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SEPARATION OF SOYBEAN HEMICELLULOSES BY PAPER ELECTROPHORESIS

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Fractionation of soybean polysaccharides were made by KAWAMURA and NARASAKI[1] by the use of water, ammonium oxalate, and 0.2, 5, and 15% sodium hydroxide solutions as successive extracting agents. Rhamnose, fucose, xylose, arabinose, mannose, glucose, galactose, and galacturonic acid were found in the hydrolyzate of polysaccharides purified through copper-complex formation. This is the first report on simultaneous existence of rhamnose and fucose in soybean hemicelluloses. It is of interest to study whether all of these component sugars are actual constituents of the same polysaccharide molecule or these sugars are distributed in some polysaccharides containing one or a few component sugars.

The separation of individual polysaccharides present in a mixture is a very difficult and tedious process which can only be successful in a particular case on the macro-scale. GELDMACHER-MALLINCKRODT and WIENLAND[2] first employed paper electrophoresis for the separation of glycogen and galactan. They used veronal-acetate buffer. PREECE and HOBKIRK[3] examined water-soluble polysaccharides of rye and oats by paper electrophoresis with borate buffer. Rye polysaccharide was separated clearly into two bands and oats polysaccharide was fractionated into three bands. FULLER and NORTHCOTE[4] published a detailed report on a micro method for the separation and determination of polysaccharides by zone electrophoresis. They used filter paper, silk, or glass paper as a strip support and showed that the electrophoretic movement of neutral polysaccharides is dependent upon the use of borate buffer. In 1957, LEWIS and SMITH[5] showed that many polysaccharides hitherto assumed to be essentially homogeneous were separated into two or more components by the use of electrophoresis on glass-fiber paper and 2 *N* sodium hydroxide solution as solvent. The results seem to indicate that the heterogeneity in polysaccharides, especially those of fairly high molecular weight, is the rule rather than the exception.

Now, paper electrophoresis was applied for the separation of soybean hemicelluloses and some hemicelluloses were clearly fractionated into some components having simple sugar compositions.

Experimental

1. Fractionation of Soybean Hemicelluloses by Successive Extraction

1. 1. Sample

The defatted soybean flake used as the raw material was donated by Nippon Koyu Kogyo K.K. through the courtesy of Mr. Torao Sakakihara, Director of the Mizushima Factory, Kurashiki, Okayama-ken.

1. 2. Fractionation of Hemicelluloses

The defatted soybean flakes were pulverized and sieved to make free from hulls and hypo-

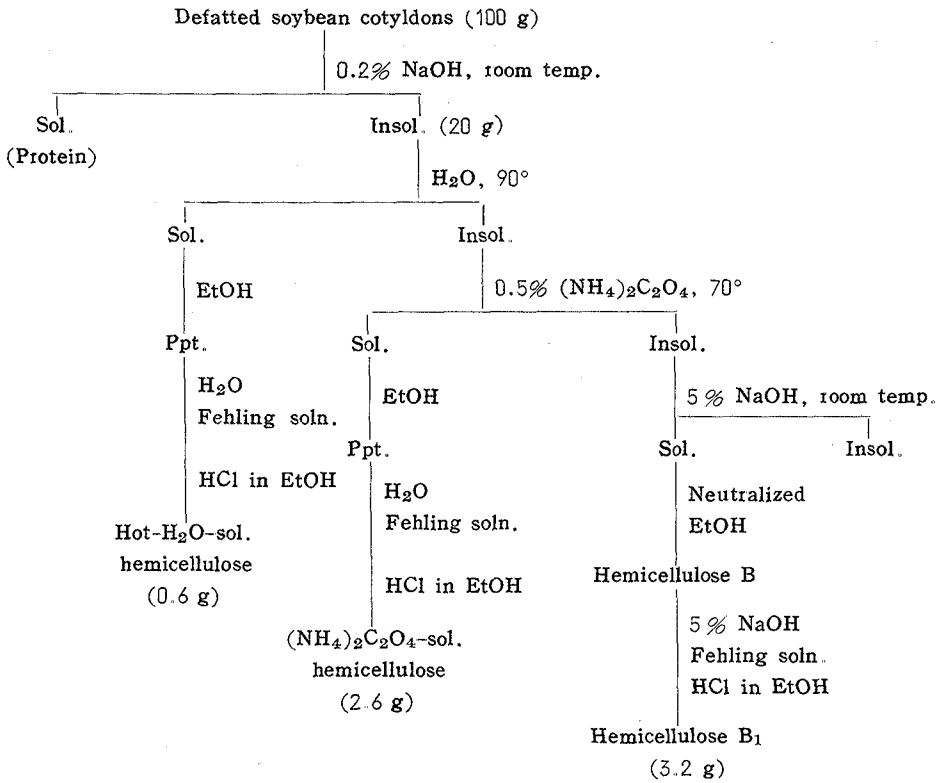

Fig. 1. Fractionation of hemicelluloses

cotyl. The cotyldons were subjected to fractionation of hemicelluloses according to a scheme outlined in Fig. 1.

1. 2. 1. *Preparation of Protein-Extraction Residue* About 100 g of defatted cotyldons was extracted with 2 L 0.2% NaOH at room temperature for about 4 hrs. After about ten repeated extractions, when biuret reaction of the extract became negative, the insoluble residue was washed with cold water to remove NaOH and then dried by washing with ethanol and ether. The yield was about 20 g.

1. 2. 2. *Preparation of Hot-Water-Soluble Hemicellulose* The protein-extraction residue (20 g) was extracted with 200 ml water at 90°C for 1 hr. The extraction was repeated four times. The combined extract was concentrated to a half volume *in vacuo* below 40°C. To the concentrated solution three volumes of ethanol was added to precipitate polysaccharide. The dried precipitate was dissolved in 100 ml water and the hot-water-soluble hemicellulose was obtained by purification with Fehling solution as reported by KAWAMURA *et al.*[6]. The yield was about 0.6 g.

1. 2. 3. *Preparation of Ammonium Oxalate-Soluble Hemicellulose* The residue from hot-water extrdction was further extracted with 200 ml 0.5% $(NH_4)_2C_2O_4$ solution at 70°C for 10 hours. After four repeated extractions, the soluble part was concentrated to a half volume and polysaccharide was precipitated with ethanol and then purified by Fehling solu-

tion as above. The yield of the ammonium oxalate-soluble hemicellulose was about 2.6 g.

1. 2. 4. *Preparation of Hemicellulose* B_1 The residue was extracted with 200 ml 5% NaOH at room temperature for 24 hrs. The four extracts were combined and neutralized with acetic acid to pH 6.0. After concentration to a half volume, hemicellulose B was precipitated by adding three volumes of ethanol and then the hemicellulose B_1 was obtained by purification by copper-complex formation. The yield was 3.2 g.

1. 3. Detection of Component Sugars of the Fractionated Hemicolluloses by Paper Chromatography

Each of the adove three hemicelluloses (0.2 g) was hydrolyzed with 20 ml *N* HCl in a sealed glass tube by heating in boiling water for 2 hrs. The hydrolyzate was concentrated to dryness *in vacuo* below 40°C and the residue was taken up in 0.5 ml water to be subjected to paper chromatography. Two-dimensional ascending paper chromatography was carried out with phenol water (4:1) and *n*-butanol-pyridine-water (3:1:1.5) as the solvent systems and *p*-anisidine hydrochloride as the spraying reagent. The results are shown in Table 1. Both the hot-water-soluble and the ammonium oxalate-soluble hemicelluloses consisted

Table 1. Component sugars of the soybean hemicelluloses

Hemicellulose soluble in	Rhamnose	Fucose	Xylose	Arabinose	Glucose	Galactose	Galacturonic acid
Hot-water	±	±	+	+++	+	+++	+
0.5% $(NH_4)_2C_2O_4$	−	−	++	+++	±	+++	++
5% NaOH	++	++	+++	++	+	+++	+

of mainly arabinose and galactose. The hemicellulose B_1 contained considerable amounts of rhamnose and fucose. Mannose was not detected from any of the three hemicelluloses.

2. Paper Electrophoresis of the Soybean Hemicelluloses

2. 1. The Methods of Experiments

2. 1. 1. *Apparatus and Conditions of Electrophoresis* An open strip horizontal paper electrophoresis apparatus Toyo CS-II was employed. The constant voltage applied from anode to cathode was 500 v and the effective length of the paper strip was about 35 cm. Therefore, the field strength was approximately 14 v/cm. Paper strip of Toyo No. 54 (5–10 x 40 cm) was used. A buffer solution employed throughout the experiments was 0.02 *M* $Na_2B_4O_7 \cdot 10H_2O$. The hemicelluloses were applied as 1% aqueous solutions in the above buffer solution.

2. 1. 2. *Colorimetric Determination of Eluate Polysaccharide* After electrophoretic runs, paper strips (5 x 40 cm) were dried at room temperature. The dried paper was cut into 1 cm segments and each segment was eluted with 1 ml water at 25°C for 1 hr. The eluates were filtered through a sintered glass filter No. 3. To 0.5 ml of the eluate 4.5 ml 89% H_2SO_4 was added by cooling with ice water, and then the mixture was heated for 5 min in a boiling water bath. After cooling with tap water, 0.2 ml of a freshly prepared 2% alcoholic α-naphthol solution was added and the mixture was shaken thoroughly. A purple color

developed after 2 hrs was read by a photoelectric colorimeter at 580 mμ with 10 mm cells.

2. 1. 3. *Detection of Component Sugars of Electrophoretic Fractions* Electrophoresis was performed under the conditions giving the best separation. The air-dried paper strips (10 x 40 cm) were washed with methanol to remove borax. The dried paper was cut into bands by reference to the corresponding electrophoretic pattern. The bands were eluted with 10 ml water at 25°C for 1 hr. The elution was repeated two times. The combined eluate was hydrolyzed with *N* HCl in a sealed glass tube by heating in a boiling water bath for 2 hrs.

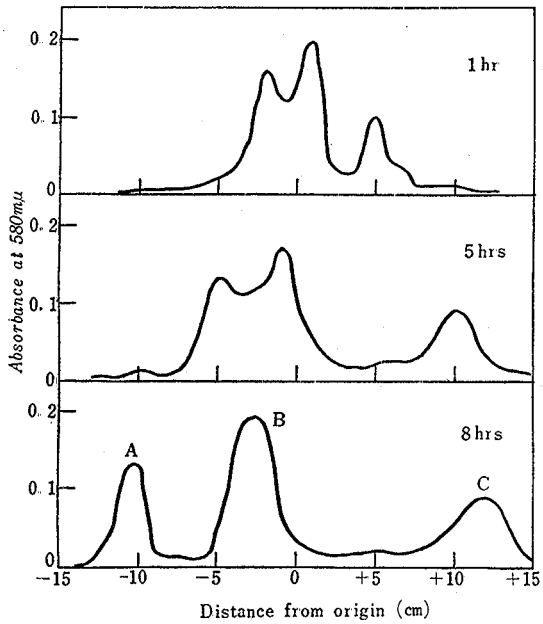

Fig. 2. Electrophoretic pattern of the hot-water-soluble hemicellulose

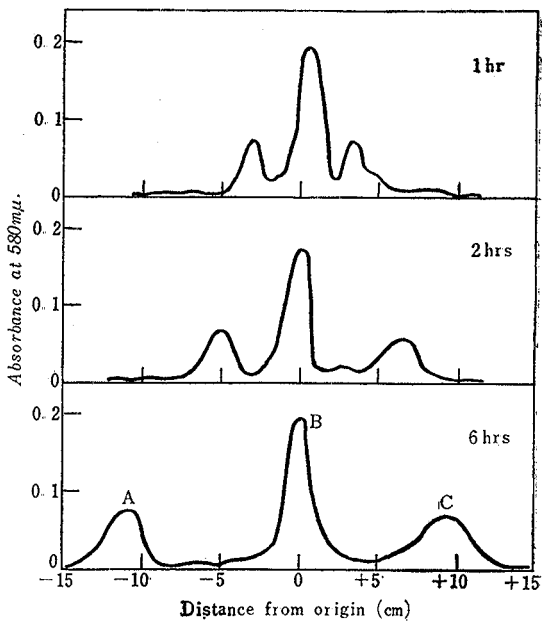

Fig. 3. Electrophoretic pattern of the ammonium oxalate-soluble hemicellulose

The hydrolyzate was concentrated to dryness *in vacuo* below 40°C and the residue was dissolved in 0.1 ml water to be tested by ascending one-dimensional paper chromatography with *n*-butanol-pyridine-water (3:1:1.5) and *n*-butanol-acetic acid-water (4:1:2) as the solvent systems.

The spraying reagent was *p*-anisidine hydrochloride.

2. 2. Electrophoretic Patterns of the Hemicelluloses

Electrophoretic patterns of the three hemicelluloses are shown in Figs. 2-4.

Both the hot-water-soluble and the ammonium oxalate-soluble hemicelluloses

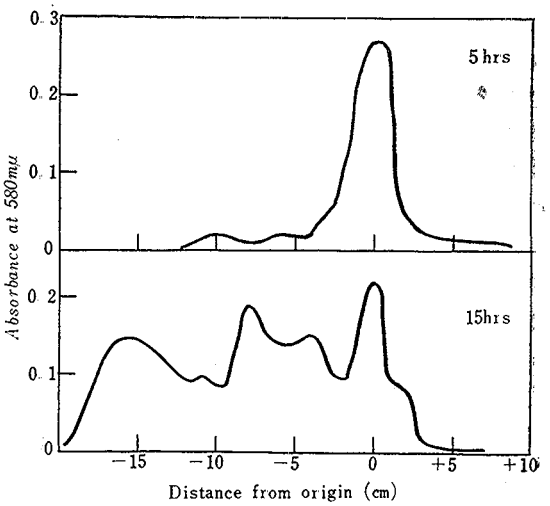

Fig. 4. Electrophoretic pattern of the hemicellulose B_1

were clearly separated into 3 bands; one moved to cathode, one remained near origin, and one moved to anode. The best separation was attained by 8 hrs' run for the hot-water-soluble hemicellulose and by 6 hrs' run for the ammonium oxalate-soluble hemicellulose, respectively. The hemicellulose B_1 moved only very slowly and gave no clear separation into distinct bands.

2. 3. Component Sugars of Electrophoretic Fractions of the Soybean Hemicelluloses

The hot-water-soluble hemicellulose was run for 8 hrs and the ammonium oxalate-soluble hemicellulose was run for 6 hrs. After electrophoretic runs, the bands corresponding to A, B, and C in Figs. 2 and 3 were examined for their component sugars. The results are given in Table 2. The bands which moved to cathode consisted of galactose and arabinose.

Table 2. Component sugars of electrophoretic fractions

Hemicellulose soluble in	Electrophoretic fractions*	Rhamnose	Fucose	Xylose	Arabinose	Glucose	Galactose	Galacturonic acid
Hot-H_2O	A	−	−	−	++	−	+++	−
	B	−	−	−	++	+	++	+
	C	−	−	−	+++	−	−	±
0.5% $(NH_4)_2C_2O_4$	A	−	−	−	+++	−	+++	−
	B	−	−	++	+++	−	+++	++
	C	−	−	−	+++	−	±	±

* See Figs 2 and 3 for the sign of electrophoretic fractions

The bands which traveled to the anode contained practically only arabinose. The bands which remained near the origin included almost all of the component sugars detected in the original hemicelluloses. The electrophoresis of the hemicellulose B_1 was unsuccessful but some results indicated the presence of a fraction consisting of xylose and galacturonic acid.

Discussion

The electrophoretic separation of the soybean hemicelluloses revealed the heterogeneity of the hemicelluloses which have been purified through copper-complex formation. Both the hot-water-soluble and the ammonium oxalate-soluble hemicelluloses gave the bands which moved to cathode and consisted of galactose and arabinose. Whether the galactose and the arabinose in these fractions arise from arabogalactan or from a mixture of araban and galactan remains to be determined. However, the presence of the other bands which moved to anode and contained only arabinose seems to suggest that the above galactose and arabinose came more probably from arabogalactan than from a mixture of araban and galactan.
Thus, the existence of arabinose residues in at least two forms of combination, araban and arabogalactan, seems to be established.

The evidence that the hot-water-soluble hemicellulose gave an electrophoretic behaviour very similar to that of the ammonium oxalate-soluble hemicellulose seems to indicate that fractional extraction has only a limitted effectiveness in isolating polysaccharides having same component sugars and similar molecular weight.

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ダイズヘミセルロースのろ紙電気泳動法による分別

楢崎丁市，藤本和昭

要旨 ダイズの子葉部から抽出されるヘミセルロースは，ラムノース，フコース，キシロース，アラビノース，マンノース，グルコース，ガラクトース，およびガラクツロン酸を含んでいた。これら多数の構成糖が単一のヘミセルロース分子として存在するのか，それともより単純な構成糖からなる数種のヘミセルロースが混在するのかは不明である。そこで脱脂ダイズ子葉部から分別抽出した熱水可溶性，修安可溶性，およびアルカリ可溶性ヘミセルロースのろ紙電気泳動を試みた。熱水可溶性および修安可溶性ヘミセルロースは容易に3区分に分離できたが，ヘミセルロースB_1（アルカリ可溶性）ははっきりした分離を示さなかった。熱水可溶性および修安可溶性ヘミセルロースの陰極側に移動した区分はアラビノースとガラクトースを含み，陽極側に移動した区分はほとんどアラビノースのみから成っていた。又原点附近に留っていた区分は泳動前のヘミセルロースの構成糖をすべて含んでいた。

これらの結果からダイズヘミセルロース中には少なくともアラバンとアラボガラクタンが存在するものと推定した。

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