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A method for tracing the six geographical indication (GI) jujube species by crude polysaccharide characterization

Kai Mao*, Chun Yang*, Weiyang Ding, Jiangning Zhang, Zheng Ye, Jiming Han and Ling Zhang

Abstract

Background: Jujube resources are abundant in China, and Shanxi Province in particular is one of the places where they originated. The most famous 6 geographical indication (GI) jujube species in Shanxi are often masqueraded by nonoriginal jujube species, which seriously undermines the brand image. However, the current national quality standards, which have simple indicators, cannot completely trace the botanical and geographical origin of GI jujubes. Compared with small biomolecules and genes, polysaccharides possess a complicated structure and are sensitive to their geographical location and botanical origin, and these characteristics are important for botanical and geographical traceability. Therefore, we selected the 6 most famous GI jujube species in Shanxi Province, measured and analyzed their crude polysaccharide characterizations, and then selected efficient tracing indicators.

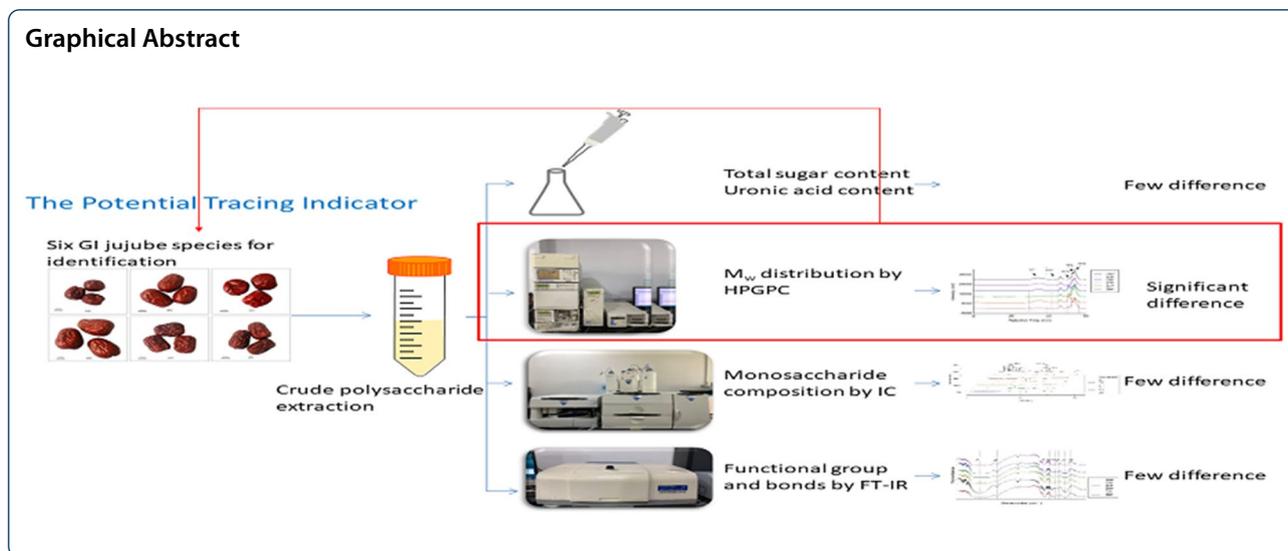
Results: The crude polysaccharides from 6 GI jujube species of Shanxi Province were tested with several parameters, including their purity (the content of polysaccharides), uronic acid content, M_w distribution, monosaccharide composition, functional groups and bonds. In the assays that measured purity and uronic acid content, the purity varied within a very narrow band (96–99%) and presented no negative or positive correlation with uronic acid content. In the experiment that measured the M_w distribution by HPGPC, five peaks (RT1, RT2, RT3, RT4, RT5) were almost observed in 6 jujube species, and the RT3 content (240–250 Da), which showed a significant correlation to the content of RT4 and RT5, exhibited significant differences among 6 jujube species ($RSD = 1.28 > 1.00$). The monosaccharide composition indicated that the polysaccharides from 6 jujube species were rich in glucose (55–75%), arabinose (10–15%) and galactose acid (10–20%), with small amounts of rhamnose, glucosamine hydrochloride, galactose, xylose, and mannose (less than 5%). The FT-IR spectrum showed that crude polysaccharides from six jujube species shared similar functional groups and chemical bonds.

Conclusions: The results above indicated that in the 6 GI jujube species, both homogeneity and differences were observed in the M_w distribution of crude polysaccharides. First, five peaks (RT1, RT2, RT3, RT4, RT5), which represent the M_w of 186,646–285,262 Da (RT1), 4634–17,296 Da (RT2), 240–250 Da (RT3), 98–103 Da (RT4) and 57–64 Da (RT5), respectively, could be detected in all 6 jujube species. Second, the RT3 contents exhibited significant differences among the 6 jujube species ($RSD = 1.28 > 1.00$) and showed a significant correlation with RT4 and RT5. Therefore, the M_w distribution may be treated as a potential distinguishing indicator among the 6 jujube species.

Keywords: Geographical indication (GI), Botanical and geographical origins, Jujube species, Crude polysaccharide, Molecular weight (M_w) distribution, Monosaccharide composition, Functional groups and bonds.

*Correspondence: maokai@sxau.edu.cn; yangchunzls@126.com

Shanxi Institute for Functional Food, Shanxi Agricultural University,
Taiyuan 030001, People's Republic of China



Background

Jujube or Chinese jujube (*Zizyphus jujuba* Mill.) is a traditional Chinese herb that is widely distributed in Asia (especially in northern China), Europe and Australia [1]. As illustrated by animal and cell experiments, positive bioactivities were found in different jujube organs, including the leaves, flowers and fruits [2]. Comparatively, jujube fruit, which is known as Chinese date or red date, contains various bioactive substances, such as vitamin C [3], phenolics [4], flavonoids [5], triterpenic acids [6, 7], and polysaccharides, and possesses a potential nutritional value that is significant. Due to its considerable nutritional and nutraceutical values, jujube fruit is also utilized as a traditional Chinese medicine (TCM) to treat anorexia or fatigue and to nourish the blood, etc. [8].

Previous studies revealed that more than 700 cultivars have been found in China, and Shanxi Province, one of the origins of jujube in northern China, has contributed to the largest amounts of geographical indications (GI) for Chinese jujube species [9]. Among them,

Zizyphus jujuba cv. Banzao, *Zizyphus jujuba* cv. Junzao, *Zizyphus jujuba* cv. Tuntunzao, *Zizyphus jujuba* cv. Hupingzao, *Zizyphus jujuba* cv. Youzao and *Zizyphus jujuba* cv. Muzao are the most famous 6 GI jujube species with the largest export volume. With an increase in demand for jujube, nonoriginal jujube species are often used to impersonate GI species, and this masquerade seriously undermines the brand image.

The establishment of quality standards that are both scientific and national for the 6 GI jujube species is the best way to protect the jujube industry from counterfeit products. However, according to the current Chinese national quality standards for the 6 GI jujube species, the following indicators are employed: appearance, edibility, total sugar, water content and vitamin C; furthermore, the quality requirements of each indicator are similar (Table 1) except for the indicator of appearance, which is also not significantly different to dried jujube fruit (Fig. 1). As a result, we cannot detect small diagnostic differences with the 6 GI jujube species by the indicators mentioned above, and the national quality

Table 1 Four indicators with similar quality requirements in the national quality standards of 6 GI jujube species (with dried jujube fruit)

	Edibility rate %	Total sugar %	Water content %	Vitamin C mg/100 g
Jishanbanzao	≥ 85	≥ 70	≤ 25	≥ 15
Jiaochengjunzao	≥ 90	≥ 60	≤ 25	≥ 10
Pinglutuntunzao	≥ 90	≥ 70	≤ 25	≥ 10
Taiguhupingzao	≥ 92	≥ 60	≤ 25	≥ 15
Baodeyouzao	≥ 90	≥ 70	≤ 25	≥ 10
Linxianmuzao	≥ 90	≥ 70	≤ 25	≥ 15



Fig. 1 Photos of the dried fruits of **a** *Ziziphus jujuba* cv. Banzao, **b** *Ziziphus jujuba* cv. Junzao, **c** *Ziziphus jujuba* cv. Tuntunzao, **d** *Ziziphus jujuba* cv. Hupingzao, **e** *Ziziphus jujuba* cv. Youzao, **f** *Ziziphus jujuba* cv. Muzao

standards of the 6 jujube species cannot effectively protect their legal rights.

More valid indicators should be determined for jujube fruit regarding its botanical and geographical traceability; however, its small biomolecules and genes do not seem to work. Jujube fruits are rich in small biomolecules, such as vitamin C and flavonoids [8], but these biomolecules cannot be used as composite indicators for botanical and geographical traceability due to their simple structures. Although genes are biomacromolecules, genetic identification methods are precise and efficient and 622 unique genotypes can be selected from 962 jujube species by 24 simple sequence repeats (SSR) markers [10], genes are difficult to vary under different geographical environments due to their stable structures.

Polysaccharides, unlike small biomolecules and genes, possess both a complicated structure and sensitivity to geographical location and botanical origin [11], which are important characteristics for botanical and geographical traceability. To date, some work has been performed on tracing methods by polysaccharide characterization, but such studies are very scarce. Dr. Zhao [12] found that the molecular weight (M_w) distribution and monosaccharide composition analysis of polysaccharides could be employed as tracing indicators to classify the 4 species of *Polygonatum* spp. into 2 different groups, reflecting the two different botanical origins.

As a result, we chose crude polysaccharide as the indicator for tracing. Jujube fruits are rich in crude polysaccharide (20% w/w, by dried fruits) [13] and there are many characterization indicators for polysaccharides, such as their purity (the content of polysaccharides), M_w distribution, monosaccharide composition, functional groups and bonds.

For a comprehensive study of polysaccharide characterization, we utilized the high-performance gel permeation chromatography (HPGPC) method, which has a greater sensitivity than that of the high-performance liquid chromatography (HPLC) method for profiling the M_w distribution. The ion chromatography (IC) method was used to measure the monosaccharide composition with 16 monosaccharide standards (which was more than that in most papers because 5 monosaccharide standards were purified by in own laboratory).

To our knowledge, there have been no studies on the botanical and geographical traceability of GI jujube species by polysaccharide characterization. Hence, we selected the 6 GI jujube species from the Shanxi Province mentioned above, measured and analyzed their crude polysaccharide characterizations, and then selected efficient tracing indicators.

Materials and methods

Materials

Six GI Chinese jujube species were collected at the red and ripened stages from their origin regions in Shanxi Province during September to October in 2020 (Table 2).

Crude polysaccharide preparation

The fresh fruits were washed, halved, and oven-dried at 70 °C for 12 h. The dried fruit (50 g) bodies were pulverized into powder that could pass through a 60-mesh sieve and were extracted twice with distilled water (1:10 w/v) at 90 °C (3 h each time). Subsequently, the supernatant was obtained by filtration using a Brinell funnel with medium speed filter paper (pore size of 50 μm) and was concentrated to one-tenth of the original volume at 65 °C by rotary evaporation [14]. The concentrated solution was deproteinated by the Sevag method [15] and precipitated with four volumes of ethanol at 4 °C for 24 h. Then, the crude polysaccharides were obtained by collecting precipitates with centrifugation (5000 rpm for 15 min, 4 °C), dissolution in distilled water, and lyophilization.

The crude polysaccharides of six jujube species (ZBP, ZJP, ZTP, ZHP, ZYP, ZMP) were all extracted with the methods described above.

Total sugar and uronic acid content

The total sugar content was determined by the phenol–sulfuric acid method [16]. The extracted crude polysaccharides of 6 jujube species were subjected to concentrated sulfuric acid and phenol to produce an orange–yellow compound, after which the absorbance at 490 nm was measured and the total sugar content was calculated by combining the standard curve.

The uronic acid content should be determined individually because the phenol–sulfuric acid reagent can also react with uronic acid (or toluene derivatives), thus interfering with the accurate determination of polysaccharide purity [12]. The uronic acid content was determined by the hydroxybiphenyl method with galacturonic acid (GalA) as the standard [17]. The extracted crude polysaccharides were heated to 100 °C in concentrated sulfuric

Table 3 Gradient elution procedure of IC

Time min	A %	B %	C %
0–20	98.8	1.2	0.0
20–30	50.0	50.0	0.0
30–46	0.0	0.0	100.0
46–50	0.0	100.0	0.0
50–85	98.8	1.2	0.0

acid/tetraborate. The product was further reacted with meta-hydroxydiphenyl to form a pink derivative, which was measured at 520 nm.

M_w distribution

The 6 prepared crude polysaccharides (ZBP, ZJP, ZTP, ZHP, ZYP, ZMP) were analyzed by high-performance gel permeation chromatography (HPGPC) on a Shimadzu LC-10A system equipped with a BRT104-102 column (8 mm × 300 mm) and a refractive index detector (RI-10A). The mobile phase (0.05 M sodium sulfate solution) was set at a constant rate of 0.6 mL/min at 40 °C. The samples were prepared as a 5 mg/L solution and were centrifuged at 12000 rpm for 10 min. The supernatant was filtered through a 0.22 μm membrane and then transferred to 1.8 mL injection vials. The standard curve of molecular weight was prepared by dextran standards (M_w 1152, 5000, 11600, 23800, 48600, 80900, 148000, 273000, 409800, 667800 Da) (Sigma). The molecular weights of the samples were determined according to the lgM_w -RT calibration curve of dextran standards.

Monosaccharide composition

The monosaccharide composition was measured by IC with 16 standard monosaccharides, including mannose (Man), manuronic acid (Man A), rhamnose (Rha), galacturonic acid (Gal A), galactose (Gal), glucose (Glc), glucuronic acid (Glc A), arabinose (Ara), xylose (Xyl), fucose (Fuc), glucosamine hydrochloride, N-acetyl-D-glucosamine, D-fructose (D-Fru), D-ribose (D-Rib), galactosamine hydrochloride, and guluronic acid. According to

Table 2 Details on the six GI jujube species from Shanxi Province

Popular name	Species	Codes	Geographical origin	Climatic condition
Jishanbanzao	<i>Ziziphus jujuba</i> cv. <i>Banzao</i>	ZB	Jishan county	Well-lit and little rain
Jiaochengjunzao	<i>Ziziphus jujuba</i> cv. <i>Junzao</i>	ZJ	Jiaocheng county	Well-lit and little rain
Pinglutuntunzao	<i>Ziziphus jujuba</i> cv. <i>Tuntunzao</i>	ZT	Pinglu county	Warm and rainy
Taiguhupingzao	<i>Ziziphus jujuba</i> cv. <i>Hupingzao</i>	ZH	Taigu county	Warm and rainy
Baodeyouzao	<i>Ziziphus jujuba</i> cv. <i>Youzao</i>	ZY	Baode county	Well-lit and rainy
Linxianmuzao	<i>Ziziphus jujuba</i> cv. <i>Muzao</i>	ZM	Lin county	Well-lit and little rain

Table 4 Purity and uronic acid content of crude polysaccharides in each sample

Codes	Total sugar content (Purity) %	Uronic acid content %
ZBP	97.5	11.9
ZJP	96.9	22.1
ZTP	96.1	15.0
ZHP	98.0	16.4
ZYP	98.0	19.5
ZMP	99.0	22.1

the procedure by Wang [18], 10 mg crude polysaccharides (ZBP, ZJP, ZTP, ZHP, ZYP, ZMP) were placed in an ampoule, added to 10 mL TFA (3 M), and then hydrolyzed for 3 h at 120 °C. The acid hydrolysate was transferred into a tube, dried with N₂, and mixed with 10 mL of distilled water. Next, 100 µL of solution was added to 900 µL of deionized water and centrifuged at 12,000 rpm for 5 min. The supernatant was analyzed by an ICS5000 ion chromatograph (Thermo Fisher) equipped with an electrochemical detector. The chromatographic conditions were set as follows: column, DionexCarbopacTMPA2 (3*150); mobile phase, A: H₂O, B: 15 M NaOH, C: 15 M NaOH and 100 mM NaOAc; flow rate, 0.3 mL/min; the gradient elution procedure was performed according to Table 3; and column temperature, 30 °C.

Fourier transform infrared (FT-IR) spectrum

The 6 samples (ZBP, ZJP, ZTP, ZHP, ZYP, ZMP) (2 mg) were pressed into 1 mm pellets with KBr powder (200 mg). Then, they were analyzed by an FT-IR650 spectrophotometer (Tianjing Gangdong Sci. & Tech. Co., Ltd.) in the vibration region of 4000–500 cm⁻¹.

Statistical analysis

The analysis of each sample was repeated three times and the average value was presented in the manuscript and used for calculation of relative standard deviation (RSD) value and correlation analysis. The correlation analysis was assessed with SPSS 19.0 (SPSS, Inc.) by Pearson correlation coefficient method. In addition, the RSD value was calculated using the equation below:

$$RSD = \frac{\sqrt{\frac{\sum_i^n (C_i - \bar{C})^2}{n-1}}}{\bar{C}}$$

where C_i is a monosaccharide's molar percentage (or a M_w content) in one of the 6 GI jujube species; \bar{C} is the average of a monosaccharide's molar percentage (or a M_w content) of the 6 GI jujube species; n equals 6 for 6 species of GI jujube.

Results and discussion

Total sugar and uronic acid content analysis

The phenol–sulfuric acid method and hydroxybiphenyl method were used in this experiment, with glucose and GalA as standards. As a result, the total sugar content of the crude polysaccharides (ZBP, ZJP, ZTP, ZHP, ZYP, ZMP) was in the range of 96–99%, and 10–23% uronic acid was found in each sample (Table 4).

The states above showed that the purity varied within a very narrow band and presented no negative or positive correlation with uronic acid content. Therefore, the purity and uronic acid content might not be suitable tracing indicators.

M_w distribution analysis

Generally, the M_w distribution is an important biochemical parameter to characterize the homogeneity of species [19]. For detection purposes, HPLC is generally used in M_w measurements. However, in our study, the M_w distribution was measured by the HPGPC–RID method, which has a greater sensitivity than that of the HPLC method for profiling the M_w distribution.

As shown in Fig. 2 and detailed in Table 5, five peaks represented different M_w values in the range of 186,646–285,262 Da (RT1), 4634–17,296 Da (RT2), 240–250 Da (RT3), 98–103 Da (RT4) and 57–64 Da (RT5). Obviously, RT3, RT4 and RT5 had M_w values that were characteristic of oligosaccharides or monosaccharides, and the fact that these values differed significantly among the studied samples indicated that M_w is a potential tracing indicator.

In the HPGPC profiles, five peaks (RT1, RT2, RT3, RT4, RT5) were almost observed in the 6 jujube species (the RT3 peak was absent only in ZYP), which might be due to the close relationship of the 6 jujube species.

According to the RSD and correlation analysis (Tables 5 and 6), the RT3 contents exhibited significant differences

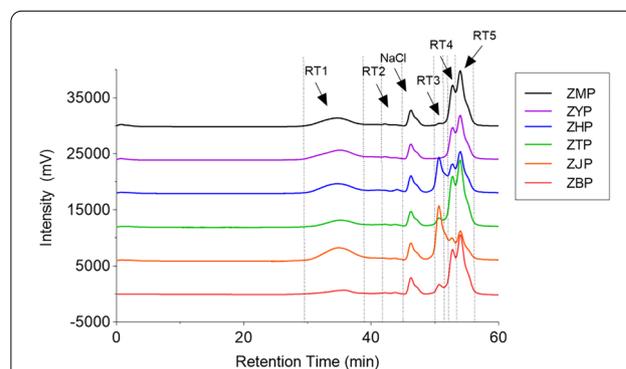


Fig. 2 HPGPC profiles of ZBP, ZJP, ZTP, ZHP, ZYP and ZMP. RT1–RT5 represented 5 peaks in the HPGPC profiles, and the peak at approximately 46 min was the solvent peak of NaCl

Table 5 M_w distribution by the HPGPC method and RSD analysis

RT (min)	Peak area ratio/contents %						RSD
	ZBP	ZJP	ZTP	ZHP	ZYP	ZMP	
RT1 (34.7–35.6)	11.488	33.453	16.458	23.235	36.930	26.647	0.39
RT2 (41.0–44.0)	2.743	2.161	2.079	3.378	2.824	2.183	0.20
RT3 (50.6–50.7)	4.913	35.709	3.772	25.393	0	0.809	1.28
RT4 (52.6–52.8)	28.244	9.225	26.672	15.411	19.389	23.617	0.35
RT5 (53.9–54.0)	52.613	19.453	51.019	32.583	40.857	46.744	0.31

For example, RT1 (34.7–35.6) is retention time 1 (the peak time ranged from 34.7 to 35.6 min); RSD is the relative standard deviation

Table 6 Correlation analysis of polysaccharide contents of different M_w values

	RT1	RT2	RT3	RT4	RT5
RT1					
Pearson correlation	1	− 0.026	0.247	− 0.714	− 0.653
Significance		0.961	0.637	0.111	0.160
RT2					
Pearson correlation		1	0.136	− 0.145	− 0.098
Significance			0.798	0.784	0.853
RT3					
Pearson correlation			1	− 0.851*	− 0.895*
Significance				0.032	0.016
RT4					
Pearson correlation				1	0.993**
Significance					0.000
RT5					
Pearson correlation					1
Significance					

* $P < 0.05$ significant

** $P < 0.01$ highly significant

among the 6 jujube species ($RSD = 1.28 > 1.00$) and showed a significant correlation with RT4 and RT5. The contents of RT3 were negatively correlated with the contents of RT4 and RT5, while that of RT4 showed a significant positive correlation with that of RT5.

Taken together, the result indicated that the M_w distributions of the 6 jujube species not only presented homogeneity with each other but also exhibited significant differences among the 6 jujube species. Therefore, M_w distribution might be a potential tracing indicator for the 6 jujube species.

Monosaccharide composition analysis

Monosaccharide composition can be measured by HPLC–RID, gas chromatography–mass spectrometry (GC–MS) and IC. A previous study showed that uronic acid may not be detected with a normal alkaline mobile

phase by the HPLC–RID method [20], and to our knowledge, up to 7 monosaccharides can be detected by the GC–MS method [21].

At present, ion chromatography is an essential method for determining the most types of monosaccharides, and the method has several advantages, including the lack of uronic acid reduction, simple sample treatment and high resolution. Normally, more than 13 monosaccharides can be detected [18], and the uronic acid content can be determined without a special derivatization reaction. Therefore, ion chromatography was used to determine the monosaccharide composition in this article.

As shown in Fig. 3 and Table 7, the same monosaccharide composition (Rha, Ara, GlcN, Gal, Glc, Xyl, Man, GalA) was detected in all polysaccharides by ion chromatography. The polysaccharides from 6 jujube species were rich in glucose between 55 and 75%, the arabinose and galactose acid contents were almost in the range of 10–20%, and the other 5 monosaccharides were less than 5%.

Generally, a RSD value greater than 1.0 indicates that most of the values in a set of data are significantly different from their mean. Therefore, the RSDs of each monosaccharide content were all less than 0.5 (Table 7), indicating that the monosaccharide composition could not distinguish the 6 jujube species.

FT-IR spectrum analysis

The functional groups of jujube polysaccharides can be characterized by FT-IR spectroscopy [22, 23]. As shown in Fig. 4 and Table 8, the functional groups and chemical bonds of crude polysaccharides from the 6 jujube species seemed roughly similar. Stretching vibrations of -OH were observed at approximately $3600\text{--}3200\text{ cm}^{-1}$ for all the samples, and these vibrations are located in the typical absorption band of polysaccharides.

The absorption peak at $2920\text{--}2935\text{ cm}^{-1}$ indicated that there were C–H stretching vibration in the sugar ring [24–26]. The absorption peaks at approximately $1500\text{--}1740\text{ cm}^{-1}$ and $1050\text{--}1260\text{ cm}^{-1}$ were attributed

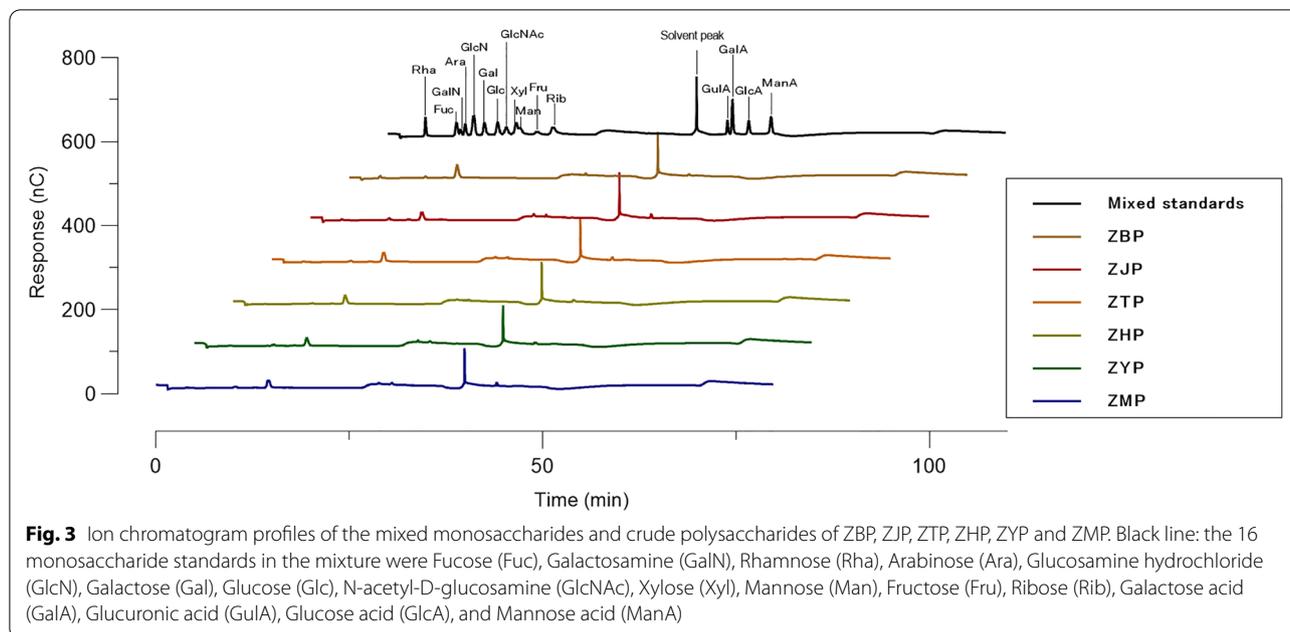


Table 7 Molar contents of different monosaccharides in ZBP, ZJP, ZTP, ZHP, ZYP and ZMP

Monosaccharide content %	ZBP	ZJP	ZTP	ZHP	ZYP	ZMP	RSD
Fuc							
GalN							
Rha	2.5	2.5	1.8	1.9	2.5	1.9	0.16
Ara	7.1	12.6	9.5	9.9	14.9	12.5	0.25
GlcN	0.1	0.4	0.3	0.4	0.4	0.3	0.37
Gal	3.0	3.9	2.3	3.5	4.4	3.2	0.22
Glc	75.7	56.1	70.4	64.8	60.1	62.4	0.11
GlcNAc							
Xyl	0.8	1.5	1.0	1.1	0.6	0.5	0.40
Man	0.7	1.0	0.8	1.0	1.1	0.8	0.17
Fru							
Rib							
GalA	10.1	20.1	14.0	17.4	16.0	18.4	0.22
GuIA							
GlcA							
ManA							

to C=O stretching vibrations and O–H bending vibrations, demonstrating the existence of uronic acid [27, 28]; these results corresponded to the results of the uronic acid assay in this manuscript. The absorption peak of 1635–1640 cm^{-1} was due to bound water [29]. The bands at 1335–1340 cm^{-1} and 1070–1450 cm^{-1} might be attributed to symmetric C=O stretching vibrations and C–O stretching vibrations, respectively.

Peaks at 910–920 cm^{-1} and 864–900 cm^{-1} indicated the existence of pyranose sugar [29] and β -type glycosidic bonds [20, 30, 31].

However, through IR spectroscopy, the C–H bending vibration (1455–1460 cm^{-1}) could be observed clearly in ZBP, ZJP, ZTP and ZMP but not in ZHP and ZYP. This distinction resulted in no significant difference, which was not enough to trace the 6 jujube species.

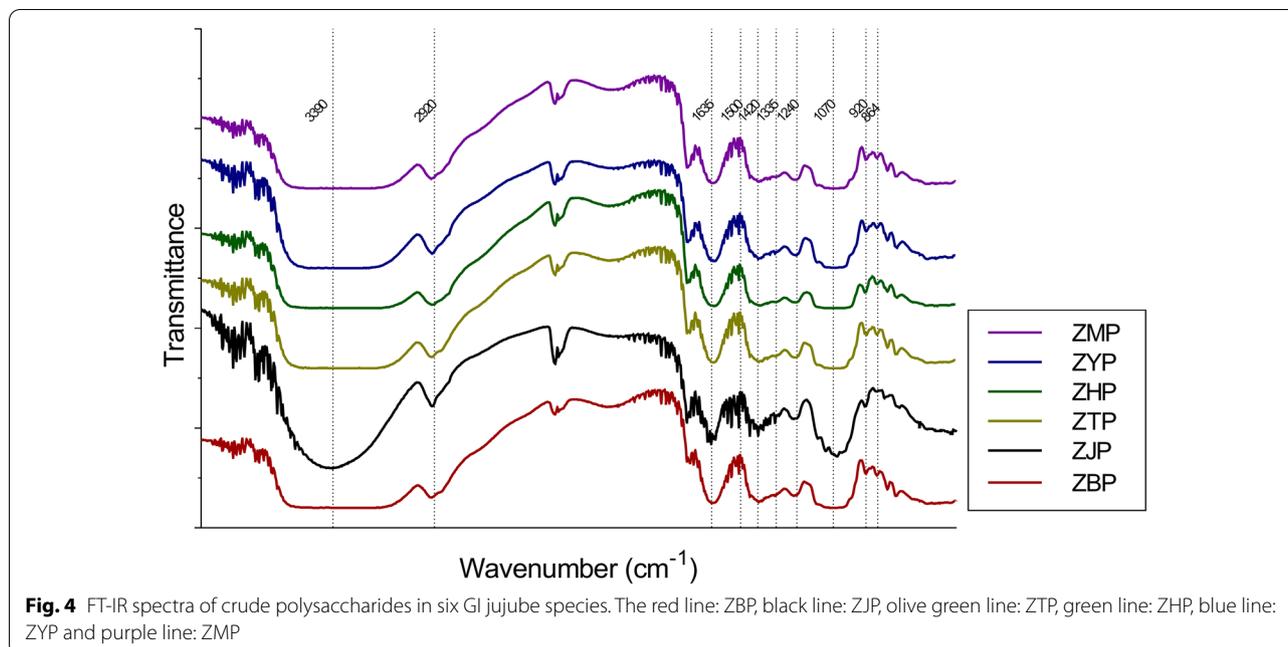


Table 8 Functional groups and chemical bonds of polysaccharides from 6 jujube species

Functional groups or chemical bonds by IR	ZBP	ZJP	ZTP	ZHP	ZYP	ZMP
Typical absorption peaks of polysaccharides (3200–3600 cm^{-1})	✓	✓	✓	✓	✓	✓
Existence of a sugar ring (2920–2935 cm^{-1})	✓	✓	✓	✓	✓	✓
Existence of uronic acid (C=O stretching vibration and O–H bending vibration) (1500–1740 cm^{-1} and 1050–1260 cm^{-1})	✓	✓	✓	✓	✓	✓
Bound water (1635–1640 cm^{-1})	✓	✓	✓	✓	✓	✓
C–H bending vibration (1455–1460 cm^{-1})	✓	✓	✓	×	×	✓
C=O symmetric stretching vibration (1335–1340 cm^{-1})	✓	✓	✓	✓	✓	✓
C–O stretching vibration (1070–1450 cm^{-1})	✓	✓	✓	✓	✓	✓
Existence of a pyranose sugar (910–920 cm^{-1})	✓	✓	✓	✓	✓	✓
β -Configuration of pyranose sugar units (864–900 cm^{-1})	✓	✓	✓	✓	✓	✓

Conclusions

Polysaccharides, as rich contents and abundant bioactive factors in jujube fruit, have been the focus of numerous studies. In a previous study, polysaccharides from different jujube species shared dissimilar characteristics and bioactivities [3]. Therefore, we aimed to identify an efficient indicator to trace the botanical and geographical origin of GI jujube species with crude polysaccharides.

This study demonstrated that the 6 GI jujube species shared similar monosaccharide compositions, functional groups and bonds. In the data regarding the monosaccharide composition, the existence of rhamnose and the abundance of galacturonic acid lead to the inference that pectin might be the main component

for the crude polysaccharides from the 6 GI jujube species [12, 32]. The FT-IR spectrum analysis revealed the existence of bond waters, which might explain the existence of M_w 240–250 Da, which is located between free monosaccharides (150–180 Da) and disaccharides (282–342 Da).

However, the M_w distribution exhibited both homogeneity and significant differences among the 6 GI jujube species. In the HPGPC profiles, five peaks (RT1, RT2, RT3, RT4, RT5) were almost observed in 6 jujube species, and through RSD and correlation analyses, the content of RT3 (240–250 Da), which had a positive and negative correlation to the contents of RT4 and RT5, respectively, was observed to have significant differences.

In summary, the crude polysaccharides of the 6 GI jujube species of Shanxi Province exhibited homogeneity and significant differences in M_w distribution but few differences in purity, uronic acid content, monosaccharide composition, and functional groups and bonds. Therefore, the M_w distribution may be a potential tracing indicator for the 6 GI jujube species.

To explore whether this method can be widely used in tracing most GI jujube species, we should set up a continuously updated and open database of M_w distribution in different jujube species, not just the six GI jujube fruits mentioned in this article.

Abbreviations

GI: Geographical indication; TCM: Traditional Chinese medicine; M_w : Molecular weight; HPGPC: High-performance gel permeation chromatography; IC: Ion chromatography; NMR: Nuclear magnetic resonance; HPLC–RID: High-performance liquid chromatography; GC–MS: Gas chromatography–mass spectrometry; FT-IR: Fourier transform infrared; SSR: Simple sequence repeats; RSD: Relative standard deviation; ZBP, ZJP, ZTP, ZHP, ZYP and ZMP: Crude polysaccharide of *Ziziphus jujuba* cv. *Banzao*, *Ziziphus jujuba* cv. *Junzao*, *Ziziphus jujuba* cv. *Tuntunzao*, *Ziziphus jujuba* cv. *Hupingzao*, *Ziziphus jujuba* cv. *Youzao*, and *Ziziphus jujuba* cv. *Muzao*; RT1–5: Retention time 1–5.

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Author contributions

MK and YC made substantial contributions to the conception and design of the work; DW and ZJ collected the jujube samples; HJ and YZ analyzed and interpreted the M_w data; MK drafted the work; and YC and ZL revised the draft. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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