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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), antitumor (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

Table 1
Some information of total samples used in this work.

ID	Origin	Variety	Contents of protein (%)	Contents of total sugar (%)	Molecular weight (Da)	Area per centage (%)
1#	Zunyi, Guizhou Province, China	Tai tea Small leaf; autumn green tea	5.00 ± 1.40	59.48 ± 0.13	755,661	6.01
					22,845.4	93.99
2#		Fuding big leaf; autumn green tea	13.05 ± 0.47	51.95 ± 0.12	760,978	16.91
					71,640.3	56.68
					23,086.9	0.35
					5458.46	23.2
					3821.95	2.87
3#		Tai tea small leaf; spring green tea	13.29 ± 2.70	52.36 ± 1.08	745,137	11.87
					25,409.3	84.82
					7841.75	1.2
					3903.2	1.33
4#	Fuan, Fujian Province, China	Fuyun VII; autumn green tea	5.75 ± 1.03	57.31 ± 0.20	760,978	11.17
					36,332.5	88.15
					3790.73	0.68
5#		Fuyun VII; spring green tea	4.34 ± 0.75	57.57 ± 0.04	732,190	13.24
					31,320.6	86.46
					4397.32	0.3
6#	Anxi, Fujian Province, China	Tieh-Kuan-Yin; summer green tea	7.78 ± 3.70	52.63 ± 0.24	751,275	8.79
					30,561.3	88.87
					3817.4	2.35
7#	Ningde, Fujian Province, China	Fuding dahao; autumn green tea	3.05 ± 3.68	55.33 ± 0.24	760,978	13.06
					34,509.9	86.04
					4591.74	0.9
8#		Fuding dabaicha; spring green tea	0.64 ± 0.53	52.38 ± 0.23	751,275	18.04
					30,383.9	81.89
					4051.83	0.01
9#		Fuding dahao; spring green tea	5.75 ± 0.31	55.85 ± 0.20	744,250	14.03
					31,948.3	85.96
					3592.5	0.01
10#	Yichuang, Hubei Province, China	Local small leaf; summer green tea	7.30 ± 2.76	50.21 ± 2.64	756,562	33.43
					71,389.5	37.39
					34,509.9	16.64
					5889.52	4.87
					3733.75	7.67
11#		Fuding big leaf; summer green tea	2.03 ± 0.07	54.89 ± 0.15	772,645	4.93
					15,280.3	95.07
12#		Local small leaf; summer green tea	0.01 ± 2.52	54.76 ± 0.77	750,380	8.81
					28,491.8	90.37
					7268.32	0.03
					4402.57	0.20
13#		Local big leaf; spring green tea	7.13 ± 4.87	56.86 ± 0.20	746,864	15.89
					29,132.2	82.52
					3939.77	1.59
14#	Enshi, Hubei Province, China	Fuyun VII; autumn green tea	2.61 ± 2.88	59.13 ± 1.77	750,380	95.07
					28,095.1	9.62
					7353.41	88.03
					3773.23	0.55
15#		Fuyun VII; spring green tea	1.57 ± 0.09	56.03 ± 0.13	748,646	12.13
					175,151	67.07
16#		Fuding dabai; spring green tea	2.19 ± 0.07	58.58 ± 0.59	753,914	17.94
					166,377	82.06
17#		Local big leaf; spring green tea	2.65 ± 0.13	54.89 ± 0.15	779,884	10.92
					31,393.1	89.08
18#		Local tea; spring green tea	1.96 ± 1.14	54.55 ± 1.86	750,380	20.64
					30,561.3	78.18
					5071.03	1.19
19#	Yidu, Hubei Province, China	Local small leaf; autumn green tea	3.02 ± 1.60	54.67 ± 1.96	747,754	6.53
					26,687.6	89.76
					6035.84	1.28
					3755.54	2.44
20#	Zigui, Hubei Province, China	Local big leaf; summer green tea	3.42 ± 0.11	53.08 ± 0.19	777,154	6.95
					27,414	93.05
21#	Wufeng, Hubei Province, China	Fuyun VII; spring green tea	4.32 ± 0.85	56.40 ± 0.55	768,107	17.85
					30,561.3	81.18
					4366.6	0.97
22#	Yichang, Hubei Province,China	Local small leaf; spring green tea	3.61 ± 0.17	53.16 ± 0.23	765,419	8.78
					23,440.9	91.22
23#		Eucommia ulmoides leaf	5.43 ± 0.12	46.23 ± 0.43	–	–
24#		Gastrodia elata Bl	0.09 ± 0.03	63.10 ± 0.43	–	–
25#		*Ginkgo biloba* leaf	0.85 ± 0.08	58.75 ± 0.12	–	–
26#		Green tea flower	3.64 ± 0.06	62.84 ± 0.26	1,075,450	0.37
					778,954	5.92
					26,969.7	93.7
27#		Green tea seed	6.72 ± 0.08	47.58 ± 0.13	895,217	8.21
					11,252.2	82.5
					3685.89	9.3

coefficient formula. The more near to 1.0 the value of $\cos\theta$ or R is, the more similar are the two vectors. Similarities of two spectrograms or chromatograms were expressed as angle cosine formula ($\cos\theta$).

$$\cos\theta = \frac{A \cdot B}{|A| \times |B|} = \frac{\sum_{i=1}^{n} A_i B_i}{\sqrt{\sum_{i=1}^{n} A_i^2} \times \sqrt{\sum_{i=1}^{n} B_i^2}} \quad [\theta \in (0, \pi/2)]$$

And correlation coefficient formula (R)

$$R = \frac{\sum_{i=1}^{n} (A_i - \bar{A})(B_i - \bar{B})}{\sqrt{\sum_{i=1}^{n} (A_i - \bar{A})^2} \times \sqrt{\sum_{i=1}^{n} (B_i - \bar{B})^2}}$$

where A_i, B_i are the ith variate of two different spectrograms or chromatograms, and $\bar{A}$, $\bar{B}$ are the mean values of them, respectively. n is the number of components (Zhang et al., 2007; Zhou et al., 2011).

3. Results and discussion

3.1. Components of polysaccharide samples

The main nutrition components in 27 samples were determined and shown in Table 1. As shown in Table 1, the content of total sugar and protein of TPS were 50.21–59.48% and 0.01–13.29%, the average content were 55.09% and 4.57%, respectively. There was no significant difference in total polysaccharide content of samples from different areas. However, a notable difference was observed in protein contents. This indicated that a certain similarity had been existed in the total sugar content of TPS which were prepared by the same process but it should not be considered as a main basis for evaluation. Total sugar content was superior to protein content, indicating that the extractions by this method could be recognized as polysaccharides. The total molecular weight distribution of polysaccharide samples ranged from 1.13×10^4 Da to 3.63×10^4 Da (Table 1) of which the mainly area percentage ≥80%, further validating that these were polysaccharide samples by the extracted method of this paper.

3.2. UV spectra fingerprint of polysaccharide samples

The UV spectra fingerprint of polysaccharide samples were shown in Fig. 1. In Fig. 1(a), the UV spectra of 22 batches of tea polysaccharide samples were clearly emerged a stronger absorption peak at 200–250 nm. This showed that the samples might contain unsaturated carbonyl, carboxyl, etc. Obviously these were the structures of polysaccharide. Fig. 1(b) was a referential fingerprint which was based in the average vector of the same wavelength of the 22 UV spectra. Two stronger absorption peaks at 260 nm or 280 nm indicated that the samples containing nucleic acid or conjugated protein. The otherness between tea polysaccharide samples and other five polysaccharide samples were visible from Fig. 1(c). Fig. 1(c) showed the absorptions peak at 200–280 nm between the referential fingerprint of TPS and other five polysaccharide samples had no crucial difference but it was not as well as peak areas. So the difference in the UV spectra fingerprint of TPS could be told by peak areas which were represented concentrations of some compounds from other polysaccharide samples.

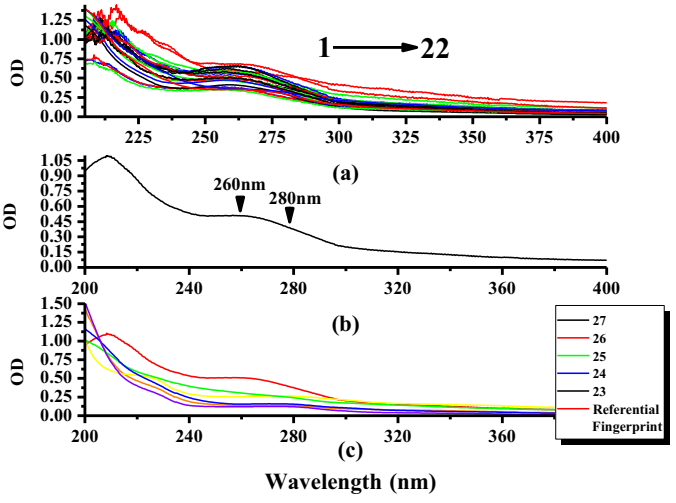

Fig. 1. The UV spectra fingerprint of polysaccharide samples: (a) twenty-two batches of tea polysaccharide samples; (b) referential fingerprint from (a); (c) other five polysaccharide samples and (b).

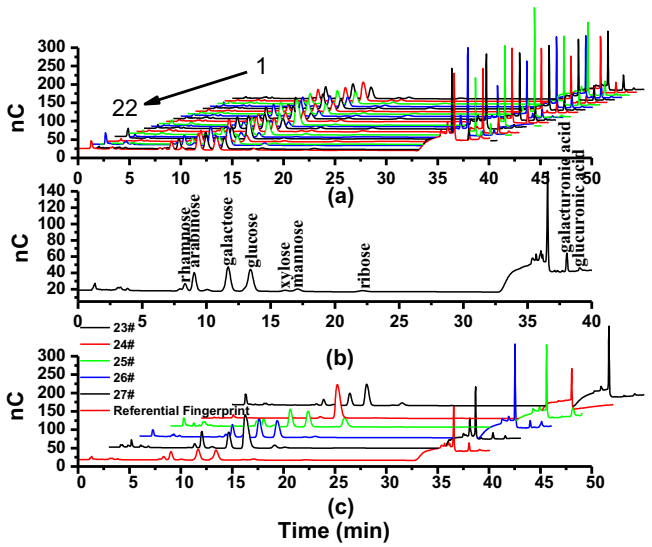

Fig. 2. The IC of polysaccharide samples: (a) twenty-two batches of tea polysaccharide samples; (b) referential fingerprint from (a); (c) other five polysaccharide samples and (b).

3.3. IC fingerprint of polysaccharide samples

The IC fingerprint of polysaccharide samples were shown in Fig. 2. In Fig. 2(a), ion chromatography of 22 tea polysaccharide samples were similar, thinking that there were a certain universality. Fig. 2(b) was a referential fingerprint which was formed from the average vector of the same time of the 22 IC, the basic monosaccharide of TPS were consisted of rhamnose, mannose, xylose, ribose, arabinose, glucose and galactose, GalA, GulA, but the molar ratio of each IC fingerprint was not same, the contents of arabinose, glucose, galactose and GalA were higher than others. The otherness between tea polysaccharide samples and other five polysaccharide samples were visible from Fig. 2(c). Fig. 2(c) showed monosaccharide compositions of different types of plant polysaccharides samples had no significant difference but the 24#. The glucose content of the 24# was abnormally higher than other four plants and 22 polysaccharides samples and had not contained GalA and GulA. And according to Table 1, the contents of total sugar also confirmed the result. In order to compare the IC fingerprint scientifically, calculations carried out using the cosine ($\cos\theta$) values and correlation coefficients (R) of the IC fingerprint of 22 batches of tea polysaccharide samples were shown in Table 2. As followed: cosine values were ranged from 0.8781 to 1.0000, the mean 0.9768, the minimum value was 0.8781; correlation coefficients were ranged from 0.5291 to 1.0000, the mean 0.8802, the minimum value was 0.5291. The cosine values and correlation coefficients of the IC between

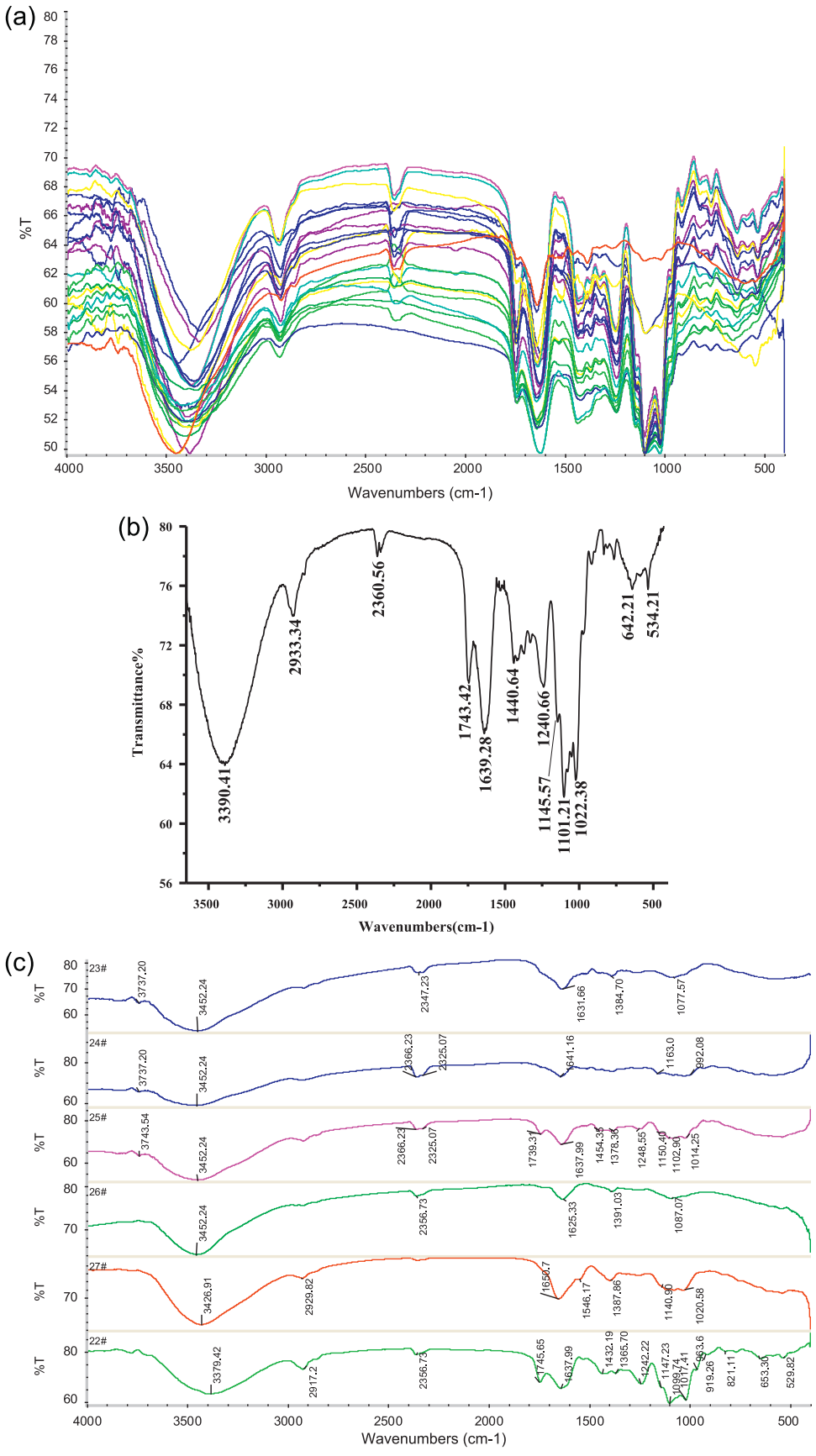

Fig. 3. (a) The IR spectra fingerprint of 22 batches of tea polysaccharide samples. (b) The IR spectra referential fingerprint from (a). (c) The IR spectra fingerprint of other five polysaccharide samples and 22#.

Table 2
The cosine ($\cos\theta$) values and correlation coefficients (R) of the IC of 22 batches of tea polysaccharide samples, upper triangular were $\cos\theta$ and lower triangular were R.

Samples	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#	12#	13#	14#	15#	16#	17#	18#	19#	20#	21#	22#
1#	1.0000	0.9925	0.9844	0.9909	0.9972	0.9867	0.9936	0.9914	0.9888	0.9811	0.9569	0.9790	0.9995	0.9847	0.9894	0.9571	0.9126	0.9971	0.9805	0.9441	0.9931	0.9919
2#	0.9590	1.0000	0.9838	0.9825	0.9877	0.9827	0.9856	0.9764	0.9747	0.9751	0.9831	0.9662	0.9903	0.9799	0.9934	0.9427	0.9045	0.9812	0.9739	0.9408	0.9904	0.9888
3#	0.9101	0.9118	1.0000	0.9864	0.9875	0.9894	0.9871	0.9845	0.9838	0.9846	0.9591	0.9797	0.9838	0.9796	0.9854	0.9626	0.9265	0.9772	0.9838	0.9599	0.9878	0.9901
4#	0.9202	0.9030	0.9485	1.0000	0.9966	0.9971	0.9987	0.9953	0.9975	0.9966	0.9467	0.9934	0.9908	0.9956	0.9788	0.9739	0.9377	0.9898	0.9952	0.9690	0.9815	0.9903
5#	0.9801	0.9270	0.9320	0.9848	1.0000	0.9919	0.9984	0.9971	0.9961	0.9888	0.9495	0.9858	0.9974	0.9921	0.9845	0.9632	0.9235	0.9954	0.9872	0.9578	0.9893	0.9931
6#	0.9511	0.9833	0.9364	0.9032	0.9243	1.0000	0.9939	0.9897	0.9924	0.9981	0.9548	0.9957	0.9864	0.9892	0.9834	0.9816	0.9461	0.9838	0.9981	0.9734	0.9841	0.9930
7#	0.9670	0.9914	0.9932	0.9271	0.9218	0.9647	1.0000	0.9972	0.9978	0.9930	0.9491	0.9876	0.9932	0.9969	0.9804	0.9648	0.9275	0.9911	0.9910	0.9636	0.9838	0.9903
8#	0.9844	0.9402	0.9836	0.9730	0.9108	0.8700	0.9519	1.0000	0.9990	0.9895	0.9306	0.9875	0.9922	0.9910	0.9749	0.9649	0.9252	0.9922	0.9880	0.9607	0.9836	0.9881
9#	0.9941	0,.9878	0.9553	0.9771	0.9855	0.9059	0.8597	0.9370	1.0000	0.9933	0.9298	0.9912	0.9895	0.9934	0.9719	0.9698	0.9326	0.9898	0.9917	0.9666	0.9798	0.9867
10#	0.9605	0.9385	0.9616	0.9883	0.9323	0.9801	0.9076	0.8588	0.8909	1.0000	0.9441	0.9956	0.9811	0.9914	0.9745	0.9787	0.9492	0.9796	0.9993	0.9788	0.9763	0.9873
11#	0.7629	0.6947	0.6992	0.7858	0.8165	0.7881	0.7747	0.8337	0.9412	0.8219	1.0000	0.9264	0.9519	0.9474	0.9808	0.9116	0.8781	0.9359	0.9421	0.9160	0.9618	0.9650
12#	0.6743	0.9722	0.9484	0.9266	0.9299	0.9722	0.9134	0.9604	0.8762	0.8047	0.8774	1.0000	0.9800	0.9807	0.9691	0.9880	0.9497	0.9820	0.9970	0.9700	0.9748	0.9841
13#	0.8739	0.8002	0.8833	0.9382	0.9548	0.9619	0.9153	0.9848	0.9448	0.9036	0.9467	0.9987	1.0000	0.9837	0.9874	0.9563	0.9145	0.9980	0.9805	0.9456	0.9935	0.9919
14#	0.9610	0.9009	0.7802	0.9599	0.9676	0.9547	0.9855	0.9485	0.9610	0.9804	0.8937	0.8966	0.9211	1.0000	0.9706	0.9541	0.9256	0.9808	0.9876	0.9665	0.9710	0.9816
15#	0.8519	0.9349	0.8314	0.9273	0,8628	0.8505	0.8674	0.8973	0.9139	0.9189	0.8876	0.9236	0.9661	0.9450	1.0000	0.9575	0.9120	0.9800	0.9744	0.9430	0.9931	0.9942
16#	0.8054	0.7905	0.7945	0.9645	0.6419	0.9112	0.8626	0.8380	0.8380	0.9280	0.8297	0.8838	0.8269	0.7318	0.8000	1.0000	0.9481	0.9611	0.9818	0.9554	0.9547	0.9699
17#	0.7697	0.5768	0.6445	0.5598	0.7432	0.4921	0.7423	0.6610	0.6250	0.6403	0.7245	0.6120	0.6855	0.6285	0.5219	0.5650	1.0000	0.9153	0.9486	0.9752	0.9162	0.9307
18#	0.5761	0.8194	0.8947	0.9002	0.9892	0.8940	0.7243	0.8808	0.9422	0.9558	0.9503	0.9051	0.9737	0.9414	0.8696	0.8970	0.9837	1.0000	0.9796	0.9417	0.9873	0.9870
19#	0.8804	0.7384	0.9282	0.8622	0.9390	0.8973	0.9807	0.7533	0.9954	0.9510	0.9300	0.9498	0.9879	0.9226	0.9713	0.9028	0.8515	0.8872	1.0000	0.9752	0.9769	0.9865
20#	0.8512	0.6679	0.8802	0.7910	0.6937	0.8233	0.6775	0.8173	0.6299	0.8735	0.8071	0.7748	0.7950	0.8395	0.7541	0.8192	0.7671	0.6709	0.6843	1.0000	0.9465	0.9624
21#	0.9646	0.9508	0.9268	0.8856	0.9359	0.8948	0.9075	0.9031	0.8769	0.8451	0.8566	0.8295	0.9601	0.8453	0.9739	0.7869	0.5533	0.9272	0.8480	0.6666	1.0000	0.9938
22#	0.9549	0.9390	0.9409	0.9415	0.9591	0.9548	0.9456	0.9306	0.9206	0.9193	0.8668	0.8964	0.9496	0.9053	0.9754	0.8664	0.6409	0.9243	0.9141	0.7728	0.9596	1.0000

Table 3
The cosine ($\cos\theta$) values and correlation coefficients (R) of the IR spectra of 22 batches of tea polysaccharide samples, upper triangular were $\cos\theta$ and lower triangular were R.

Samples	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#	12#	13#	14#	15#	16#	17#	18#	19#	20#	21#	22#
1#	1.0000	0.9997	0.9998	0.9966	0.9990	0.9985	0.9982	0.9980	0.9985	0.9994	0.9992	0.9950	0.9981	0.9977	0.9993	0.9994	0.9992	0.9998	0.9966	0.9992	0.9996	0.9980
2#	0.9452	1.0000	1.0000	0.9982	0.9997	0.9993	0.9993	0.9992	0.9993	0.9997	0.9983	0.9970	0.9993	0.9989	0.9985	0.9986	0.9982	1.0000	0.9980	0.9982	1.0000	0.9992
3#	0.9617	0.9911	1.0000	0.9979	0.9996	0.9992	0.9991	0.9990	0.9992	0.9997	0.9987	0.9966	0.9991	0.9987	0.9987	0.9989	0.9986	1.0000	0.9978	0.9985	0.9999	0.9990
4#	0.7239	0.8863	0.8784	1.0000	0.9993	0.9995	0.9992	0.9997	0.9995	0.9985	0.9948	0.9998	0.9994	0.9997	0.9950	0.9952	0.9945	0.9981	0.9998	0.9944	0.9984	0.9997
5#	0.8184	0.9462	0.9394	0.9841	1.0000	0.9999	0.9996	0.9998	0.9999	0.9998	0.9977	0.9984	0.9996	0.9997	0.9978	0.9980	0.9975	0.9997	0.9991	0.9974	0.9998	0.9998
6#	0.6888	0.8676	0.8516	0.9958	0.9732	1.0000	0.9992	0.9997	1.0000	0.9997	0.9975	0.9986	0.9994	0.9999	0.9977	0.9978	0.9973	0.9994	0.9995	0.9973	0.9993	0.9997
7#	0.9182	0.9843	0.9873	0.9303	0.9739	0.9103	1.0000	0.9998	0.9992	0.9989	0.9958	0.9988	1.0000	0.9992	0.9960	0.9963	0.9956	0.9992	0.9987	0.9955	0.9995	0.9998
8#	0.8187	0.9423	0.9386	0.9852	0.9962	0.9749	0.9756	1.0000	0.9997	0.9992	0.9962	0.9992	0.9998	0.9998	0.9964	0.9966	0.9959	0.9991	0.9994	0.9959	0.9994	1.0000
9#	0.6888	0.8676	0.8516	0.9958	0.9732	1.0000	0.9103	0.9749	1.0000	0.9997	0.9975	0.9986	0.9994	0.9999	0.9977	0.9978	0.9973	0.9994	0.9995	0.9973	0.9993	0.9997
10#	0.7428	0.8998	0.8077	0.9933	0.9903	0.9866	0.9403	0.9866	0.9583	1.0000	0.9988	0.9972	0.9989	0.9993	0.9989	0.9990	0.9986	0.9998	0.9986	0.9986	0.9996	0.9992
11#	0.8162	0.9425	0.9340	0.9666	0.9913	0.9583	0.9660	0.9836	0.9913	0.9831	1.0000	0.9926	0.9958	0.9965	0.9999	0.9999	1.0000	0.9986	0.9953	0.9999	0.9981	0.9962
12#	0.7463	0.9003	0.8944	0.9967	0.9856	0.9913	0.9418	0.9883	0.9294	0.9933	0.9695	1.0000	0.9989	0.9992	0.9929	0.9931	0.9922	0.9969	0.9994	0.9921	0.9973	0.9992
13#	0.9049	0.9834	0.9814	0.9441	0.9785	0.9294	0.9953	0.9813	0.9920	0.9472	0.9675	0.9539	1.0000	0.9994	0.9961	0.9964	0.9956	0.9992	0.9989	0.9956	0.9995	0.9998
14#	0.6809	0.8617	0.8488	0.9903	0.9685	0.9920	0.9046	0.9697	0.9735	0.9862	0.9556	0.9918	0.9214	1.0000	0.9967	0.9969	0.9962	0.9989	0.9999	0.9962	0.9990	0.9998
15#	0.8059	0.9367	0.9333	0.9839	0.9930	0.9735	0.9654	0.9915	0.9611	0.9901	0.9842	0.9914	0.9727	0.9789	1.0000	1.0000	1.0000	0.9987	0.9956	1.0000	0.9982	0.9964
16#	0.8418	0.9547	0.9532	0.9739	0.9907	0.9611	0.9771	0.9905	0.9371	0.9796	0.9808	0.9836	0.9840	0.9653	0.9972	1.0000	1.0000	0.9989	0.9957	1.0000	0.9984	0.9966
17#	0.8831	0.9745	0.9744	0.9532	0.9846	0.9371	0.9894	0.9843	0.8907	0.9633	0.9778	0.9660	0.9915	0.9411	0.9880	0.9956	1.0000	0.9985	0.9950	1.0000	0.9980	0.9959
18#	0.9392	0.9881	0.9913	0.9104	0.9564	0.8907	0.9920	0.9596	0.9871	0.9148	0.9459	0.9226	0.9939	0.8817	0.9499	0.9678	0.9823	1.0000	0.9980	0.9985	0.9999	0.9991
19#	0.6175	0.8198	0.8000	0.9777	0.9413	0.9871	0.8653	0.9451	0.9877	0.9684	0.9269	0.9785	0.8880	0.9939	0.9556	0.9383	0.9080	0.8410	1.0000	0.9950	0.9981	0.9994
20#	0.7407	0.9046	0.8895	0.9868	0.9490	0.9877	0.9362	0.9814	0.8684	0.9891	0.9749	0.9924	0.9498	0.9926	0.9892	0.9813	0.9658	0.9166	0.9831	1.0000	0.9979	0.9959
21#	0.9534	0.9919	0.9968	0.8921	0.9810	0.8684	0.9930	0.9498	0.9749	0.9035	0.9418	0.9070	0.9884	0.8635	0.9413	0.9601	0.9794	0.9964	0.8177	0.9024	1.0000	0.9994
22#	0.8187	0.9423	0.9386	0.9852	0.9962	0.9749	0.9756	1.0000	0.9866	0.9866	0.9836	0.9883	0.9813	0.9697	0.9915	0.9905	0.9843	0.9596	0.9451	0.9814	0.9498	1.0000

other five polysaccharide samples and referential fingerprint as followed: 0.8934, 0.9214, 0.8402, 0.8509, 0.7989 and 0.7364, 0.7403, 0.9293, 0.9630, 0.7827, respectively. The results showed that the similarity in monosaccharide compositions of tea polysaccharide samples was existed, and the difference between other polysaccharide samples was also found. IC fingerprints could be used as a basis to distinguish between the different polysaccharide samples.

3.4. IR spectra fingerprint of polysaccharide samples

Fig. 3(a) and (c) was clearly showed substantial overlap of each absorption spectrum of various components, each band was represented an overall overlap of some characteristic absorption peaks of functional groups in the samples. In Fig. 3(c), tea polysaccharide samples 22# was used to compare, the choice is arbitrary. Fig. 3(b) was a referential fingerprint which was based in the average vector of the same wavenumber of the 22 IR spectra, because the IR spectra in Fig. 3(a) were rather similar. Any one in Fig. 3(a) was not representative of the 22 IR spectra as a whole, and therefore mapping Fig. 3(b) on behalf of them. In Fig. 3(b), the relative peaks within the range of 3600–3200 cm^{-1}, 3000–2800 cm^{-1}, 2500–2200 cm^{-1}, 1800–1000 cm^{-1} and 800–500 cm^{-1} were the characteristic absorption peaks of TPS, which obviously differ from the spectra of Fig. 3(c) in range of 1800–1000 cm^{-1} and 800–500 cm^{-1}. Spectrum of each compound has a unique fingerprint region; in this paper, it was ranged from 1400 to 400 cm^{-1}. The cosine ($\cos\theta$) values and correlation coefficients (R) of the IR spectra of 22 batches of tea polysaccharide samples in the range of 1400–400 cm^{-1} were shown in Table 3. As followed: cosine values were ranged from 0.9921 to 1.0000, the mean 0.9985, the minimum value was 0.9921; correlation coefficients were ranged from 0.6175 to 1.0000, the mean 0.9475, the minimum value was 0.6175. The cosine values and correlation coefficients of the IR spectra between other five polysaccharide samples and referential fingerprint in the range of 1400–400 cm^{-1} as followed: 0.9984, 0.9977, 0.9987, 0.9987, 0.9982 and 0.4263, 0.1779, 0.5686, 0.4175, 0.4143, respectively. So we found that the characteristic peaks of tea polysaccharides in this paper were similar and comparison with tea polysaccharides that other five polysaccharide samples were different. The same types of polysaccharides had a highly consistency by both the methods and the different types of polysaccharides with a certain difference in correlation coefficients.

4. Conclusion

The molecular weight of TPS was mainly ranged from 1.13×10^4 Da to 3.63×10^4 Da and validated that these were polysaccharide samples by the extracted method of this paper. Regarded the components, the differences of tea polysaccharose samples and between tea polysaccharose samples and other polysaccharose samples could not be determined significantly by the total sugar content, but could be convicted of the protein content preliminary. Within the 200–250 nm of the UV spectra fingerprint, the variability could be further determined. Zhou et al. (2011) held that the correlation coefficient, included angle cosine and Euclidean distance methods were not appropriate to analyze the characteristic difference of the fingerprints, but they could be used as an auxiliary method for analyzing the similarity of the fingerprint. Also found a similar problem by calculating in this paper, and different techniques should be dealt with an appropriate data handling methods. The correlation coefficient similarities about IC fingerprint of other polysaccharose samples mean was 0.8303, that of tea polysaccharose samples was 0.8802, and the angle cosines means were 0.8610 and 0.9768, respectively. The correlation coefficient similarities about IR spectra fingerprint means were 0.4009 and 0.9475, the angle cosines means were 0.9983 and 0.9985, respectively. So the data handling method of IC fingerprint was matching the angle cosine method and that of IR spectra fingerprint was adopted for the correlation coefficient method. Combined the different monosaccharide compositions of IC fingerprint and the characteristic absorption peaks of IR spectra fingerprint with the results of correlation coefficient and angle cosine analysis, quality evaluation of tea polysaccharide samples were further determined.

The corresponding data analysis methods to the fingerprints could be readily used for the comprehensive evaluation of tea polysaccharide samples. Lucio-Gutiérrez, Coello, and Maspoch (2012) obtained a new strategy for multi-wavelength chromatographic (226,254,280 and 326 nm) fingerprinting of herbal materials by the HPLC–UV–Vis. As well as, a variety of construction methods were managed to establish a multi-fingerprint in this paper, in which a desired accuracy of quality evaluation could be enhanced. Also a multi-fingerprint provided a very precise, flexible and reliable method for quality assessment of tea polysaccharide samples.

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