DETERMINATION OF FREE MONOSACCHARIDES AND DETECTION OF SUGAR ALCOHOLS IN MATURE SOYBEAN SEEDS

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Summary. Although the oligosaccharide contents of soybeans are well documented, the exact values of monosaccharide contents have not been reported. Elaborate methods of preparative paper chromatography together with gas chromatography established the following data for one variety, Kyushu No. 12. The air-dried cotyledon part (admixed with hypocotyls) contained 0.030% glucose and 0.039% fructose. The hull part contained 0.018% galactose, 0.028% glucose, 0.023% fructose, 0.005% arabinose, and 0.002% xylose. Gas chromatograms of trimethylsilated monosaccharide fractions revealed the existence of minute amounts of sorbitol, arabitol, xylitol, and mannitol in decreasing order (about 0.03% to 0.001% of whole seeds).

Simultaneous determination of sugars is very difficult when various sugars are present in extremely different amounts. When soybeans were analyzed for mono- and oligosaccharides, their hulls gave measurable data for both, whereas their cotyledons and hypocotyls did not, since the monosaccharides were far less than the oligosaccharides. Our previous data (1) were calculated without regard for the presence of monosaccharides in cotyledons and hypocotyls. Therefore, the values for monosaccharides were too low since they were calculated only from the data of monosaccharide contents of hulls, consisting of only about 7% of total seeds. The data (average of all the nine varieties of soybeans) are shown in Table 1.

In this paper, we report the monosaccharide contents of the hulls and cotyledons (mixed with small amounts of hypocotyls) of mature soybean seeds determined by careful methods using paper partition and gas liquid chromatography.

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Table 1. Average sugar contents of nine varieties of soybeans (1).

| Sugar          | Hulls | Defatted cotyledons | Defatted hypocotyls | Whole soybeans* |
|----------------|-------|---------------------|---------------------|-----------------|
| Arabinose      | 0.02  | —                   | 0.0015              | 0.0006          |
| Glucose (+Fructose?) | 0.05  | —                   | 0.006               | 0.006           |
| Sucrose        | 0.60  | 6.6                 | 7.0                 | 5.0             |
| Raffinose      | 0.13  | 1.4                 | 1.9                 | 1.1             |
| Stachyose      | 0.41  | 5.3                 | 7.7                 | 3.8             |

Trace amounts of verbascose were also present.

* Calculated from the yield of the three parts. On dry, full-fat basis.

The latter showed the presence of sugar alcohols besides monosaccharides which were identified by retention time of the authentic samples.

MATERIALS AND METHODS

Soybeans. Mature seeds used were obtained from soybeans, Glycine max cultivar, Kyushu No. 12, which were planted in July 1969, at the University Farm of our faculty. They were separated into the two parts, cotyledons (hypocotyls present in about 2% of the total seeds were not removed) and hulls. They were pulverized to pass through a 32-mesh sieve.

Extraction of sugars. (a) For the cotyledon part. Eleven parts of 80% ethanol were added to one part of cotyledons and refluxed 1 hr in a boiling-water bath. About four parts of ether were added to the ethanolic extracts and the upper layer discarded after settling. This defatting process was repeated several times. Lead acetate and then sodium carbonate were added to remove proteins. The concentrated filtrate was passed through a column consisting of Amberlite IRC-50 and Amberlite IR-45 (1:2) to remove remaining salts, and the eluate was concentrated to about a 10% sugar solution. (b) For the hull part. The procedure is the same except the omission of the defatting process.

Separation of monosaccharides from oligosaccharides. Preliminary experiments showed that the carbon column chromatography according to WHISTLER and DURSO (2) was the most suitable, when compared with ion exchange chromatography with Dowex-50 (K+ type), cellulose column chromatography, and gel filtration with Sephadex G-15. Chromatography on a column of Darco G-60 and Celite (1:1) (2) gave monosaccharide fractions which were concentrated to 1 or 2 ml.

Qualitative paper chromatography. Since 5 monosaccharides were detected in soybean seeds by preliminary gas chromatography, a search was made for the most convenient solvent system to clearly separate the five sugars, i.e., glucose, fructose, galactose, arabinose, and xylose. A solvent system consisting of ethyl acetate, pyridine, acetic acid, and water (30:11:1:6) was selected since the time necessary for chromatography was relatively short and five repetitions were satisfactory to separate all five sugars. Toyo filter paper No. 50 was used. A p-
anisidine hydrochloride spray was used and the colors after heating at 110° were observed.

**Preparative paper chromatography.** The concentrated sugar solution was chromatographed on Toyo filter paper No. 514 (40 cm × 40 cm). The five parts of the filter paper corresponding to the five sugars (as evidenced by *R*<sub>f</sub> values and colors by spraying *p*-anisidine hydrochloride on the paper strip simultaneously chromatographed) were cut and each sugar was extracted with water.

**Gas liquid chromatography.** The aqueous extract containing only one sugar was dried under reduced pressure. The dried sugar was trimethylsililated according to Brobst and Lott (3) with hexamethyilsilazane and trifluoroacetate. The supernatant (1 µl) after reaction was subjected to a gas chromatograph, Hitachi K-23 with flame ionization detector, with 2 stainless steel columns (inner diameter 2 mm, length 1 m) with 10% SE-30 (methyl silicone oil) on Chromosorb WAW. The flow speeds of the carrier gas (nitrogen) and hydrogen gas were 24 and 20 ml/min, respectively. The instrument was equipped with a QPD<sub>23</sub>-type electronic recorder. The recorder span was 1 mv, and the chart speed was 5 mm/min. The temperature of columns was constant at 168°C and 148°C for hexoses and pentoses, respectively.

**Determination of monosaccharides.** Each monosaccharide was characterized by retention time of the gas chromatogram. The mixed sugar solution was subjected to preparative paper chromatography quantitatively, and the aqueous solution was analyzed for reducing sugar by the improved Somogyi method by measuring absorbance at 530 nm.

**RESULTS AND DISCUSSION**

**Qualitative analysis of cotyledon and hull parts for monosaccharides by paper chromatography**

Reference to the *R*<sub>f</sub> values and colors showed that the cotyledon part contained glucose and fructose, whereas the hull part contained galactose, glucose, fructose, arabinose, and xylose. Our previous result (Table 1) was incorrect concerning monosaccharides: cotyledons (plus hypocotyls) do contain the two monosaccharides, glucose and fructose, and hulls contain not only arabinose, glucose, and fructose, but also galactose and xylose.

**Identification of monosaccharides and sugar alcohols by gas chromatography**

a) **Sugar solutions from the cotyledon part.** The gas chromatogram of the glucose fraction (Fig. 1) gave four peaks; No. 13 and No. 17 represent α- and β-glucose, respectively; No. 16 corresponds to sorbitol. All the sugar alcohols were identified by comparison with the retention time of the authentic samples. The largest peak No. 10 is D-pinitol. Its existence in soybeans (about 0.6% of full-fat sample) was first reported by Honig *et al.* (4). The identification is based on
Fig. 1. Gas chromatogram of the glucose fraction of the cotyledon part. Peaks Nos. 10, 13, 16, and 17 correspond to pinitol, α-glucose, sorbitol, and β-glucose, respectively.

The retention time of the pinitol kindly supplied by them. However, their preparation is not completely pure. We are now trying to crystallize this O-methyl-inositol.

The gas chromatogram of the fructose fraction (Fig. 2) gave five peaks: No. 7 and No. 12 represent α- and γ-fructose, respectively. No. 5 and No. 6 are xylitol and arabitol, respectively.

Fig. 2. Gas chromatogram of the fructose fraction of the cotyledon part. Peaks Nos. 5, 6, 7, 10, and 12 correspond to xylitol, arabitol, α-fructose, pinitol, and γ-fructose, respectively.

Fig. 3. Gas chromatogram of the glucose fraction of the hull part. Peaks Nos. 10, 13, 15, 16, and 17 correspond to pinitol, α-glucose, mannitol, sorbitol, and β-glucose, respectively.

b) *Sugar solutions from the hull part.* The gas chromatogram of the glucose fraction (Fig. 3) gave five peaks. No. 10, 13, 16, and 17 are D-pinitol, α-glucose, sorbitol, and β-glucose, respectively; they are similar to the peaks in Fig. 1. No. 15, not found in Fig. 1, corresponds to mannitol.

The gas chromatogram of the fructose fraction (Fig. 4) is simpler than that from the cotyledon part (Fig. 2). Only three peaks appeared: No. 6 (arabitol), No. 7 (α-fructose), and No. 10 (pinitol).

The galactose fraction (Fig. 5) gave four peaks: No. 8 (γ-galactose), No. 11 (α-galactose), No. 14 (β-galactose), and No. 16 (sorbitol). The peak No. 10
Fig. 4. Gas chromatogram of the fructose fraction of the hull part. Peaks Nos. 6, 7, and 10 correspond to arabitol, α-fructose, and pinitol, respectively.

Fig. 5. Gas chromatogram of the galactose fraction of the hull part. Peaks Nos. 8, 11, 14, and 16 correspond to γ-galactose, α-galactose, β-galactose, and sorbitol, respectively.

Fig. 6. Gas chromatogram of the arabinose fraction of the hull part. Peaks Nos. 1, 2, 3, and 6 correspond to α-, β-, and γ-arabinose, and arabitol, respectively. No. 10 (not shown in the figure) is pinitol.

Fig. 7. Gas chromatogram of the xylose fraction of the hull part. Peaks Nos. 4, 5, and 9 correspond to α-xylose, xylitol, and β-xylose, respectively.

(pinitol) could not be found. The reason is not known. Sorbitol (No. 16) found in the glucose fraction (Figs. 1 and 3) appeared also here in the galactose fraction.

The arabinose fraction (Fig. 6) gave five peaks: No. 1, No. 2, and No. 3 represent α-, β-, and γ-arabinose, respectively, No. 6 is arabitol, and No. 10 (not shown in the figure) is pinitol.

The xylose fraction (Fig. 7) showed four peaks: No. 4 (α-xylose), No. 5 (xylitol) No. 9 (β-xylose), and No. 10 (pinitol) (not shown in the figure).

Quantitative analysis of cotyledons and hulls for monosaccharides by paper chromatography

It may be possible to determine monosaccharides from the area or height of the corresponding peaks of gas chromatograms. However, the presence of the large peak of pinitol and of various anomers of monosaccharides make this method very difficult. Thus determination of monosaccharides was made by paper chro-
**Table 2. Absorbance (530 nm) of the authentic sugars (100 μ/ml) by the modified SOMOGYI method.**

| Sugars       | Galactose | Glucose | Fructose | Arabinose | Xylose |
|--------------|-----------|---------|----------|-----------|--------|
| Absorbance (530 nm) | 0.274     | 0.411   | 0.450    | 0.326     | 0.420  |

**Table 3. Monosaccharide contents of soybeans (% on air-dry basis).**

|                | Galactose | Glucose | Fructose | Arabinose | Xylose |
|----------------|-----------|---------|----------|-----------|--------|
| Cotyledonsa    | —         | 0.030   | 0.039    | —         | —      |
| Hulls          | 0.018     | 0.028   | 0.023    | 0.005     | 0.002  |
| Whole soybeansb| 0.0013    | 0.0298  | 0.0379   | 0.0004    | 0.0001 |

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**Approximate estimation of sugar alcohols from gas chromatograms**

Sugar alcohols were identified by retention time, but they were not determined quantitatively. However, their approximate amounts may be estimated from the corresponding peak areas. The values are shown in Table 4. As shown in Fig. 7 the xylose fraction of hulls contain a considerable amount of an unidentified compound (possibly a sugar alcohol) with a low retention time. Anyway the air-dried whole soybeans contain about the same order of free sugar alcohols with free...
monosaccharides: about 0.03% sorbitol, 0.01% arabinol, 0.002% xylitol, and 0.001% mannitol.

In conclusion we obtained reliable data for free monosaccharides present in mature soybean seeds. At the same time we discovered that soybeans contained sugar alcohols, though in very small amounts.

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