, e.g. Tieguanyin, Fenghuangdancong and Dahongpao with the lightest, intermediate and the highest degree of fermentation respectively, it is important for us to know whether fermentation degree affected the production and their bioactivities of tea polysaccharide. In the present study, three polysaccharides named TTPS, FTPS and DTPS were isolated from these three typical kinds of oolong teas in the above order respectively. Physicochemical characteristics, in vitro antioxidant activity and alpha-glucosidase inhibitory effect of TTPS, FTPS and DTPS were studied. Analysis of the chemical compositions (protein, rot neutral sugars and uronic acid) revealed that they all belonged to acid heteropolysaccharides bound with protein. The contents of protein and uronic acid but rot neutral sugars proportionally increased with degree of fermentation, highest in DTPS, which significantly correlated with their potencies in antioxidant and alpha-glucosidase inhibitory activities. Analysis of neutral sugar compositions showed that they all contained seven monosaccharides with different molar ratio. Measurements of their molecular weight by high performance gel permeation chromatography indicated that 92.9% of TTPS and 94.4% of DTPS had a peak of molecular weight of 0.82×10^6 and 2.64×10^6 respectively, but that of FTPS had 34.2% with 0.93×10^6 and 68.8% with 0.01×10^6 respectively. Fourier Transform IR spectra exhibited that they all had similar characteristic absorption bands of uronic acid, protein, polysaccharide and pyran-glycosides respectively. Furthermore, measurements of antioxidant activities by assays of DPPH, ABTS and ferric-reducing antioxidant power (FRAP) showed that DTPS was the most potent, FTPS the next and TTPS the least respectively. Similarly, DTPS possessed the strongest inhibitory potential against alpha-glucosidase activity, followed by FTPS and then TTPS. The potencies of the bioactivities of these three OTPS were apparently proportional to their contents of protein and uronic acid, suggesting that the higher degree of fermentation of oolong teas produced higher contents of protein-bound acid heteropolysaccharides accompanied with higher antioxidant and alpha-glucosidase inhibitory activities. Perhaps, DTPS had a potential to play a role in prevention of type 2 diabetes mellitus. Further studies to confirm this contention is in progression.

1. Introduction

Tea polysaccharides (TPS) are one of important bioactive macromolecules in tea (*Camellia sinensis* L.). In the past two decades, it has been reported that TPS possess various biological activities, such as antioxidant activity, antidiabetic effect, hypolipidemic, immunomodulatory activity and anticancer activity (Chen, Zhang, Qu, & Xie, 2008; Nie & Xie, 2011). Among these bioactivities, antidiabetic potential of TPS has been concerned because tea drink is recognized as a popular folk medicine for management of diabetes in China and Japan. Tea polysaccharides have been considered to be the most distinctive compounds in the tea leaves (Wang, Zhou, Li, Hou, & Sun, 2008). To date, most studies on the antidiabetic effect of polysaccharides were focused on green tea (Chen et al., 2010; Zhou, Wang, & Sun, 2007). Only a few studies explored tea polysaccharides from oolong and black teas (Chen, Qu, Fu, Dong, & Zhang, 2009). Previous studies on the physicochemical properties and bioactivities of tea polysaccharides isolated from different kinds of teas revealed that they are considerably different (Nie & Xie, 2011; Chen, Qu et al.,

* Corresponding author. Tel./fax: +86 571 88982217.
** Corresponding author. Tel.: +886 2 2356 2213; fax: +886 2 2391 8949.
E-mail addresses: tyfwang@gmail.com (Y. Wang), jklin@ntu.edu.tw (J.-K. Lin).
[1] These authors contributed equally to this work.

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.11.027

2009). Factors affecting these properties may be due to the distinctive manufacturing processes of teas such as the degree of fermentation, extraction and purification conditions (solvents, enzyme used and temperature) (Chen et al., 2008; Chen, Qu et al., 2009). Previous studies have mostly focused on the polysaccharides rot neutral sugars from the unfermented green tea (Chen et al., 2008; Monobe, Ema, & Kato, 2008; Nie, Xie, Zhou, & Cao, 2007), and less attention has been paid on the polysaccharides from other special kinds of teas, like oolong (semi-fermentation) and black (full fermentation) teas.

Oolong tea is classified as (semi-fermented tea) and is widely consumed in Japan and China for its unique flavor and health benefit (Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). In a recent study, oolong tea polysaccharides (OTPS) were found to have remarkable antioxidant activity and inhibitory effect on alpha-glucosidase in vitro (Chen, Qu et al., 2009). Whereas oolong teas are in considerable difference among themselves and are divided into three typical kinds which are represented by Tieguanyin, Fenghuangdancong and Dahongpao, based on the degree of fermentation during manufacturing. Tieguanyin and Dahongpao are the oolong teas with the lightest and the highest degree of fermentation, respectively, and between them is Fenghuangdancong with the moderate level of fermentation. Because it is important for us to elucidate the differences of polysaccharides in these three typical kinds of oolong teas, therefore, in this study, we attempted to isolate three polysaccharides named TTPS, FTPS and DTPS from three typical kinds of oolong teas including Tieguanyin, Fenghuangdancong and Dahongpao, respectively. Their physicochemical properties, in vitro antioxidant activity and alpha-glucosidase inhibitory effect of the three OTPS were comparatively studied.

2. Materials and methods

2.1. Chemicals

Three typical oolong teas including Tieguanyin, Fenghuangdancong and Dahongpao were purchased from the local tea market of Hangzhou, China. Dialysis membrane (7000 Da), galacturonic acid, dextran, acarbose, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), seven standard monosaccharides (L-rhamnose, D-fucose, L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose) and baker's yeast α-glucosidase (EC 3.2.1.20) were obtained from Sigma Chemical Co. (Missouri, USA). Coomassie brilliant blue G-250 and bovine serum albumin were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). *p*-Nitrophenyl-α-D-glucopyranoside (*p*NPG) was obtained from Xibao Co. (Shanghai, China). All other chemicals were analytical grade and purchased from Shanghai Boer Chemical Reagent Co. (Shanghai, China).

2.2. Extraction and isolation of the OTPS

Three OTPS (TTPS, FTPS and DTPS) were isolated by the modified procedure as described previously (Chen, Zhang, & Xie, 2004). Briefly, dry meshed tea powders (100 g) were mixed with 1 L of ethanol (80%, v/v) for 24 h to remove most of the polyphenols and monosaccharide. After the supernatant was removed, the residues were dried in air and then extracted with hot water at 70 °C for 60 min (3 times). The aqueous extracts were concentrated and then precipitated with 4-fold vol of 95% ethanol. The precipitate that formed was collected by centrifugation at 3000×*g* for 10 min and repeatedly washed sequentially with ethanol, acetone, and ether, respectively for 3 times. The precipitate was dissolved in hot water (70 °C) and excluded protein with Sevag method (Sevag & Miller, 1948), and dialyzed against distilled water for 48 h with dialysis tubing (molecular weight cut-off, 7000 Da) to remove low-molecular weight matters, and then concentrated and precipitated with 4-fold vol of 95% ethanol to obtain the polysaccharide fraction. The fraction was dissolved in water (60 °C) to remove the rest of ethanol in a rotary evaporator under reduced pressure, and lyophilized to yield OTPS, finally.

2.3. Composition analysis

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

2.4. Monosaccharide composition analysis

The three OTPS were hydrolyzed respectively in 2 M trifluoroacetic acid at 120 °C for 2 h. The hydrolysate was converted into its respective alditol acetate by reduction with $NaBH_4$ and acetylation with acetic anhydride (Erbing, Jansson, Widmalm, & Nimmich, 1995), which was analyzed by Agilent-6890 GC (Waldbronn, Germany) equipped with an Elite-17 ms column (30 m×0.32 mm×0.25 μm) and a flame-ionization detector (FID). The temperatures of injector and detector were 250 °C and 230 °C, respectively. The column temperature program was set to hold for 3 min at 190 °C, then increased to 230 °C at 4 °C/min. N_2 was used as carrier gas at a flow rate of 1.2 ml/min. The standard monosaccharides (L-rhamnose, D-fucose, L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose) with myo-inositol as the internal standard were measured following the same procedure.

2.5. Molecular weight (MW) determination

MWs of the three OTPS were determined by high performance gel permeation chromatography (HPGPC) with equipments of a Waters 515 chromatography, a TOSOH BIOSEP G4000SWXL column (7.8×300 mm, Tokyo, Japan) and a Waters 2410 RI detector. Fifty microliters of the samples was injected and eluted at a buffer (0.1 M $NaNO_3$ solution) flow rate of 0.8 ml/min at 40 °C. The HPGPC system was precalibrated with various standard dextrans of different molecular weights (10.5 kDa, 43.2 kDa, 76.9 kDa, 473 kDa and 2000 kDa).

2.6. Infrared spectral analysis

The IR spectra of the OTPS were determined using a Fourier Transform Infrared spectrophotometer (Avatar 370, Thermo Nicolet, Madison, USA) equipment. The polysaccharides were grounded with KBr powder and then pressed into pellets for FTIR measurement in the frequency range of 4000–400 cm^{-1}.

2.7. DPPH assay

The DPPH free radical scavenging activity of the OTPS was determined by the method of Mohsen and Ammar (2009), with a slight modification (Mohsen & Ammar, 2009). One milliliter of the tested samples at various concentrations (0.25–8 mg/ml) was added to 3 ml of ethanolic DPPH solutions (0.1 mM). Discolorations were measured at 517 nm after incubation for 30 min at 30 °C in the dark. BHT was used as the positive control. The DPPH scavenging effect was calculated as follows:

$$\textit{DPPH scavenging effect}\ (\%) = \left(1 - A_{\text{samp}}/A_{\text{cont}}\right) \times 100$$

where A_{samp} and A_{cont} are defined as absorbance of the sample and the control, respectively.