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DETERMINATION OF POLYSACCHARIDE CONTENTS IN CHINESE FRUIT BY GAS CHROMAROGRAPHY

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ABSTRACT

This paper analyzes fifteen common fruits in northernChina,such as five varieties of apples, pears, peaches, and melons,as well as three varietiesof grapes, for non-digestible polysaccharides (NDPS) content. Through the use of the acid hydrolysis-GC method, the results show that the total polysaccharides (TPS)content of the fruits ranges from 0.08% (grape) to 1.30% (kiwi fruit). Furthermore,the content of water-soluble polysaccharides (SPS) in TPSrange from 17.7% (kiwi) to 86.3% (peach). The content of non-digestible polysaccharides (NDPS)in the various fruits is also significantly different.

Keywords: Fruit, non-digestible-polysaccharides, polysaccharide, gaschromatography.

INTRODUTION

Non-digestible polysaccharides (NDPS) are the main constituents of dietary fiber. Studies have shown that NDPS may help to decrease the risk of health problems such as constipation, diabetes, cardiovascular diseases, diverticulosis, and obesity (Spiller, 2001). The health-promoting functions of NDPS are associated with their plant sources, monosaccharide compositions, and bonding structures.Earlier research regardingNDPS focused on herbs and other non-edible plant materials. Studies on edible sources covered cereals and legumes. However, fruits are another important resource of NDPS, and are also soluble in water (Yuan and Zheng,2002).

The total dietary fiber (TDF) content of pineapples,hawthorns, mandarin oranges, apples, guavas, kiwis, apricots, and pearshave been determined using the non-enzyme-weight method (Wang *et al*.,2009). Indian scholars have used the AOAC method to discern the dietary fiber (DF) content of 25 kinds of Indian fruits (Ramulu and Rao, 2003). However, strategies used in previous studies are not applicable to the analysis of the NDPS content of different fruits in China. Thus, the purpose of the present study is to determine the contents of NDPS in fruits from northern China with a reliable method.

MATERIALS AND METHODS

Materials and reagents: Apples (Fuji, Meixia, Meiba, Rattan wood, Gala), pears (Snowflake, Banana pear, pear, Tropical pear, Golden pear), grapes (Kyoho, Milk, Grapes, Red grapes), peaches (Okubo, Jinghong, Well jade, Oulu, Peach), apricots, plums, persimmons (Disc), jujube (Fuping jujube), hawthorns (Large gold star), kiwis, strawberries, mulberries, melons (Watermelon, Cantaloupe, Feng lei, Sweet melon, Elizabeth), oranges, and bananas were purchased from a fruit market in Baoding, Hebei Province, located in northern China. The sugar standards, rhamnose, xylose, arabinose, mannose, glucose, galactose, and galacturonic acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO,-USA). Fucose was purchased from Johnson Matthey Co. All other reagents were of analytical grade.

Instruments: Gas chromatography (GC) analysis was carried out on an Agilent 6890 GC-equipped with a SGH-300 type high purity hydrogen generator (Oriental essence Yuan Technology Co., Beijing, China) and a SGK-2LB low noise air pump (Oriental essence Yuan Technology Co., Beijing, China). An analytical balance (TB-215D; Sartorius Co., Germany),an electric blast oven (101-2AB; Taisite Instrument Co., Tianjin, China), and a high-speed smash medicine machine (Standing Tools Co., Wuyi County, China) were purchased.

Sample preparation: The preparation of the total polysaccharides (TPS) was

followed by 1594-2008 (NY-T 2008). Each sample was cut into small pieces that were then blended into a pulp. Four volumes of 95% ethanol were added to the pulp, and the mixture was left overnight. The precipitate was collected by centrifugation and respectively washed twice with 78% ethanol, washed once with acetone, and then dried. After being put through a 100 mesh screen, the TPS powder was obtained. The powder had a particle size of less than 1.0 mm. Each sample of TPS (2.0 g) was dissolved in 100 mL warm water (60℃). The solution was stirred for 1 h, then centrifuged before being washed with 78% ethanol, 95% ethanol, and acetone. 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 (IPS). The supernatant was concentrated and then added to 4 volumes of 95% ethanol. The content was kept at room temperature for 1 h, then centrifuged and washed with 78% ethanol, 95% ethanol, and acetone. It was then dried for preparing soluble polysaccharides (SPS) from fruit samples.

Preparation of acetylation reaction of nitrile derivatives of sugar: Each TPS sample of 50 mg was dissolved in 5 mL sulfuric acid (2 mol/L) and placed in boiling water for 3 h. After cooling to ambient temperature, the content was neutralized by the excess sulfuric acid with 5% Ba $(OH)_2$, and transferred into a 25 mL volumetric flask. It was then filled with distilled water. The portion of content (10 mL) was taken and centrifuged (3000 r/min, 10 min) using. The standards and prepared samples (2.5 mL) were dried using a vacuum evaporator at 60℃. Then 11 mg hydroxylamine hydrochloride and 0.5 mL pyridine were mixed in a stoppered tube and then put into a hot air oven for 40 min at 90℃. After cooling to room temperature, acetic anhydride (1.0 mL) was added to the samples, which were then put into a hot air oven at 90℃for 10 min. Finally, the derivatives were vacuum-dried at 60℃ and dissolved with 1.0 mL $CHCl_3$.

GC conditions: The GC separation was performed using a fused silica capillary column KB-1 (0.5 μm ×0.32 mm×30 m; Thermo scientific, New York, USA) and a flame ionization detector (FID). High purity nitrogen served as the carrier gas. The column flow rate was set at 1 mL/min with a split ratio of 20:1. The injector temperature was 280℃ and the detector temperature was 250℃. The GC conditions were programmed as follows: initial oven temperature 180℃, held for 8 min, followed by a temperature ramp of 10℃/min to 220℃, held for 3 min, then followed by a temperature ramp of 10℃/min to 240℃, and held for 3 min.

Calibration curves and limits of detection: The standard solutions of rhamnose, arabinose, xylose, fucose, mannose, glucose, galactose, and galacturonic acid were prepared at 10 mg/mL. The solutions were stored at 4℃ and then diluted into 4, 3, 2, 1, 0.5, 0.1, 0.08, 0.06, and 0.04 mg/mL solutions. The standards were then dried by a vacuum evaporator at 60℃and then analyzed by the GC. The calibration curve of each standard was generated using the different concentrations. The correlation coefficient was determined using a linear regression model. The limits of detection (LODs) under the present chromatographic conditions were determined by diluting the standard solution when the signal-to-noise ratios (S/N) of the samples were about 3.

Precision and accuracy: Intra-day variations were chosen to determine the precision of the developed method and were investigated by obtaining eight samples in six replicates during a single day. Variations of the peak area were taken as the measures of precision and expressed as percentage relative standard deviations (RSD).

Therecovery test was used to evaluate the accuracy of this method. The test involved adding accurate amounts of the eight standards separately into a certain amount of dietary fiber content of hawthorns. The spiked samples were then extracted, processed, and quantified according to the above methods. Four replicates were used for the test. The average recovery percentagewas calculated by the formula: recovery (%) = (observed amount - original amount)/spiked amount × 100.

RESULTS AND DISCUSSION

Chromatogram of standard and samples (Figure 1 and Figure 2): The gas chromatogram of the derivertized monosaccharides from the standard containing 8 different monosaccharides can be seen in Figure 1. The derivatized monosaccharide from plant polysaccharides was well separated by the present method. Figure 2 displays the gas chromatogram of the derivatized monosaccharides from fruit samples.

GC method validation: The proposed GC method for quantitation analysis was validated through linearity, LOD, precisions, and accuracy. All standard calibration curves have strong linearity (r^2> 0.9995) in the test ranges (Table 1). Additionally, the overall LODs are between 0.002 and 0.008 mg. The method shows a wide range of linearity (0.04-10.0 mg) and high detection sensitivity (about 2.0 ng) (Table 1). These properties completely meet the requirement of determination.

The precision results for the dietary fiber of hawthornsreveal that the RSD values of intra-day variations of the 8analyteswere less than 2% (Table 2). The developed method was reproducible with good precision.

The results of the recovery experiments showed that all of the overall recoveries were between 90.1% and 107.3% with RSD less than 2.27% (Table 2).This demonstrates that the developed method was reliable and performed well in terms of both recovery and accuracy.

Analysis of Polysaccharide content: The AOAC method for determining the dietary fiber content in generalwas complicated(Li and Yang,2007). Furthermore, the resulting values may include those derived from lignins and the coexistence of inorganic constituents, meaning

that they would not provide information regarding the structure of polysaccharides. A chemical method was performed in the present study to analyze the content of each monosaccharide from the polysaccharide matrix in plant cells. The TPS, SPS, and IPS from each fruit sample were properly extracted and determined by the suggested method. This method not only measures the polysaccharide content, but also reveals the polysaccharide composition of different samples. However, the result of each monosaccharide content was lower than that by the weight method.In this condition, lignin and other non-carbohydrate parts could not be detected (Xie *et al*., 2006). In addition, the polysaccharides also underwent a loss due to the hydrolysis process, which could lower the results of the actual content (Jin *et al*., 2006).

The results of the TPS, SPS and IPS content and the ratio of SPS/TPS of the fifteen different varieties of fruits by the suggested method can be found in Table 3. In the fifteen varieties of fruit, kiwis showed the highest TPS content (1.30% in fresh weight)(Table 3). Furthermore, thepeaches (1.17%) and persimmons (1.06%) were also shown to be a rich source of polysaccharides. We can thus conclude that the SPS was the predominant proportion in the fruit from northern China. The ratios of SPS/TPS in peaches and hawthorns accounted for 86.3% and 75%, respectively, and those of grapes, bananas, persimmons, melons were all over 65%. In addition to kiwis, plums and jujubes were below 30%.The ratios of SPS/TPS of the remaining fruits were more than 30% (Table 3), suggesting greater health benefits to eating these fruits.

Eachmonosaccharide content obtained by thesuggested method was lower than that obtained by the enzymatic-gravimetric method or the weight method, both of which were lower than what was reported in the Indian study. At the same time, the ratio of SPS/TPS of strawberries, bananas, pears, apples, melons, peaches, jujubes, and grapes were higher than that reported in the Indian study. Plumes were also lower than what the Indian researchers reported. Except for the different analysis methods, the varying species, geographical locations, agro-climatic, soil, and storage conditions may have contributed to these differences, which also reflect finedistinctions in different fruits.

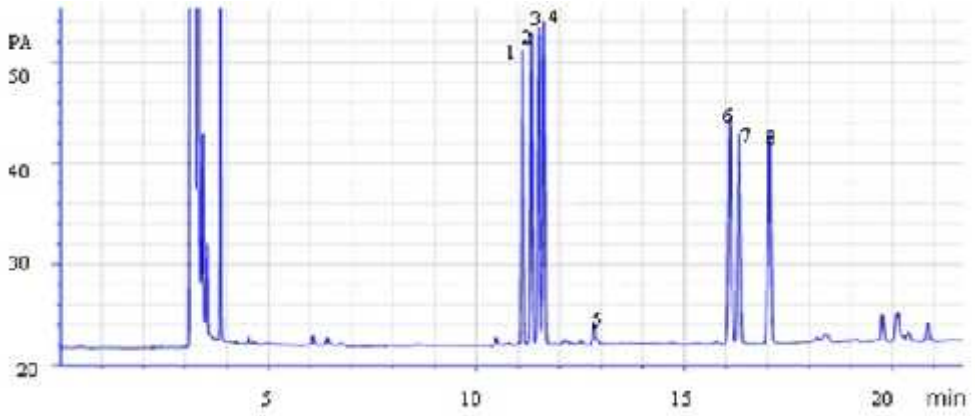

Figure 1.Gas chromatogram of derivertized monosaccharides from the standard1.L-Rhamnose; 2.L-Arabinose; 3.D-Xylose; 4. D-Fucose; 5.D-Galacturonic Acid; 6.D-Mannose; 7.D-Glucose; 8.D-Galactose

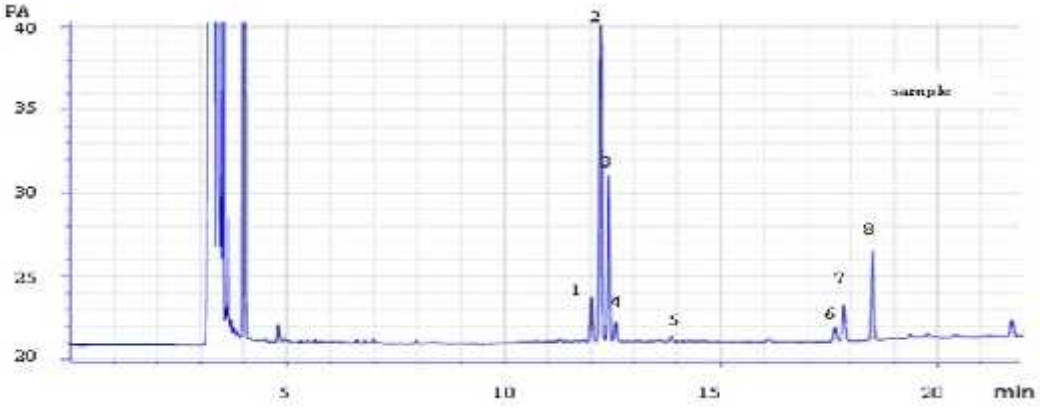

Figure 2.Gas chromatogram of derivertized monosaccharides from pear TPS 1.L-Rhamnose; 2.L-Arabinose; 3.D-Xylose; 4.D-Fucose; 5.D-Galacturonic Acid; 6.D-Mannose; 7.D-Glucose; 8.D-Galactose

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

Analytes	Calibration curves	r^2	Linear range(mg)	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

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

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

fruit	Variety	TPS	SPS	IPS	SPS as % TPS
strawberry	Star	0.30 ±0.005	0.15±0.005	0.15±0.004	50.0±0.25
hawthorn	Largegold star	0.76±0.012	0.57±0.002	0.19±0.005	75.0±0.20
Apricot	Gold sun	0.26±0.001	0.10±0.002	0.16±0.005	38.5±0.04
Pear	Banana pear	0.22±0.001	0.10±0.003	0.12±0.006	45.5±0.50
	Golden pear	0.40±0.007	0.20±0.003	0.20±0.005	50.0±0.02
	Pear	0.31±0.004	0.12±0.001	0.19±0.001	38.7±0.02
	Tropicalpear	0.41±0.002	0.12±0.001	0.29±0.002	29.3±0.03
	Snowflake	0.40±0.001	0.29±0.001	0.11±0.002	72.5±0.01
Mulberrie	Black pearl	0.79±0.021	0.40±0.001	0.39±0.002	50.6±0.69
	Red grapes	0.10±0.0001	0.050±0.0012	0.050±0.0007	50.0±0.05
Grape	Milk grapes	0.18±0.002	0.11±0.001	0.07±0.001	61.1±0.03
	Kyoho	0.10±0.002	0.040±0.0001	0.060±0.0006	40.0±0.06
Banana	Hainan banana	0.75±0.001	0.53±0.001	0.22±0.007	70.7±0.03
Persimmon	Disc	1.06±0.006	0.69±0.003	0.37±0.006	65.1±0.02
Kiwi	Su hong	1.30±0.011	0.23±0.004	1.07±0.006	17.7±0.02
	Watersmelons	0.078±0.0013	0.034±0.0011	0.044±0.0003	43.6±0.05
	Cantaloupe	0.16±0.001	0.05±0.001	0.11±0.005	31.3±0.06
melon	Sweet melon	0.20±0.001	0.13±0.0003	0.070±0.0008	65.0±0.04
	Fengli	0.13±0.001	0.078±0.0003	0.052±0.0004	60.0±0.06
	Elizabeth	0.18±0.001	0.13±0.0001	0.05±0.0006	72.2±0.02
jujube	Fuping jujube	0.34±0.002	0.10±0.001	0.24±0.007	29.4±0.01
	Okubo	1.17±0.09	1.01±0.009	0.16±0.004	86.3±1.30
	Peach	0.83±0.004	0.63±0.002	0.20±0.006	75.9±0.05
peach	Well jade	0.89±0.002	0.28±0.001	0.61±0.004	31.5±0.05
	Oulu	1.29±0.001	0.89±0.003	0.40±0.003	69.0±0.02
	Jinghong	0.52±0.007	0.37±0.003	0.15±0.001	71.2±0.03
	Fuji,	0.33±0.002	0.13±0.005	0.20±0.004	39.4±0.30
	Meixia	1.15±0.002	0.27±0.001	0.88±0.001	23.5±0.06
apple	Meiba	0.76±0.001	0.24±0.001	0.52±0.001	31.6±0.05
	Rattan wood	1.04±0.008	0.32±0.001	0.72±0.002	30.8±0.05
	Gala	0.66±0.003	0.30±0.005	0.36±0.001	45.5±0.02
plum	Ruby	0.97±0.005	0.22±0.002	0.77±0.003	22.7±0.01
orange	Sweet orange	0.43±0.003	0.21±0.003	0.22±0.005	48.8±0.01

Conclusions: The GC method used in this experiment was sensitive, efficient and accurate. This method has shown to obtain more reliable results for DPS content. Based on the polysaccharide content and monosaccharide composition of the different varieties of fruits determined by the proposed method, it can be seen that fruit is a very rich dietary fiber material, of which the ratio of water-soluble polysaccharide is high in the fruit polysaccharides. Because lignin and other components were not included by the proposed method, the results of the total content of the major eight monosaccharidesis lower than that by the weight method from the AOAC. The data showed the relative polysaccharide content of fruits from northern China, including fruit nutritional value, which could be used as a reference for future study. Based on these data regarding the monosaccharide content in fruits, we further obtaina different percentage of monosaccharides in fruit polysaccharides.

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