Korean J. Crop Sci. 50(5): 319~324(2005)

Determination of Soluble Carbohydrates in Soybean Seeds

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ABSTRACT : This study was conducted to identify the soluble carbohydrates in soybean seeds using on-line HPLC-RID-ES/MS and HPLC behavior, and to determine their contents for high quality soybean breeding. The monosaccharide (glucose) and three oligosaccharides (sucrose, raffinose, and stachyose) were identified in Korean soybeans by their chromatographic behavior and results of on-line HPLC-RID-MS with Electrospray Ionization mode. On the basis of HPLC with a RID detector, the 32 Korean major soybeans contain 0.37 ± 0.26% glucose, 4.55 ± 0.91% sucrose, 1.19 ± 0.19 % raffinose, and 2.72 ± 0.37% stachyose on a dry basis. In 468 soybean germplasms, the ranges of glucose, sucrose, raffinose, and stachyose were 0.03 - 0.98%, 2.33 - 6.96%, 0.08 - 1.87% and 0.75 - 3.18%, respectively. Among 500 soybean samples, oligosaccharide contents of 32 Korean major cultivated soybeans and 468 soybean germplasms were varied 5.83 - 10.06% and 3.66 - 10.32%, respectively. The composition of glucose, sucrose, raffinose, and stachyose in soluble carbohydrates of 500 soybean samples were 2.07 ± 1.75%, 58.01 ± 5.82%, 10.13 ± 2.28% and 29.80 ± 4.54%, respectively. Sucrose appeared to be most prevalent in soybean soluble carbohydrates.

Keywords: soybean, soluble carbohydrate, HPLC-RID-ES/MS, oligosaccharide

Soybean (*Glycine max*), a legume crop, is an excellent food and feed source all over the world, with a total production in 2001 of about 130 million tons (American Soybean Association, 2001). The primary constituents of its seed are protein, oil, and soluble carbohydrates (Kim *et al.*, 2005). On the average, protein and oil together constitute about 60% of dry soybeans. The remaining dry matter is composed of mainly carbohydrates (about 35%) and ash (about 5%) (Liu, 1999).

The term carbohydrate, also known as saccharide, refers to a class of compounds with general chemical formula $C_n(H_2O)_m$ and their derivatives. It includes simple sugars (mono- and disaccharides), oligosaccharides, and polysaccharides. Polysaccharides are also known as complex carbohydrates, commonly including starch and some cell wall structural compounds (cellulose, hemicellulose, and pectin). Based on solubility in water, carbohydrates can also be grouped into water soluble and water insoluble. Because water solubility decrease as the number of sugar units increase, in general, compounds consisting of one to several sugar units are water soluble whereas polysaccharides are water insoluble (Liu, 1999).

The chemistry of soybean carbohydrates was briefly reviewed by Aspinall (1988). On average, moisture-free soybeans contain about 35% carbohydrates, and mature soybeans contain about 10% soluble carbohydrates. Soy carbohydrates are the second largest component in soybeans. However, the economical value of soybean carbohydrates is considered much less important than soybean protein and oil. As a results, relatively fewer efforts have been made to study soybean carbohydrates and their potential utilization.

Because research has shown the health benefits of dietary soy oligosaccharides as well as a link between the consumption of dietary fiber and reduced risk of colon cancer and other disease (Tomomatsu *et al.*, 1994; Burkitt & Trowell, 1975), the value of soy soluble carbohydrates is under further exploration.

Therefore, the objective of this study was to identify the soluble carbohydrates for sugar analysis in soybean seeds using on-line HPLC-RID-ES/MS and HPLC behavior, and to determine their contents in soybean seeds for high quality soybean breeding.

MATERIALS AND METHODS

Plant Materials

The 32 Korean major cultivated soybeans (Table 2) and 468 soybean germplasms which were distributed to National Gene Bank, Rural Development Administration were used in this study. The soybeans were grown at the experimental field of Yeongnam Agricultural Research Institute, Milyang, Korea in 2003.

The soybean seed samples were ground with ball mill and sieved with a 1.0 mm screen. The ground samples were well mixed and used for the analysis of soluble carbohydrates.

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Chemicals

Pure water (18 MΩ cm^{-1}) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA). Stachyose trihydrate, raffinose, sucrose, glucose, fructose, and Ca-EDTA were obtained from Sigma Chemical Co. (St. Louis, MO). All laboratory chemicals used in this study were of reagent grade.

Instrumentation and Conditions

On-line HPLC-RID-ES/MS analyses were carried out on a Thermo Finnigan AQA single-quadrupole mass spectrometer system (Thermo Electron Inc., Somerset, NJ), equipped with a Spectra system P-4000 HPLC system (Thermo Electron Inc., Somerset, NJ). The analytical HPLC system consisted of a G1311A Agilent 1100 quaternary pump (Agilent Technologies, Wilmington, DE), a G1313A Agilent auto sampler equipped with a 20 μL sample loop, and a Shodex RI-71 detector (Shodex Ltd., Tokyo). The chromatographic data were processed by a Donam dsCHROM 2000 Software.

Extraction and analysis of soluble carbohydrates in soybean seeds

The 1.0 g soybean sample was extracted with 80% EtOH (10 ml) at room temperature for 24 h. The 80% EtOH extract was filtered with TOYO 5B filter paper and 0.45 μm membrane filter. For elimination of non-polar compounds, the filtered extraction was passed through the Sep-Pak C18 plus cartridge, and then the 80% EtOH extract was diluted with distilled water (80% EtOH extract 1 ml : water 1ml). The 20 μL of diluted extract was injected into HPLC. The HPLC conditions are shown in Table 1.

The moisture content was analyzed by oven-dry method with 105°C for 2 h, and then all soluble carbohydrate contents were converted to dry matter base.

The soybean soluble carbohydrate contents were calculated by HPLC peak areas compared with external standard calibration curves. The linear standard calibration curves (r = 0.999**) were generated by injection of 0.5% to 4.0% standard solutions.

RESULTS AND DISCUSSION

Identification of soluble carbohydrate in soybean seeds

Fig. 1 shows the HPLC chromatogram of soluble carbohydrates in soybean seeds. The soluble carbohydrate peaks showed good separation and resolution.

A total of 6 peaks were resolved with in 12 min by employing a isocratic solvent system of deionized water containing 0.0001 M calcium EDTA. This solvent system was chosen on the basis of a instrument manual of Sugar-Pak I column (Waters Assoc.).

The 6 soluble carbohydrate peaks were identified by *m/z* value of on-line HPLC-RID-ES/MS and comparison of retention time of the reference standards (Fig. 2).

The peak 1 and 6 were eluted at 5.3 min and 10.5 min, and showed the molecular ion at *m/z* 217.07 and *m/z* 249.13, respectively. These data were not corresponding to any authentic sugar standards by comparison of molecular

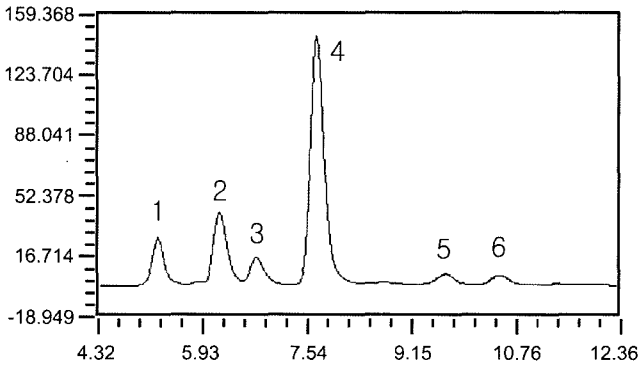

Fig. 1. HPLC chromatogram of the soluble carbohydrates in soybean seeds.
(peak 1=Unidentified, peak 2=stachyose, peak 3=raffinose, peak 4=sucrose, peak 5=glucose, and peak 6=Unidentified)

Table 1. HPLC conditions for analysis of soluble carbohydrates in soybean seeds.

Parameter	Condition
Column	Waters Co. Sugar Pak I (6.5×300 mm)
Detector	Shodex RI-71 refractive index
Sensitivity	×8
Temperature	90°C
Mobile phase	Deionized water(containing 0.0001M calcium EDTA)
Flow rate	0.5 ml/min.

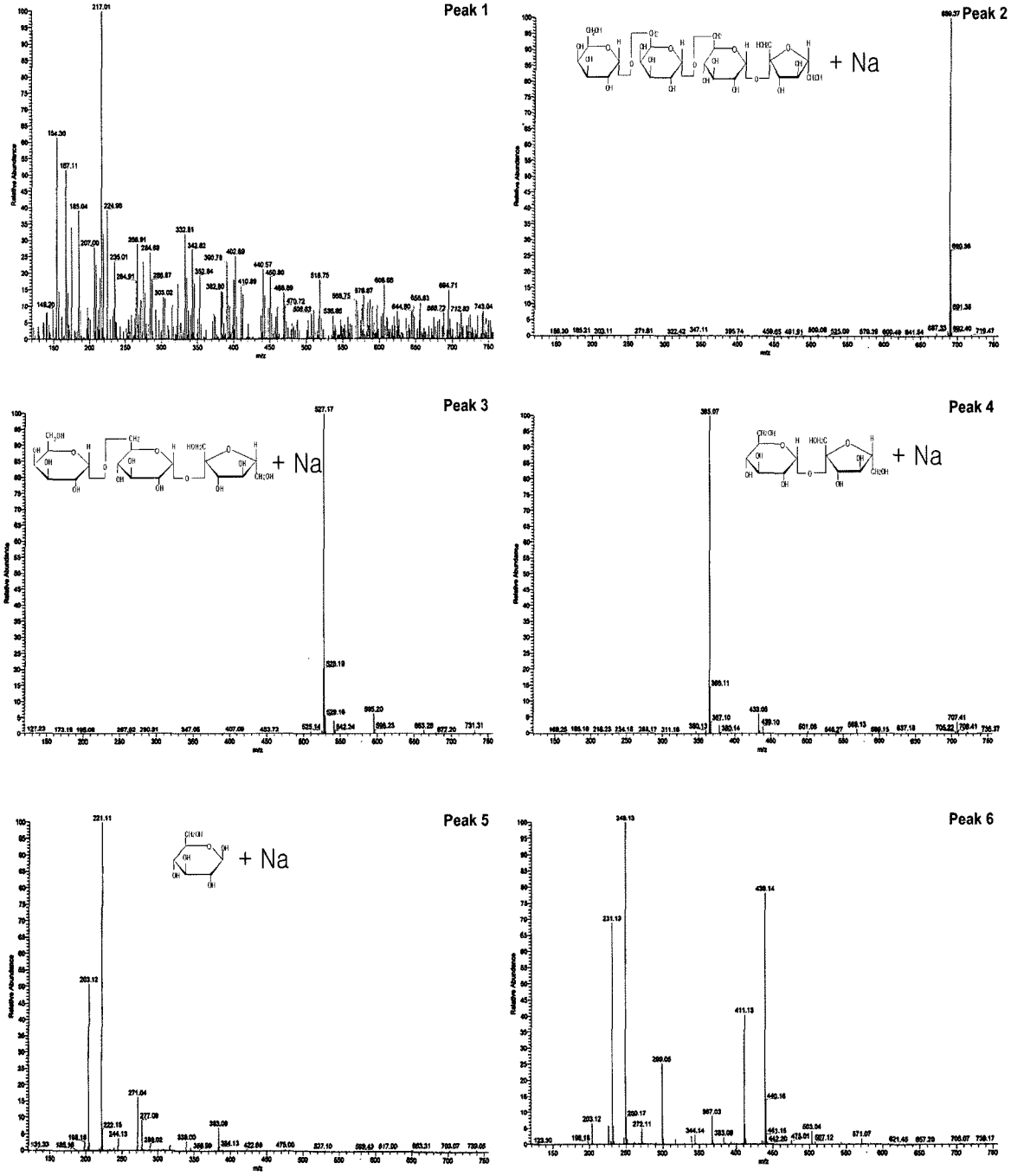

Fig. 2. Mass spectra of soluble carbohydrates in soybean seeds. (peak 1=Unidentified, peak 2=stachyose, peak 3=raffinose, peak 4=sucrose, peak 5=glucose, and peak 6=Unidentified)

weight and chromatographic behavior. So, We could not identify these 2 peaks.

The retention times of peak 2 - 5 were 6.2 min, 6.8 min, 7.8 min, and 9.5 min, and their molecular ions were *m/z* 689.37, *m/z* 527.17, *m/z* 365.07, and *m/z* 203.12, respectively.

The *m/z* of peak 2 ($C_{24}H_{42}O_{21}$ + Na = 689.55), peak 3 ($C_{18}H_{32}O_{16}$ + Na = 527.42), peak 4 ($C_{12}H_{22}O_{11}$ + Na = 365.28), and peak 5 ($C_6H_{12}O_6$ + Na = 203.14) revealed the structure to be stachyose, raffinose, sucrose, and glucose, respectively. These molecular ions were confirmed by the presence of adduct ions $[M + Na]^+$. Also, the retention times of peak 2 - 5 were corresponded to authentic sugar standards.

Table 2. Comparison on the soluble carbohydrate contents in 32 Korean major soybean seeds.

Cultivar	Content (%)				
	Glucose	Sucrose	Raffinose	Stachyose	Total
Hwangkeumkong	0.40	4.03	1.06	2.85	8.35
Eunhakong	1.31	2.68	0.92	2.23	7.14
Jangsukong	0.24	4.37	1.32	2.54	8.46
Danweonkong	0.47	4.56	1.47	2.34	8.83
Malikong	0.21	5.23	1.26	2.68	9.38
Shinpaldalkong	0.58	4.17	1.41	2.66	8.82
Taekwangkong	0.29	6.79	1.04	2.23	10.35
Samnamkong	0.21	4.34	1.16	2.77	8.48
Keunolkong	0.18	4.15	1.09	2.88	8.30
Bukwangkong	0.29	4.43	1.05	2.88	8.66
Duyoukong	0.28	5.40	1.36	2.69	9.74
Kwangankong	0.13	4.88	1.06	3.01	9.09
Hwasungputkong	0.19	4.58	1.29	2.92	8.98
Hwaeomputkong	0.16	4.83	1.29	2.85	9.12
Geomjeongkong 1	0.26	4.32	1.78	2.79	9.16
Soyangkong	0.20	4.28	1.14	2.90	8.51
Seokyangputkong	0.19	4.34	1.19	2.63	8.35
Jinpumkong	0.13	5.40	1.15	3.19	9.88
Iksannamulkong	0.12	5.82	0.99	2.71	9.65
Pungsannamulkong	0.22	5.75	1.23	2.40	9.61
Ilpumgeomjeongkong	0.72	3.37	1.07	2.23	7.39
Saeolkong	0.25	4.88	1.49	2.59	9.20
Ilmikong	0.19	5.59	1.06	2.84	9.68
Daehwangkong	0.59	5.00	1.06	3.04	9.69
Jinyulkong	0.44	2.87	1.51	3.32	8.13
Heugcheongkong	0.44	3.51	1.03	2.99	7.97
Galmikong	0.65	4.34	1.08	2.95	9.02
Saebyeolkong	0.73	3.97	0.86	2.48	8.04
Cheongjakong	0.22	5.41	1.18	3.09	9.89
Seonnogkong	0.65	2.86	1.20	3.03	7.73
Sorokkong	0.23	4.35	1.24	3.04	8.85
Geomjeongkong 4	0.64	5.15	1.20	1.36	8.35
Minimum	0.12	2.68	0.86	1.36	7.14
Maximum	1.31	6.79	1.78	3.32	10.35
Mean	0.37	4.55	1.19	2.72	8.84
S D	0.26	0.91	0.19	0.37	0.77

In the result, soybean seeds contain monosaccharide, such as glucose, and di- (sucrose) and oligosaccharides (raffinose and stachyose).

Soybean oligosaccharides are very important as a nutritional component of food. Greater than 90% of the sugars in ripe seeds are present as sucrose, raffinose, and stachyose (Kawamura, 1954). These three are important determinants for consumer acceptance of soybean products.

Although soybean oligosaccharides have long been considered undesirable components that cause flatulence (Omo-

Table 3. Statistical values of soluble carbohydrate contents in 468 soybean germplasms.

Parameter	Content (%)				
	Glucose	Sucrose	Raffinose	Stachyose	Total
Minimum	0.03	2.33	0.08	0.75	4.64
Maximum	0.98	6.96	1.87	3.18	10.47
Mean	0.15	4.65	0.78	2.33	7.90
S D	0.08	0.83	0.17	0.30	0.87

saiye *et al.*, 1978; Borejszo & Khan, 1992), they are also a safe, 'probiotic material' approved by the FDA for use in the USA (Hoover, 1993).

Because of their economic value to the food and feed industries, attempts have been made to recover them from defatted soybean meal (Kim *et al.*, 2003). For example, in the sweetener industry, new technologies have been applied that high-oligosaccharide syrups can be commercially produced from the soybean (Koga *et al.*, 1993).

Determination of soluble carbohydrate contents in soybean germplasms

The soluble carbohydrate contents were determined in 32 Korean major cultivated soybean samples. The cultivars contained the same constituents, but each soybean samples contained soluble carbohydrates in the different concentration (Table 2). Sucrose would appear to be most prevalent in soybean soluble carbohydrates. Total soluble carbohydrate contents ranged from 7.14% to 10.35%. Among 32 Korean major cultivated soybean samples, total soluble carbohydrate content of cv. Taekwangkong (10.35%) was the highest among that of other cultivated soybeans. When the relative total soluble carbohydrate content was set to 100 in cv. Taekwangkong it was 85 in cv. Sinpaldalkong and 68 in cv. Eunhakong.

The quantitative distribution of stachyose, raffinose, sucrose, glucose, and total soluble carbohydrate contents in the 468 soybean germplasms is given in Table 3. The average contents of stachyose, raffinose, sucrose, glucose, and total soluble carbohydrate were 2.33 ± 0.30, 0.78 ± 0.17, 4.65 ± 0.83, 0.15 ± 0.08, and 7.90 ± 0.87, respectively. On the other hand, the range of soluble carbohydrate contents among 468 soybean germplasms has been investigated to be: stachyose, 0.75 (IT 905788) - 3.18% (IT 153437); raffinose, 0.08 (IT 108998) - 1.87% (IT 025465); sucrose, 2.33 (IT 171044) - 6.96% (IT 177813); glucose, 0.03 (IT 025465) - 0.98% (IT 905788); total soluble carbohydrates, 4.64 (IT 905788) - 10.47% (IT 153437). The results were agreement with those reported by Hymowitz *et al.* (1972). Also, the composition ratio of soluble carbohydrates in soybean seeds was high in the order of sucrose (58.01 ± 5.82%) > stachyose (29.80 ± 4.54%) > raffinose (10.13 ± 2.28%) > glucose (2.07 ± 1.75%).

Oligosaccharide have shown the health benefits of multiple functions such as reducing toxic metabolites (Tomomatsu, 1994), suppress the activity of putrefactive bacteria (Masai *et al.*, 1987), reducing blood pressure (Koga *et al.*, 1993; Choung & Lee, 2003), and anti-carcinogenic effects (Hill *et al.*, 1971). Therefor, soybean seeds are an abundant source of oligosaccharide for functional food.

ACKNOWLEDGMENTS

This study was conducted by sponsoring of research assistant fund of Samcheok National University.

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