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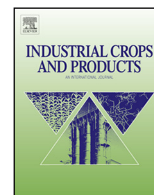

Functional groups and antioxidant activities of polysaccharides from five categories of tea

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ABSTRACT

Ten polysaccharides were extracted from five categories of tea with hot water and further fractionated by stepwise ethanol precipitation. The polysaccharides were characterized by Fourier transform infrared spectroscopy (FT-IR) and the components including sulfuric radical, aminohexose and uronic acid were determined. All analyzed polysaccharides had antioxidant activities, with polysaccharides-II being stronger than polysaccharides-I and the antioxidant activity of the tea polysaccharides was closely related with the contents of functional groups in the polysaccharides. Our results suggest that the tea polysaccharides could be explored as a potential antioxidant agent for use in medicine or functional foods. On the other hand, the different antioxidant activities of samples provided an important reference for choosing the suitable kind of tea in application.

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1. Introduction

As a daily beverage, tea is widely consumed in the world and is usually classified into unfermented (green tea – GT), slight-fermented (white tea – WT), semifermented (oolong tea – OT), fermented (black tea – BT), and post-fermented (dark green tea – DGT) forms (Tanaka et al., 2012; Zhu et al., 2002). Due to its validated as well as assumed beneficial effects on human health, it is planted as an important commercial crop in many areas in the world especially in China. In addition, tea has been used as a pharmaceutical to treat coma, to improve blood flow, and to eliminate toxins for many years (Balentine et al., 1997; Dufresne and Farnworth, 2000; Hara, 2011; Sewell, 1864). Recently, interest in tea is growing because of many other beneficial bioactivities, including its roles as an antioxidant (Wang et al., 2012c; Yang et al., 2012a), effects on angiogenesis (Maiti et al., 2003), and possible therapeutic values against cancers (Carvalho et al., 2010; Fan et al., 2011), mutagenesis (Geetha et al., 2004; Kuroda and Hara, 1999), hypertension (Antonello et al., 2007), and hyperglycemia (Han et al., 2011). These biological activities and health benefits are associated with the constituents of tea such as polysaccharides, polyphenols, caffeine, theanine and so on (Nie and Xie, 2011; Wang et al., 2012a).

In recent years, the association of bioactivities with structure of natural polysaccharides has caused great interest among researchers (Kishk and Al-Sayed, 2007; Klaus et al., 2011). Tea polysaccharides (TP) are among the most important components of tea. Although many of the bioactivities of tea mentioned above have been associated with TP, to date most of previous studies have been focused on green tea polysaccharides (Nie and Xie, 2011). Notably, the bioactivities and structures of the constituents from different categories of tea can be affected by the remaining enzymes originally contained in the leaves after different manufacturing processes (Tanaka et al., 2009, 2012). However, so far to our knowledge no comparative studies have been reported on the antioxidant activities and structures of TP from five categories of tea.

In this study, we analyzed the functional groups of ten TP samples from five types of tea by Fourier transform infrared (FT-IR) spectroscopy analysis, determined the contents of sulfuric radical, aminohexose and uronic acid, and investigated the antioxidant activities. Our work suggests that the tea polysaccharides, as potent antioxidant agents, have great potential for use in medicine or functional foods. Additionally, our results of different antioxidant activities of teas treated by different manufactural processes provide important guideline information for choosing the suitable kind of tea in different applications.

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2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH•) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Vitamin C (Vc) was purchased from the Sinopharm Chemical Reagent Co. (Beijing, China). Ultra-pure water was used throughout the experiments.

2.2. Plant materials

All categories of tea (GT, DGT, OT, WT and BT) were purchased from the local market (Harbin Tea City). They were ground into fine powder using a pulverizer (WND-200, Weinengda Instrument Co., Ltd., Zhejiang, China). The materials were stored at room temperature (25 °C) in a desiccator until use.

2.3. Preparation of tea polysaccharides

Polysaccharides were extracted from five different categories of tea by hot-water and stepwise ethanol precipitations. Each material was extracted with 85% ethanol at 60 °C for 5 h three times to defat and remove colored materials, oligosaccharides, and small molecule impurities under reflux. The pretreated samples were separated from the solvent by filtration and air dried. Each dried pretreated sample was extracted with distilled water (1:5, w/v) at 80 °C for 3 h. The supernatants and residues were separated by vacuum filtration and the residues were re-extracted three times. The combined supernatants were concentrated to 1/4 of the original volume with rotary evaporator and then were deproteinated by the Sevag method for three times (Kuang et al., 2011; Sevag et al., 1938). And then the extracts were intensively dialyzed for two days against distilled water (cut-off Mw 3500 Da). Dehydrated ethanol was slowly added to the retentate portion to the final ethanol concentration of 30% (v/v), and the mixture was left for 12 h at 4 °C. The precipitates (TP-I) were obtained by centrifugation at 4000 rpm for 15 min and then were dried by lyophilization. Into the supernatant, ethanol was added to the final concentration 60% (v/v) and TP-II was obtained.

2.4. Preliminary characterization of tea polysaccharides

2.4.1. Determination of contents of sulfuric radical, aminohexose and uronic acid

The contents of sulfuric radical and aminohexose were determined according to the reported methods (Amarowicz et al., 2012; Dodgson and Price, 1962; Rondle and Morgan, 1955; Yang et al., 2012b). The content of uronic acid was determined according to the method of Dische by using D-glucuronic acid as the standard (Dische, 1947; Yang et al., 2012b).

2.4.2. Fourier transform infrared spectroscopy analysis

The Fourier transform infrared (FTIR) spectra of samples were recorded on an FTIR spectrophotometer (IRPresting-21, Shimadzu Co., Japan). The dried sample was ground with potassium bromide powder and pressed into pellet for spectrometric measurement in the frequency range of 4000–400 cm^{-1} at the resolution of 4 cm^{-1} and a maximum source aperture.

2.5. Assay of DPPH free radical scavenging activity

The scavenging activity of the polysaccharides on DPPH free radical (DPPH•) was determined as reported (Ye and Huang, 2012) with modifications. Briefly, 1.0 ml of 0.1 mM DPPH-methanol solution was added to 3.0 ml of the polysaccharides at various concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) in water. The mixture was incubated at 25 °C for 30 min with shaking in the dark. Then the absorbance was measured at 517 nm (UV-2550, Shimadzu Co., Japan) against a blank (water instead of test sample and DPPH• solution). Vc was used as the positive control. The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity } (\%) = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100$$

where A_0 is the absorbance of the control (water instead of sample solution), A_1 is the absorbance of the sample and A_2 is the absorbance of the sample under identical conditions as A_1 with water instead of DPPH• solution.

2.6. Assay of superoxide anion radical scavenging activity

Superoxide anion radical was generated in the system of pyrogallol's autoxidation in an alkalescent condition (Zou et al., 1986). The scavenging activity of the polysaccharides on superoxide anion radical ($O^{2\bullet}$) was measured as described previously (Chen et al., 2012) with a minor modification. Briefly, 5.0 ml of 50 mM Tris–HCl buffer (pH 8.2) and 1.0 ml of samples at different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) were mixed with 0.2 ml of 7 mM pyrogallol. The reaction mixture was incubated at 25 °C for 20 min and then the change speed of absorbance (A/min) of the reactive solution was measured at 325 nm, against a blank (water instead of sample and pyrogallol solution), with Vc as the positive control. The scavenging activity of the polysaccharides on the superoxide anion radical was calculated by the following formula:

$$\text{Scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the change speed of absorbance of the control group (water instead of sample solution) and A_1 is the change speed of absorbance of the test sample.

2.7. Assay of hydroxyl radical scavenging activity

The hydroxyl radical (•OH) scavenging activity of the polysaccharides was measured according to Fenton's reaction (Jiang et al., 2010). The hydroxyl radical was generated in a mixture of 1.0 ml of 5 mM 1, 10-phenanthroline, 1.0 ml of 0.05 M sodium phosphate buffer (pH 7.4), 0.5 ml of 7.5 mM $FeSO_4$ and 0.5 ml of H_2O_2 (3%, v/v). After addition of 2.0 ml sample solution, the mixture was incubated at 37 °C for 1 h. The absorbance was measured at 510 nm. Deionized water and Vc were used as the blank and positive control respectively. The scavenging activity on •OH was calculated by the following equation:

$$\text{Scavenging activity } (\%) = \frac{A_1 - A_2}{A_3 - A_2} \times 100$$

where A_1 is the absorbance of the sample, A_2 is the absorbance of the deionized water instead of sample and A_3 is the absorbance of the deionized water instead of H_2O_2 and sample.

3. Results and discussion

3.1. Preliminary characterization of tea polysaccharides

3.1.1. Contents of sulfuric radical, aminohexose and uronic acid

The contents of sulfuric radical, aminohexose and uronic acid in all samples were shown in Table 1 and there were significant differences among them. All kinds of TP-I had higher content of sulfuric radical than TP-II. On the other hand, all kinds of TP-II had higher contents of aminohexose and uronic acid than TP-II.

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