# POLYSACCHARIDES OF SOYBEAN HULLS

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Extensive studies have been made in this university by KAWAMURA and co-workers on the soybean carbohydrates[1-7]. Main attention of these studies was focussed on the polysaccharides of soybean cotyldons.

WHISTLER and SAARNIO[8] have shown that soybean hulls contained a galactomannan soluble in cold water. They suggested that the soybean hull galactomannan possessed a structure similar to guaran and other galactomannans from the seeds of leguminous plants. ASPINALL and WHYTE[9] isolated two galactomannans from soybean hulls by extracting soybean hulls with water at room temperature and at 60° C.

In this report, soybean hull polysaccharides were fractionated by successive extraction with water, 0.5% ammonium oxalate, and 5% sodium hydroxide and the separated polysaccharides were examined for their component sugars by paper chromatography after hydrolyzing with sulfuric acid. Further, the hot-water-soluble polysaccharide was hydrolyzed with Taka-diastase and the formed oligosaccharides were examined for their component sugars and it was presumed that xylose in the hot-water-soluble polysaccharide was not a mere contamination but an actual component of galactomannan.

## Experimental

### 1. Fractionation of Soybean Hull Polysaccharides by Successive Extraction

#### 1.1. *Sample*

The defatted soybean flake used as the raw material was donated by Nippon Koyu K. K. through the courtesy of Mr. Torao Sakakihara, Director of the Mizushima Factory, Kurashiki, Okayama-ken. This flake contained 10% moisture and 1.5% crude oil[10].

#### 1.2. *Fractionation of Plysaccharides*

The defatted soybean flakes were pulverized and sieved to collect seed hulls. The contamination of hypocotyls was removed by the use of controlled current of air. Then the purified hulls were subjected to the fractionation of polysaccharides according to a scheme given in Fig. 1.

#### 1.3. *Detection of Component Sugars by Paper Chromatography*

Each of the polysaccharides (0.2 g) was hydrolyzed with 2 $N$ $H_2SO_4$ in a sealed glass tube by heating in a boiling water bath for 4 hrs. The hydrolyzate was neutralized with $BaCO_3$ and the formed precipitate was removed by centrifugation and filtration through Toyo No. 5 C filter paper. The filtrate was concentrated to dryness under reduced pressure below 40° C and the residue was taken up in 1 ml water to be examined by paper

chromatography. Two-dimensional paper chromatography was carried out with phenol-water (4 : 1) and *n*-butanol-pyridine-water (6 : 4 : 3) as the solvent systems and 0.3% *p*-anisidine-HCl in water-saturated *n*-butanol as the spraying reagent. The results are shown in Table I.

The hot-$H_2O$-soluble polysaccharide A gave xylose, mannose, galactose, and galacturonic

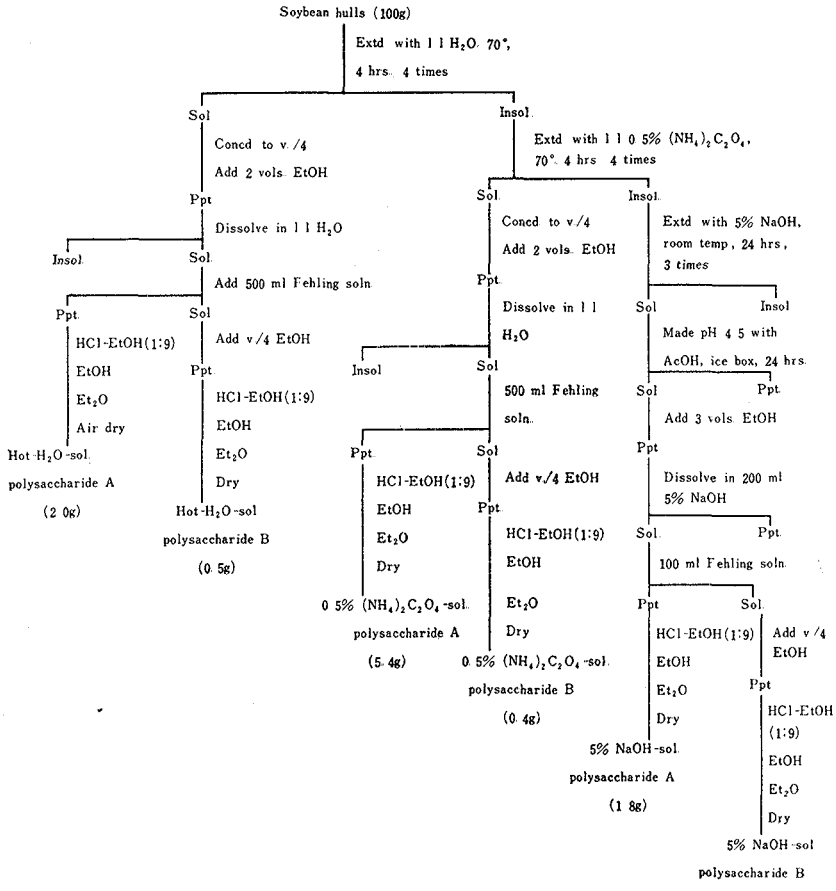


Fig. 1. Fractionation of polysaccharides

Table I. Component sugars of the hull polysaccharides

| Polysaccharides soluble in | | Rhamnose | Fucose | Xylose | Arabinose | Mannose | Glucose | Galactose | Galacturonic acid |
|---|---|---|---|---|---|---|---|---|---|
| Hot-$H_2O$ | A | ± | − | + | ± | +++ | − | ++ | + |
| | B | ± | − | + | ++ | + | − | ++ | + |
| 0.5%$(NH_4)_2C_2O_4$ | A | + | ± | ± | ++ | + | − | ++ | + |
| | B | − | − | ± | ++ | ± | − | ± | +++ |
| 5% NaOH | A | ± | ± | +++ | ± | + | ± | ++ | + |
| | B | ± | ± | +++ | +++ | − | ± | ++ | + |

acid in the ratio of 1 : 1.5 : 1 : 1 together with a trace of arabinose and rhamnose. This seemed to be very similar to the galactomannan of WHISTLER and SAARNIO[8], but the presence of xylose and galacturonic acid was a marked difference of the two polysaccharides. The 0.5% $(NH_4)_2C_2O_4$-soluble polysaccharide B appeared to be a typical polygalacturonic acid. The presence of pectin in the soybean hulls was reported by SASAKI and FUJI[11]. The 5% NaOH-soluble polysaccharide A seemed to be a xylan. All the polysaccharides contained comparatively large amounts of arabinose and galactose as in the case of soybean cotyldons. These two sugars were presumed to be in the forms of araban, galactan, and arabogalactan as in the case of the cotyldon hemicelluloses.[7]

## 2. Hydrolysis of the Hot-$H_2O$-Soluble Polysaccharide A by Taka-Diastase and Paper Chromatography of the Hydrolyzate

### 2.1. *Preparation of Enzyme Solution*

A commercial Taka-diastase (50 g) was dissolved in 500 ml $H_2O$. $(NH_4)_2SO_4$ was added to saturation and the precipitate formed after 4 hrs. was collected by centrifugation and washed with saturated $(NH_4)_2SO_4$ solution. This $(NH_4)_2SO_4$ precipitation was repeated three times and finally the precipitate was dissolved in 100 ml $H_2O$ and dialyzed against deionized water for 4 days. The dialyzed enzyme solution was centrifuged to remove a precipitate and filled up to 150 ml with deionized water to be used as an enzyme solution.

### 2.2. *Hydrolysis of the Polysaccharide and Paper Chromatography of the Hydrolyzate*

The polysaccharide (1.5 g) was dissolved in 150 ml $H_2O$ by heating. MCILVAINE buffer at pH 4.5 (50 ml) and 150 ml of the enzyme solution were added to the polysaccharide solution. The mixture was covered with small amount of toluene and incubated for 24 hrs. at 35°C. The hydrolysis degree was 70% as shown in Fig. 2.

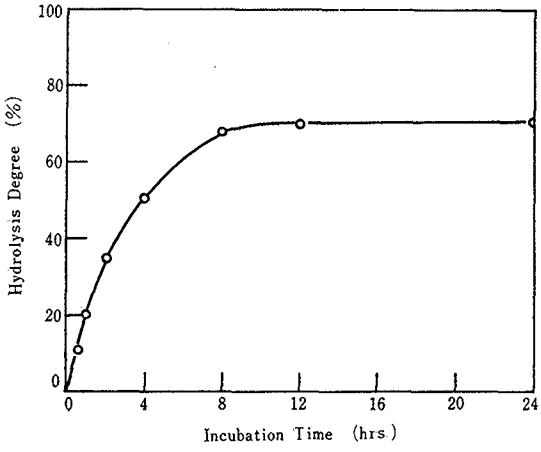


Fig. 2. Hydrolysis of the hot-$H_2O$-soluble polysaccharide by Taka-diastase

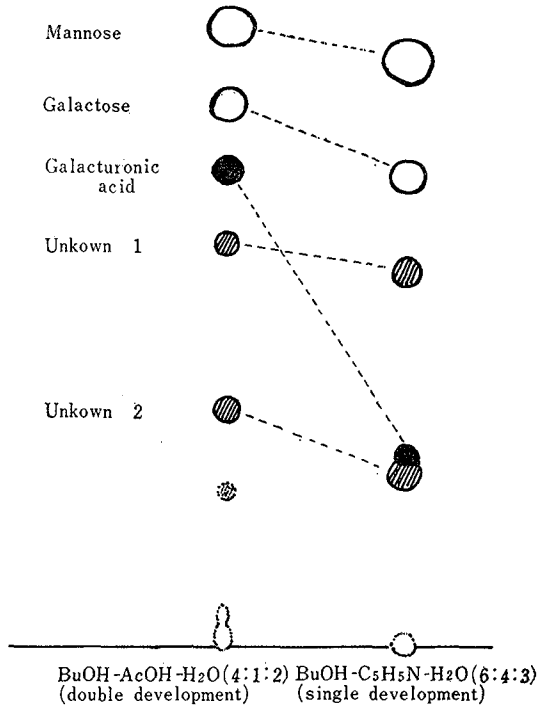


Fig. 3. Paper chromatograms of the enzymatic hydrolyzate of the hot-$H_2O$-soluble polysaccharide

After the incubation, 2 vols. of ethanol was added to precipitate the enzyme and the unhydrolyzed polysaccharide. The clear hydrolyzate was concentrated to dryness *in vacuo* and the residue was dissolved in 5 ml $H_2O$. One-dimensional paper chromatograms of the hydrolyzate are given in Fig. 3. An unknown spot 1 gave xylose and mannose in the ratio of 1 : 1 by hydrolysis with $H_2SO_4$. The other unknown spot 2 gave xylose, mannose, and galactose in the ratio of 1 : 1 : 1. These results seemed to indicate conclusively that xylose was chemically combined with mannose in the polysaccharide. ASPINALL and WHYTE[9] showed that the purified soybean hull galactomannan contained traces of arabinose and xylose and these trace components were contaminations. The findings of xylose-containing oligosaccharides from the enzymatic hydrolyzate of the polysaccharide suggest that xylose was not a mere contamination but an actual component of the hot-$H_2O$-soluble polysaccharide of soybean hulls.

## Summary

(1) Most part of the soybean hull polysaccharides were shown to be precipitated by Fehling solution in the absence of ethanol.

(2) Xylose in the hot-$H_2O$-soluble polysaccharide was proved to be an actual component of the polysaccharide and to combine with mannose residue of the polysaccharide.

(3) Soybean hulls contained a polygalacturonic acid containing arabinose.

## Acknowledgment

The authors wish to thank prof. Akira KAJI, Laboratory of Fermentation Chemistry, this University, for his kind advice. A part of expenditure was defrayed by the research fund donated by the Ministry of Education to T. NARASAKI (Enzymatic hydrolysis of soybean hemicelluloses, 1962) and to A. KAJI (Improvement of the utilization of agricultural and horticultural products by the use of enzymes, 1962–1963).

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(Received October 30, 1965)

# ダイズ種皮の多糖類

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**要旨** 著者等はダイズ子葉部の多糖類に関して一連の研究を行い，いくつかの新知見を発表してきた。最近ダイズ種皮から，かなり純粋なガラクトマンナンが得られることが報告された[8-9]．しかしこれらの研究は種皮多糖類の極一部のみを取扱っているものでより総括的研究が必要であると考えられた．そこで著者等は，ダイズ種皮より分別抽出法により多糖類を分離しその構成糖を調べた．種皮の多糖類は子葉部のものと異なり，アルコールを加えなくても大部分がフェーリング液により銅複合体として沈でんする。熱水可溶性区分はキシロース，マンノース，ガラクトース，ガラクッロン酸からなり，キシロースとマンノースからなる二糖とキシロース，マンノースとガラクトースからなる三糖がタカジアスターゼの加水分解物中から得られるので，キシロースはガラクトマンナンの構成分であることが推定された。また，修安可溶性区分からはペクチンが検出された。5％カセイソーダ可溶性区分からはキシランの存在が推定された．全体に各区分ともアラビノースとガラクトースが多く，ガラクッロン酸もすべての区分に検出された。

本研究を行うにあたり有益な助言を与えられ，又実験上種々便宜を計って下さった本学発酵化学研究室梶明教授に深く感謝する．なお研究費の一部は文部省科学研究費（昭和37年度各個研究費，楢崎丁市，ダイズヘミセルロースの酵素分解；昭和37，38年度機関研究費，梶明，酵素による農園芸産物の高度利用に関する研究）により支払われた。