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Multi-fingerprint and quality control analysis of tea polysaccharides

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ABSTRACT

High performance gel permeation chromatography (HPGPC), ultraviolet–visible spectra (UV), infrared absorption spectra (IR) and ion chromatography (IC) techniques were used in fingerprint analysis of tea polysaccharides (TPS). Multi-fingerprint was applied to assess TPS consistency and to discriminate other polysaccharides in order to achieve the quality control of TPS. The experimental data for spectrogram and chromatogram were used for similarity calculation, included angle cosine method and correlation coefficient method. The results showed that the UV absorption spectra, IR absorption spectra and IC of 22 batches of TPS had a high degree of similarity, respectively, and the similar indexes were up to 0.9985 and 0.9475. Other five polysaccharides were compared with the referential fingerprint, which had an obvious difference. It could conclude that some differences were really existed between TPS and other polysaccharides; and multi-fingerprint is a more useful means to control the quality of TPS than one simple fingerprint. This analytical method is highly rapid, effective, visual and accurate for polysaccharides research.

1. Introduction

The quality control has always been the key issue in the commercialization of polysaccharide products. Polysaccharide products are similar to herbal medicines, which have a complicated composition led to a limited numbers of specific components does not availably reflect the real qualities (Liu, Sun, Lv, & Chan, 2006). Fingerprint technique is a powerful tool for the quality control of multi-component herbal medicines (The State Drug Administration of China, 2000; WHO, 1991). In recent years, the fingerprint of the relative amounts of various active ingredients has been shown to be a convenient and effective method for the standardization and quality control of various herbal materials, especially when there is a lack of authentic standards for the identification of all the active components present in these complex natural products (Di, Chan, Leung, & Huie, 2003). Therefore, it is a trend that fingerprints are used in quality control of polysaccharide products. And the development of polysaccharide products will benefit from the fingerprint technique of traditional Chinese medicine, emphasizing a principle component analysis and a fuzzy information analysis.

There have been a number of reports regarding the use of HPLC, CE, TLC, NIR, IR fingerprints on the quality assessment of some herbal medicines and their raw materials (Yang, 2002). Those techniques have been used in the study of polysaccharide fingerprint. HPLC technique is applied to establish the carbohydrates fingerprint of traditional Chinese medicines (TCM) and the serum fingerprint of herbal medicinal polysaccharide (Wang, Lv, Li, & Zhang, 2008; Xia et al., 2008). High-performance thin-layer chromatography (HPTLC) is developed for a fingerprint analysis of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of Lingzhi (Di et al., 2003). Fourier-transform infrared spectra (FT-IR) technique is used to build polysaccharide fingerprint of *Ganoderma lucidums* (He, Shao, & Sun, 2010) and two-dimensional correlation infrared spectra (2D-IR) technique is used to establish polysaccharide fingerprint of three purified polysaccharides (sugar 30%, 60%, 90%) from a same tea sample (Zhou, 2007). These proved that polysaccharide fingerprints can be fully applied to the quality control of polysaccharide and have a great potential. Although fingerprint is employed to quality control of polysaccharides, but too limited technique is adopted to an uncertain conclusion. Therefore, multi-fingerprint which can be reduced this uncertainty will be the future trend of polysaccharides research.

Green tea (*Camellia sinensis*) has been used as the second most consumed beverage for thousands of years in the world next to water and has caused great interests among researchers (Tsubaki, Iida, Sakamoto, & Azuma, 2008; Wang et al., 2010; Xiao et al., 2008). And some studies have found that TPS was a proteoglycan. TPS from green tea has been found to be an important water soluble polysaccharide with certain bioactivities in the late 1980s (Guo, Du, Lan, & Liang, 2010; Kardosova & Machova, 2006; Lv et al., 2009). Such as immunostimulation (Warrand, 2006; Yamada, 1994), anti-tumor (Zha, Luo, Luo, & Jiang, 2007), antioxidant activities (Liu et al., 2007), anti-inflammatory (Wang & Luo, 2007), hypoglycemic (Wu, Cui, Tang, Wang, & Gu, 2007). The structure of TPS is very complex, and a great discrepancies are existed among the different sources

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of polysaccharides, and the activity of TPS is the result of its overall. Therefore in the quality standard of TPS, only the content of total sugar and protein are quantified and used as marker compounds cannot reflect the real and comprehensive active constituents and inadequate to control the quality of TPS.

The present study aimed at developing the multi-fingerprint of TPS which is combined with ultraviolet–visible spectra (UV) fingerprint, infrared absorption spectra (IR) fingerprint and ion chromatography (IC) fingerprint to control the quality of TPS. The fingerprints of other polysaccharides can be discriminated by similarity matching from the corresponding fingerprints of TPS. Then fingerprint models can accurately reflect the quality and differentiate the TPS from other polysaccharide products. The methodologies described in this thesis would be potentially useful to establish suitable quality control models to control the quality of TPS. In this paper, there are two innovations. One is that the fingerprint of traditional Chinese medicine is used to the quality control of TPS, another is that the multi-fingerprint which is build up by three techniques can be achieved an integrated and comprehensive quality control of TPS.

2. Materials and methods

2.1. Materials and reagents

Twenty-two batches of tea samples and other five samples were collected from various varieties and origins in different region in China plucked in different season. Among them, No. 22 has been stored for seven years, others has been stored one to two years (Table 1). Among them, No. 23 to No. 27 were all collected in Yichang City, Hubei Province, China, No. 23 is Eucommia ulmoides leaf, No. 24 is *Gastrodia elata* Bl, No. 25 is *Ginkgo biloba* leaf, No. 26 is Green tea flower, and No. 27 is Green tea seed. D-Ribose, L-rhamnose, L-arabinose, L-fucose, D-xylose, D-mannose, D-glucose, D-galactose, D-galacturonic acid, D-galacturonic acid, various standard dextrans with different molecular weight (T3, T6, T10, T40, T100, T500, and T1000) and bovine serum albumin (BSA) were purchased from Sigma (MO, USA). Phenol, methanol, trifluoroacetic acid (TFA), potassium bromide (KBr), Coomassie brilliant blue G-250 and sulfuric acids were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). All 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.

2.2. Preparation of crude polysaccharide samples

Each of the dried samples was pulverized and passed through a 30-mesh sieve. The sample powder (150 g) was immersed in 95% ethanol for four times (every 2 h) to remove most of the pigment and extracted with water (1:8, m/v) in bath at 90 °C for two times (every 4 h). Then the extractions were filtered and the combined filtrate was centrifuged at 4500 rpm for 10 min to remove the contaminants. The supernatant was concentrated to 1/20 volume in a rotary evaporator under reduced pressure and precipitated with 75% ethanol. The precipitation was collected by centrifugation and dissolved in warm water, and then the redissolution was centrifuged. The supernatant was concentrated to 1/10 volume in a rotary evaporator under reduced pressure and dialyzed to remove the small molecules. The dialyzed solution was freeze-dried to yield polysaccharides powder.

2.3. Analytical methods of components in polysaccharide samples

The total sugars were determined by the phenol-sulphuric acid method (Monobe, Ema, Kato, & Maeda-Yamamoto, 2008) with D-glucose as standard. The soluble protein (SP) was determined by the Coomassie brilliant blue G-250 method (Tatsuga et al., 2005) with bovine serum albumin as a standard.

2.4. Determination of molecular weight-HPGPC

The molecular weight of polysaccharide samples were determined by gel permeation chromatography. Sample (10 mg) were dissolved in 1 ml of 0.02 M phosphate buffer solution and centrifuged at 15,000 rpm for 10 min, and then passed through a 0.45 μm filter. Twenty-microliter of the supernatant was injected into a Shodex SB-804 HQ GPC column (300 mm × 8 mm) with a Shodex SB-G guard column (50 mm × 6 mm) from Showa Denko K.K. (Tokyo, Japan). GPC system was maintained at 45 °C and eluted with phosphate buffer solution at a rate of 0.3 ml/min. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans with different molecular weight (T3, T6, T10, T40, T100, T500, and T1000).

2.5. UV fingerprint experiment

50 μg/ml of the polysaccharide samples were measured on UV–Vis spectrophotometer in 1.00 cm quartz cell against distilled water as the blank. The scanning range was 200–400 nm at 0.4 nm intervals resulting in 500 points spectra for each sample.

2.6. IC fingerprint experiment

Polysaccharide samples (2 mg) were dissolved in 4 ml of 2 mol/L trifluoroacetic acid solution (TFA) and hydrolyzed at 110 °C for 6 h. The hydrolysate of polysaccharide was evaporated to dry under reduced pressure. Then, TFA was removed by washing with methanol (3 ml) four times in order to remove TFA absolutely. The dried hydrolysate was dissolved with ultra-pure water and diluted to 100 ml, and then measured by diluting 10-fold again (Blumenkrantz & Asboe-Hansen, 1973). IC was used for the identification and quantification of monosaccharide. IC experiment was performed on a Dionex ICS2500 chromatographic system (CA, USA) with a Dionex pulsed amperometric detector equipped with an Au electrode, a Dionex Carbopac PA20 column (150 mm × 3 mm). The temperature was kept at 30 °C and the injection volume was 25 μl. The eluents were NaOH (2 mmol/L) at a flow rate of 0.45 ml/min. L-fucose, D-galacturonic acid, D-glucuronic acid, D-mannose, D-xylose, D-ribose, D-glucose, D-galactose, D-fructose, L-rhamnose, L-arabinose were used as references.

2.7. IR fingerprint experiment

The IR spectra of polysaccharide samples were recorded with a Nicolet 5700 IR spectrometer. The sample was ground with spectroscopic grade KBr powder and then pressed into 1 mm pellets for FT-IR measurement in the frequency range of 400–4000 cm^{-1} (mid-infrared region), equipped with a DTGS detector, was used with a resolution of 4 cm^{-1}. The interference of H_2O and CO_2 was minimized when scanning.

2.8. Data processing

Data were expressed as the mean ± SD for three determinations, and the criterion for statistical significance was $P < 0.05$. Cluster analysis was performed by using statistics software of SPSS (10.0) (Xu & He, 2003). Angle cosine method and correlation coefficient method were used for evaluating the similarity between two spectrograms or chromatograms. Spectrogram or chromatogram can be treated as vector of hyperspace, and the similarity between them can be counted according to angle cosine formula and correlation