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MONOSACCHARIDE COMPOSITION IN DIETARY FIBER OF FRUITS IN NORTHERN CHINA BY GAS CHROMATOGRAPHY

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Abstract

Dietary fibers (DFs) contents and monosaccharide composition of northern eight China fruits (apple, pear, peach, apricot, hawthorn, strawberry, mulberry and jujube) were analyzed using GC method. The results indicated that total dietary fibers (TDF) contents in these fruits was between 0.26% (apricot) and 1.17% (peach), SDF (water-soluble dietary fibers)/TDF ranged from 38.5% (apricot) to 86.3% (peach). Galacturonic acid was the main component in SDF, between 33.1% (mulberry) and 91.1% (hawthorn). The insoluble dietary fibers (IDF), had a high content of galacturonic acid (4.5 and 46.5%) and also a considerable contents of arabinose (9.4 and 37.7%), galactose (8.3 and 24.2%), xylose (8.7 and 20.3%) and fucose (8.0 and 23.7%), The per cent content of glucose (Glc) varied between 0.7% (peach) and 15.6% (hawthorn), while mannose and rhamnose took a proportion of less than 6%. The monosaccharide composition of DFs among different fruits exhibited significant difference.

Introduction

The dietary fibers (DFs), in general, refer to the edible carbohydrates and their analogues that cannot be digested by human body, including the indigestible polysaccharides, oligosaccharides, and lignans, etc. (Li and Yang 2007). There is a great deal of research which demonstrated that DFs have many important healthcare physiological effects, such as to promote the intestinal peristalsis, to improve the intestinal micro-ecotope, to lower the serum cholesterol level and blood sugar, and to help lose weight, etc. (Spiller 2001). All these healthcare functions are associated with the botanical source, monosaccharide composition and the bonding structures. Plants-based foods, involving the cereals, beans, fruits and vegetables, etc. act as key sources for the human DFs. The cereal DFs mainly consist of insoluble dietary fibers (IDFs), whose healthcare effect is not as significant as that of water-soluble dietary fibers (SDFs) (Yuan and Zheng 2002). Fruits are a good source of SDF. Indian researchers have studied the SDF/IDF ratios of 25 local common fruits (Ramulu and Rao 2003).

In the case of Chinese fruits, Shen *et al.* (2004) analyzed a polysaccharide isolated from bananas, which was composed of glucose and xylose. Many researchers investigated the composition of : monosaccharides, in apple pectins (Tian *et al.* 2009), hemicellulose in apples (Chen *et al.* 2010), grape polysaccharides (Wang *et al.* 2006), the composition and the changes of polysaccharides of peach fruits at a mature stage (Jin *et al.* 2006), and the composition of hemicellulose polysaccharides of pineapples (Yan *et al.* 2008). In addition, other scholars also studied the composition of polysaccharides of longans (Jia and Li 2009), yellow peaches (Wei *et al.* 2005), jujubes (Peng *et al.* 2008), and kiwi fruits (Chu *et al.* 2007), etc. These scattered studies are of some value for revealing the composition and structure of some specific polysaccharides in the fruits, however, they always focused on the individual polysaccharide present in a single fruit.

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Due to the complex structure of the plant cytoderm, the polysaccharides extracted under a specialized experimental condition are not suitable to infer the overall situation of fruits, which is thus of less significance for assessing the overall healthcare function of the fruit DFs.

During this study gas chromatography was employed with an acid hydrolysis-sugar nitrile acylation method to analyze the composition of monosaccharides in the TDFs of jujubes, in which the highest content of galacturonic acid and the presence of some miscellaneous sugars as arabinose, xylose and galactose, etc. were found. In this study, the composition of monosaccharides in the TDFs, SDFs and IDFs of several major fruits grown in the north of China was analyzed utilizing the well-developed GC analysis method (Lv *et al.* 2010). As a part of a systematic study on the DFs composition of Chinese major fruits, it was aimed to discover the compositional features of monosaccharides in the DFs of different fruits, and offer new vital evidence for disclosing the healthcare function characteristics of different fruits in respect of the polysaccharide composition.

Materials and Methods

The materials of hawthorns (Da-Jin-Xing), apples (Fuji), pears (banana pear), peaches (Kubo), apricots, strawberries, mulberries, and jujube were all purchased from the local fruit market of Baoding, Hebei province of China. The reference standards of monosaccharides, comprising rhamnose (Rha), xylose (Xyl), arabinose (Ara), mannose (Man), glucose (Glc), galactose (Gal), and galacturonic acid (Galr A), were purchased from Sigma (USA). Fucose (Fuc) from Johnson Matthey and other reagents of analytical grade were used.

The GC spectrometer (Agilent, USA) equipped with the SGH-300 high-purity hydrogen (H_2) generator and SGK-2LB low-noise air pump (Beijing Dongfang Jingrui Science & Technology Development Co., Ltd.) were used. TB-215D balance of one over 100000 was from Sartorius AG (Germany). The high-speed pulverizer (Wuyi Standing Tools Co., Ltd.) and the electric heat drum wind drying oven (Tianjin Taisite Instrument Co., Ltd.) were used.

The extraction of the TDFs was consistent with NY-T 1594-2008 method of the U.S. Agriculture Administration (NY-T 2008). The fresh fruits (only the edible parts) were cut into pieces, and then pulped. Later 95% ethanol was added to make the alcohol content reaching 78%, and further extracted by digestion. After being centrifuged, the precipitate was then extracted by 78% ethanol by digestion for another two times. The obtained precipitate was washed by acetone, and then dried in the drying oven after vaporizing the organic solvent. The dried precipitate was grinded and filtered through a 100-mesh screen to prepare the TDFs. An aliquot of 2 g was accurately weighed, and added with 100 ml of water (60°C). The TDFs were subjected to magnetic stirring for 1 hr. We then repeated the previous procedure and dried the precipitate in a hot air oven. This was used for analyzing the content of insoluble polysaccharides (IDF). The pooled supernatant was concentrated and added with four-fold volume of 95% ethanol for further deposition for 1 hr. After being centrifuged, the precipitate was successively washed by 78% ethanol, 95% ethanol, and acetone, respectively. The left portion was then dried to obtain the SDFs.

An aliquot of 50 mg DFs was accurately weighed and added with 5 ml 2M H_2SO_4, allowing to being hydrolyzed on the boiling water bath for 3 hrs. After cooling, the surplus acid was neutralized with 5% $Ba(OH)_2$. The reaction system was then diluted to a 25 ml constant volume. A part of 10 ml was centrifuged with the precipitate reserved for the later use. 2.5 ml of the hydrolysate of the mixed monosaccharide reference standards or the test samples was placed in the stoppered test tubes, and then dried under reduced pressure on a water bath at 60°C. Then 11 mg of hydroxylamine hydrochloride and 0.5 ml of pyridine were added and then heated at 40°C in a drying oven for 40 min. After cooling down to the room temperature, 1 ml of acetic anhydride was

added, allowing the system to react in the drying oven at 90°C for 10 min. The reaction system was evaporated to dryness under reduced pressure at 60°C. The dried residue was dissolved in 1 ml of chloroform, and injected for GC analysis.

Chromatographic column: KB-1 elastic quartz capillary column (0.5 μm × 0.32 mm × 32 m, Agilent); detector: hydrogen flame ionization detector; carrier gas: high-purity nitrogen; flow rate: 1 ml/min; split ratio: 20:1; vaporizing chamber temperature: 280°C; detector temperature: 250°C; temperature programming: initial 180°C kept for 8 min, then ascending at 10°C/min to 220, 220°C kept for 3 min, and then ascending to 240°C at 10°C/min and then kept for 3 min; injection volume: 1 μl.

The gas chromatogram of the derivertized monosaccharides from the standard containing eight different monosaccharides can be seen in Fig. 1. The derivatized monosaccharide from plant polysaccharides was well separated by the present is method. Fig. 2 displays the gas chromatogram of the derivatized monosaccharides from fruit samples.

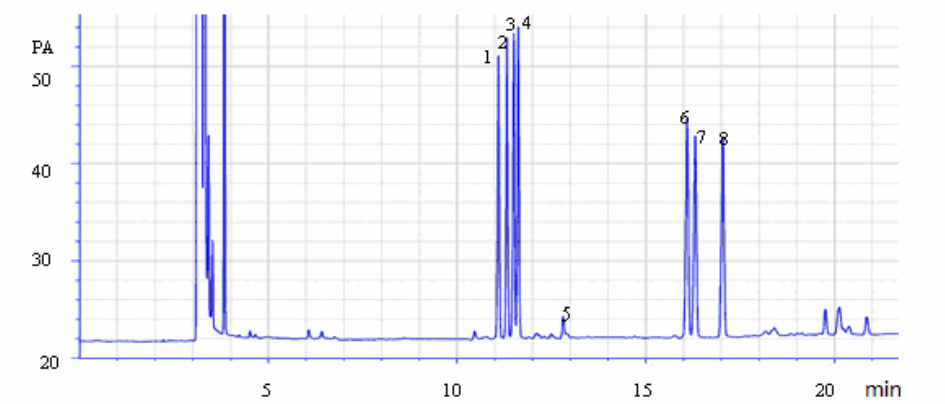

Fig. 1. Gas chromatogram of eight kinds of standard monosaccharide derivatives, 1. L - rhamnose; 2. L - arabinose; 3. D - xylose; 4. D - fucose; 5. D - galacturonic acid; 6. D - mannose; 7. D - glucose; 8. D - galactose.

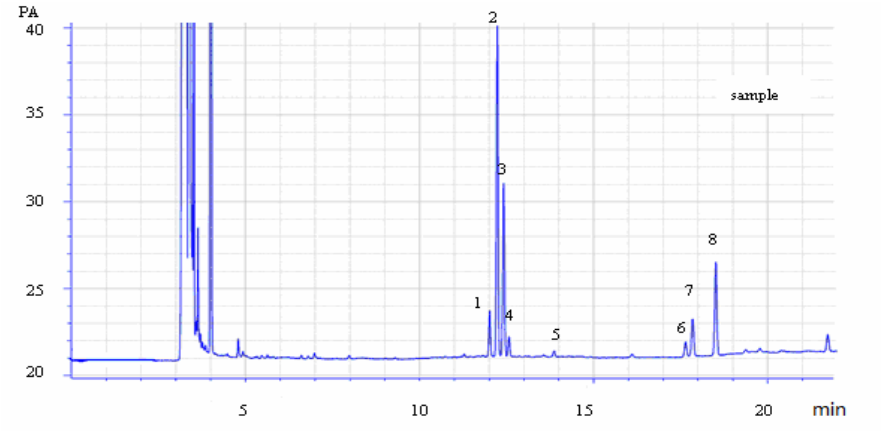

Fig. 2. Gas chromatogram of monosaccharide derivatives of pear TDF 1. L - rhamnose; 2. L - arabinose; 3. D- xylose; 4. D - fucose; 5. D - galacturonic acid; 6. D - mannose; 7. D - glucose; 8. D - galactose.

The mixed reference standard solution of all eight monosaccharides was prepared at the concentration of 10 mg/mL. Ten different volumes of the stock solution, 4, 6, 8, 10, 50, 100 µl, 200 µl, 300, 400, and 1000, were evaporated to dryness at 60°C, and then derivatized following the method described in Section 1.4 and analyzed by the GC method depicted in Section 1.5. The calibration curves were prepared by plotting the peak area (Y-axis) *versus* the mass (mg) of each reference standard (X-axis). The regression equations and correlation coefficients were calculated. The limit of detection (LOD) was determined with the signal to noise ratio set at 3. The LOD values for eight analytes were given in Table 1. The peak areas of each monosaccharide displayed good linearity against the concentration. Additionally, the established GC approach showed high sensitivity based on the LODs. The low amount up to the µg level in the test samples could be measured by use of this method. The LODs can reach the ng level if the dissolution volume of chloroform and the injection volume are adjusted.

Table 1. The detection limit, liner range, regression equation of monosaccharide.

Analytes	Calibration curves	r^2	Linear range(m)	LOD (mg)
Rhamnose	y = 62.486x + 1.6137	0.9995	0.06 - 4	0.002
Arabinose	y = 41.182x + 2.8493	0.9998	0.04 - 10	0.004
Xylose	y = 33.509x + 6.3435	0.9996	0.04 - 10	0.004
Fucose	y = 8.484x + 0.9171	0.9997	0.1 - 10	0.008
Galacturonic acid	y = 18.201x + 1.6213	0.9999	0.06 - 10	0.006
Mannose	y = 32.276x + 4.3496	0.9997	0.04 - 10	0.004
Glucose	y = 64.622x + 4.5216	0.9995	0.06 - 4	0.002
Galactose	y = 31.404x + 4.078	0.9997	0.04 - 10	0.004

Five copies of the mixed reference standard solutions of 100 µl were derivatized according the method of Section 1.4 and analyzed following the method in Section 1.5, and the peak areas were used to calculate the relative standard deviation (RSD) for each monosaccharide. The RSDs of 0.42% for rha, 1.13% for ara, 0.47% for xyl, 1.87% for galr A, 1.71% for fuc, 1.24% for man, 1.36 % for glc and 1.72% for gal, indicated the good precision for this analysis method, repeated double, and shown in Table 2.

Four copies of hawthorn TDF samples were derivatized and analyzed to calculate the contents of each of the eight monosaccharides. The average values were considered as the primary content of each monosaccharide. On the other hand, another four copies of the samples with the same mass were hydrolyzed and evaporated to dryness. Then each 100 µl of the mixed reference standards was added. The contents of each analyte were determined using the same method. The average recovery of each component was calculated as shown in Table 2. Results disclosed that the average recovery of eight monosaccharides ranged from 90.1 to 107.3%, indicating the good accuracy of the established method.

The composition and structure of the DFs are quite complex, and a desirable content analysis approach targeting the DFs is still unavailable, to date. The AOAC recommends the use of the weighing method or enzyme-weighing method since their results are highly consistent with the definition of the DFs (Li and Yang 2007), however, these methods are complicated to implement and fail to give any structural information with respect to DFs. In this study, a chemical analysis

Table 2. The precision and recovery of monosaccharide.

Analytes	RSD of intra-day (n = 6) (%)	Recovery (%)	RSD (%)
Rhamnose	0.42	92.0	0.333
Arabinose	1.13	94.2	0.627
Xylose	0.47	92.3	0.365
Fucose	1.71	90.1	1.74
Galacturonic acid	1.87	107.3	1.92
Mannose	1.24	92.2	1.03
Glucose	1.36	96.1	2.16
Galactose	1.72	98.4	2.27

method was utilized to analyze the contents of each monosaccharide of the DFs, giving the contents of the TDFs, SDFs, and IDFs of each sample. In addition to the DFs content, important information regarding the polysaccharide composition in different fruit samples was obtained as well. However, on the other hand, the results obtained by this method were lower than those acquired by the weighing method mainly due to the fact that the contained nonsugar components like the lignans could not be determined by the used chemical method (Xie *et al.* 2006). Otherwise the carbohydrates in the DFs suffered from certain loss during the hydrolysis process, contributing to the relative lower content results than the actual levels (Jin *et al.* 2006) . Table 3 displays the TDFs, SDFs, and IDFs contents and the ratio of SDF/TDF in eight different fruits. Among these common fruits grown in the northern districts of China, peaches involve the highest level of TDFs up to 1.17% of the fresh weight. Hawthorns and mulberries are also the good sources of the DFs, with the contents 0.76 and 0.79%, respectively. It was also witnessed that these northern fruits have a high proportion of the SDFs, among which peaches and hawthorns possess the ratios of SDF/TDF up to 86.3 and 75%, respectively. The ratios of SDF/TDF of the other fruits are higher than 30%.

Table 3. The total contents of monosaccharides in dietary fiber of eight kinds of fruits (FW, %).

	Strawberry	Hawthorn	Apple	Pear	Peach	Mulberry	Apricot	Jujube
TDF	0.30 ± 0.005	0.76 ± 0.012	0.33 ± 0.002	0.22 ± 0.001	1.17 ± 0.09	0.79 ± 0.021	0.26 ± 0.001	0.34 ± 0.002
SDF	0.15 ± 0.005	0.57 ± 0.002	0.13 ± 0.005	0.10 ± 0.003	1.01 ± 0.009	0.40 ± 0.001	0.10 ± 0.002	0.10 ± 0.001
IDF	0.15 ± 0.004	0.19 ± 0.005	0.20 ± 0.004	0.12 ± 0.006	0.16 ± 0.004	0.39 ± 0.002	0.16 ± 0.005	0.24 ± 0.007
SDF/T	50.0 ± 0.25	75.0 ± 0.20	39.4 ± 0.30	45.5 ± 0.50	86.3 ± 1.30	50.6 ± 0.69	38.5 ± 0.04	29.4 ± 0.01
DF%								

Table 4. The ratio of monosaccharide composition in dietary fiber of eight kinds of fruits (%).

Monosac	Strawberry	Hawthorn	Apple	Pear	Peach	Mulberry	Apricot	Jujube
TDF Rhamnose	4.2 ± 0.02	4.7 ± 0.04	3.4 ± 0.20	3.3 ± 0.05	1.3 ± 0.06	4.2 ± 0.13	4.1 ± 0.02	4.8±0.15
Arabinose	7.3 ± 0.01	11.1± 0.13	12.7±0.76	31.7± 0.11	18.6± 0.11	16.6±0.53	12.1±0.03	22.9±0.28
Xylose	8.1 ± 0.03	9.5 ± 0.26	7.8 ± 0.14	11.1± 0.57	3.2 ± 0.05	13.1 ± 0.38	8.3 ± 0.03	7.4±0.13
Fucose	10.6 ± 0.15	6.9 ± 0.42	7.8 ± 0.16	11.1± 0.26	8.1 ± 0.54	14.6 ± 0.27	7.3 ± 0.18	10.0±0.31
Galac. acid	51.5 ± 0.14	40.6 ± 0.06	53.0±0.78	18.3±0.92	50.0±0.77	19.6 ± 1.11	51.1±0.12	16.9±0.22
Mannose	2.5 ± 0.02	3.7 ± 0.14	1.9 ± 0.10	1.3 ± 0.03	1.2 ± 0.03	2.5 ± 0.08	2.4 ± 0.03	3.3±0.09
Glucose	7.0 ± 0.02	13.5 ± 0.33	5.6 ± 0.28	10.7±0.27	2.2 ± 0.16	13.0 ± 0.07	7.3 ± 0.03	4.6±0.24
Galactose	8.7 ± 0.04	10.2 ± 0.14	7.8 ± 0.15	12.5±0.22	15.4±0.23	16.3 ± 0.54	7.6 ± 0.05	30.1±0.09
SDF Rhamnose	1.6 ± 0.01	1.0 ± 0.01	1.1 ± 0.01	1.4 ± 0.08	0.7 ± 0.01	4.0 ± 0.05	1.2 ± 0.01	5.5±0.09
Arabinose	2.9 ± 0.01	1.7 ± 0.01	2.5 ± 0.01	14.5±0.20	11.7± 0.09	9.1 ± 0.11	3.7 ± 0.03	22.8±0.26
Xylose	2.7 ± 0.01	1.2 ± 0.01	1.8 ± 0.02	1.1 ± 0.02	0.4 ± 0.02	3.7 ± 0.03	1.6 ± 0.01	1.3±0.07
Fucose	2.4 ± 0.05	1.1 ± 0.07	6.3 ± 0.27	5.3 ± 0.34	4.9 ± 0.31	3.9 ± 0.13	2.4 ± 0.15	9.2±0.26
Galac. acid	82.0 ± 0.05	91.1 ± 0.07	83.3±0.25	68.8±0.56	63.8±0.25	33.1 ± 0.21	82.8±0.15	22.5±0.38
Mannose	1.0 ± 0.01	0.6 ± 0.01	0.7 ± 0.01	0.9 ± 0.02	0.9 ± 0.03	2.7 ± 0.02	1.1 ± 0.01	1.6±0.05
Glucose	2.4 ± 0.01	1.7 ± 0.01	1.5 ± 0.01	1.6 ± 0.12	2.8 ± 0.16	13.4 ± 0.07	3.0 ± 0.02	5.0±0.09
Galactose	5.0 ± 0.01	1.8 ± 0.02	2.7 ± 0.02	6.4 ± 0.19	14.9±0.15	30.2 ± 0.14	4.3 ± 0.03	32.1±0.47
IDF Rhamnose	5.2 ± 0.05	5.8 ± 0.08	3.8 ± 0.01	3.6 ± 0.12	2.9 ± 0.07	4.6 ± 0.16	4.2 ± 0.04	4.5±0.07
Arabinose	9.4 ± 0.08	12.0 ± 0.13	13.8±0.06	32.4±0.09	37.7±0.34	19.3 ± 0.07	12.9± 0.11	38.0±0.69
Xylose	14.4 ± 0.12	13.4 ± 0.18	8.7 ± 0.04	20.3±0.12	10.5± 0.11	17.0 ± 0.26	9.3 ± 0.09	7.7±0.08
Fucose	13.0 ± 0.42	9.0 ± 0.69	11.4± 0.37	11.5± 0.25	12.2±0.72	23.7 ± 0.27	8.0 ± 0.47	12.3±0.25
Galac. acid	34.3 ± 0.81	25.0 ± 0.35	46.4±0.32	4.5 ± 0.34	9.7 ± 0.63	13.3 ± 0.67	46.5±0.19	9.0±0.20
Mannose	3.6 ± 0.02	5.2 ± 0.09	2.1 ± 0.02	1.4 ± 0.05	2.0 ± 0.05	3.3 ± 0.10	2.7 ± 0.03	2.1±0.09
Glucose	7.9 ± 0.04	15.6 ± 0.21	5.7 ± 0.04	12.6±0.08	0.7 ± 0.05	5.7 ± 0.29	7.4 ± 0.07	4.4±0.28
Galactose	12.2 ± 0.16	13.9 ± 0.09	8.3 ± 0.03	13.7±0.09	24.2±0.33	13.1 ± 0.58	8.9 ± 0.08	22.0±0.11

*Monosaccharide. ** Galacturonic acid.

Some researchers have used the simplex paper chromatography (Shen *et al.* 2004) or HPLC method (Yan *et al.* 2008, Wei *et al.* 2005, Peng *et al.* 2008, Chu *et al.* 2007) to analyze the monosaccharide composition of the DFs in the fruits. In the present research HPLC method was used for the analysis of monosaccharide composition of the DFs in jujubes (Zhao *et al.* 2004). However, the capillary GC method, having better sensitivity and separation capacity than the HPLC approach, can obtain more reliable analysis results. Thereby the GC method was applied to quantitatively analyze eight monosaccharides of the DFs in eight fruits of the north of China, based

on which the total content ratio of each monosaccharide in versatile DFs to exhibit the compositional difference of the DFs in different fruits distinctively with the results given in Table 4.

As exhibited in Table 4, Galr A plays a vital role in the TDFs of the fruits, in particular as the most important ingredient in the SDFs. Galr A took a proportion up to 91.1% in hawthorns, suggesting that the major components of the SDFs in these fruits are the pectins. In contrast, the Galr A was distinctively low in the IDFs, which should be the pectins conjunct with the hemicelluloses in the cytoderm. Among these pears, peaches, and jujubes, had the lowest levels of Galr A in the IDFs, revealing the characters of the cytoderm structures. In addition, the content of Glc was not quite high, even in the IDFs. Only hawthorns and pears had Glc of 15.6 and 12.6%, which are over 10%, indicative of the low content of cellulose in the cytoderm of the fruits. The contents of the other monosaccharides in the DFs of different fruits showed difference and were discussed in the following.

Strawberries are one of the common berries in the market. The major monosaccharides of the SDFs were Galr A, with a proportion of 82.0%, in contrast to those possessing very low proportions which might be obtained by the hydrolysis of the hemicellulose molecules in the cytoderm. Aside from Galr A, Xyl, Fuc and Gal took a proportion over 10% in the IDFs, suggesting that these monosaccharides are the important structural elements of the cytoderm of strawberries.

Hawthorns are a sort of fruit products as a food and drug unique for China. An extremely high level of Galr A was present the SDFs, compared with the other neutral monosaccharides lower than 2%, indicating the good material for extracting good-quality pectins. In addition to Galr A, Glu, Gal and Xyl were also present in the IDFs of hawthorns.

Apples serve as a typical fruit species in the north of China, which possess a close pro-edge relationship with hawthorns. The composition of the DFs between apples and hawthorns were quite similar as well. Comparatively, apples had higher contents of Galr A, Fuc and Ara, consistent with the differentiated composition of the IDFs. These differences may influence the subtle differentiation of the physicochemical properties between these two different fruits. Aside from the high content of Galr A which might correlate to the degree of maturity, the content of Glc was quite low, which might be consistent with the organization structure of the hemicellulose in apples.

Pears are another sort of representative fruits mainly grown in the north of China. Besides Galr A, the SDFs possess high levels of Ara and Gal, which are difficult to obtain by the degradation of the hemicellulose. However, for the IDFs, Ara and Xyl were of high abundance. Additionally, certain contents of Gal, Glu, and Fuc, were present in the IDFs, in contrast to the rather low content of Galr A. These data indicated that, despite belonging to the same family Rosaceae, remarkable difference existed in the structure of their cytoderm.

Peaches are another sort of bulk fruits for the north of China. Aside from the Gal of high content, the SDF composition characters of peaches were quite similar to pears. However, the most prominent feature of the IDFs composition was the content of Ara and content of Gal which were the highest among the analyzed fruits. Compared with pears, peaches possessed the similar low abundance of Galr A. In addition, the content ratio of Glc no matter in the SDFs or IDFs was the lowest, indicating that the DFs of the peaches may be the good culture medium for the probiotics in the gut and thus providing the theoretical basis for the saying "Peaches benefit the health of the humans". Above data are in accordance with the report of Jin *et al.* (2006) regarding the composition of monosaccharides in the polysaccharides during the process of growing mature for the peach fruits.

Mulberries despite not belonging to the bulk fruits, as a traditional healthcare food. Results obtained indicated the obvious difference in the DFs between mulberries and other fruits as Glc took a proportion as high as 30.2% which is the highest among all the test samples. However, the

content ratio of Fuc was also the highest in the IDFs. Besides Ara, Xyl and Gla also occupied high ratios. However, the ratio of Galr A was not very high.

Apricots have comparatively modest ratios of the monosaccharides in the DFs. Galr A was very rich in the SDFs of apricots and Fuc was very rare in the IDFs.

Conclusions

The used analysis method by acid hydrolysis-sugar nitrile acylation derivatization-capillary GC was sensitive, high-efficiency, and accurate, giving the reliable analysis results for the monosaccharide content in the DFs of the fruits. By means of this method, the contents and monosaccharide composition of the DFs in eight common fruits in the north of China were analyzed. Results obtained showed that the SDFs occupy a high proportion, of which Galr A was the highest. These fruits are the good materials for extracting the DFs. Different fruits have different monosaccharides in the DFs, which not only reveals the structural features of the cytoderm, but also offers important information regarding the beneficial healthcare values for the DFs of these fruits.

This study presents partial investigational results with respect to the composition of the fruit DFs. Further studies will be performed to investigate the spatial structures of the polysaccharides in different fruits and their variations during the growing, storage and preparation, which not only relate to the fruit quality, but also have respect to the healthcare function and the exploitation/utilization of the source for the bioactive components.

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