
V. A Hemicellulose Fraction Soluble in Hot Water. Isolation and Component Sugar Determinations.

Sin'itirô KAWAMURA, Tsuweo KOBAYASHI, Mitio ÔSIMA, and Masahiro MINO

Dehulled and defatted soybean meal was treated with 0.2% sodium hydroxide to give a sugar-free protein-extraction residue, from which hot-water-soluble hemicellulose was precipitated by adding ethanol to the concentrated hot-water extract. The hemicellulose was purified through the copper complex formed by adding Fehling’s solution and acetone. Its decomposition point was 194-5° and $\alpha = 97.5°$ and it consisted of pentoses (arabinose and xylose) 17.8% (as pentosan), uronic acid (galacturonic acid) 19.2% (as lactone), and hexose (galactose) 41.9% (as galactan). The methods of analyses are described in detail.

Introduction

In the manufacture of soybean protein, two by-products are produced, one of which, the waste liquor of protein precipitation, or "soybean whey" as it is called in the U.S.A., contains sucrose, raffinose, and stachyose with the monosaccharides produced from them by hydrolysis. Soybeans or defatted soybeans contain only a trace of reducing sugar. The contents of the three above-mentioned oligosaccharides were determined by Kawamura.

The other by-product obtained during the manufacture of soybean protein is the insoluble residue from protein extraction. This residue contains sparingly soluble polysaccharides of soybeans. The separation of hemicelluloses from this residue by the method of Buston was critically examined. The result showed that hemicelluloses A and B increased only slightly by repeating the extractions with 4% sodium hydroxide, while hemicellulose C increased successively by repeating the extractions and especially abruptly upon extraction at 98°. Thus it was presumed that a polysaccharide insoluble in water or in very dilute alkali (such as 0.2% sodium hydroxide) but soluble in hot water was present in the protein-extraction residue and was admixed in the fraction of hemicellulose C.

The authors separated and studied this polysaccharide fraction soluble in hot water. It may nearly correspond with the "pectin-like substance" mentioned by Sasaki, and was considered to belong to hemicelluloses (Whistler and Smart defined hemicelluloses as water-insoluble cell wall polysaccharides of land plants except cellulose and pectin). It is called hot-water-soluble...
hemicellulose in this paper.

This study was made possible partly by the grant of the Ministry of Education (Scientific Research Fund) to Kawamura (studies on soybean hemicelluloses, 1951). Most parts of the experiments were carried out in the Laboratory of Biological Chemistry, Faculty of Agriculture, University of Tokyo, through the courtesy of Professor Yoshikazu Sahashi, Professor Yoshihiko Matsuyama, and Assistant-Professor Bunji Maruo. We wish to express our sincere thanks to them. Some later experimental works were made in the Laboratory of Biological Chemistry, Kagawa Agricultural College. Our thanks are due to Dr. Taiji Kurokami, the Director of Kagawa Agricultural College for his encouragement.

The outline of the study was presented (excluding the data described in paragraphs 5, 6 and 9-2 below) before the annual meeting of the Agricultural Chemical Society of Japan on April 7, 1953, at the Faculty of Agriculture, University of Tokyo.

**Experimental**

1. **Preparation of the protein-extraction residue of soybeans.**

Defatted soybean flakes (obtained by low-temperature extraction with petroleum naphtha) were pulverized to pass a 24-mesh sieve and were separated almost entirely from the seed hulls. About 2.8 kg of dehulled defatted soybean meal (with 8.77 % moisture) was obtained. To this 281 of 0.2 % sodium hydroxide was added and the mixture was allowed to stand for one hour.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Crude (III)</th>
<th>Purified (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Naphthol reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthrone reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ag mirror test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reduction of Fehling soln.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Seliwanoff test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phloroglucinol test</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Resorcinol test</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Aniline test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Orcinol test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Naphthoresorcinol test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Basic Pb acetate test(9)</td>
<td>...</td>
<td>+</td>
</tr>
<tr>
<td>Oxidation to mucoic acid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetone test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pptn. as Ca salt</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Iodine reaction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Melting point (decompn.,)</td>
<td>191~4°</td>
<td>194~5°</td>
</tr>
<tr>
<td>$[\alpha]_D$</td>
<td>-</td>
<td>+97.5°</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>...</td>
<td>0.17 %</td>
</tr>
<tr>
<td>Ash</td>
<td>...</td>
<td>3.1 %</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>0.68 %</td>
<td>...</td>
</tr>
</tbody>
</table>

Table I.
Studies on the Carbohydrates of Soybeans

Soybean protein-extn. residue (I) 150 g
  extd. with 2250 ml H₂O at 90° for 5 hrs.; filtered with cloth
  Residue
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1100 ml
  Residue
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1760 ml
  Residue
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1820 ml
  Residue
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1620 ml
  Residue (II) 129 g
    (moisture 18.09 %)
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1600 ml
  Residue
    extd. with 3000 ml H₂O at 90° for 5 hrs.
    Filtrate 1720 ml
  Combined filtrates 9620 ml (pH 7.2)
    filtered with paper; evapd. in vacuo
  Concd. soln. 2000 ml (pH 8.6)
    HCl (pH 5.4) added:
      4000 ml 9% EtOH added; centrifuged

Ppt.
  500 ml H₂O at 90° added; centrifuged
  Supernatant soln.

Insol. matter
  300 ml H₂O at 90° added
  Soln.

Insol. matter
  (trace)
  Soln.

Combined solns. 760 ml
  3040 ml 9% EtOH added;
  Supernatant soln.

Ppt.
  washed with 50% EtOH: 700 ml H₂O (90°) added
  Soln.
  350 ml. Fehling soln. added

Ppt.
  (Cu salt of pectic substance)
  350 ml Me₂CO added
  Soln.

Ppt. (blue-white)
  (Cu salt)
    washed with 90% EtOH
    10% HCl in EtOH added (removed from Cu++)
    washed 4 times with 92% EtOH
    washed 3 times with 99% EtOH (removed from Cl−)
  Supernatant soln.

Hot-water-sol. hemicellulose (III) 20.8 g
  Washings
  600 ml 4% NaOH added

Insol. matter (dark-colored jelly-like)
  dissolved in 100 ml 4% NaOH at 60°

Soll. (grey, opaque)
  acidified to pH 5 with AcOH
  treated with amylase (1 reaction neg. in 1 hr.)
  25 ml Fehling soln. added

Cu salt (dark grey)
  removed from Cu++; removed from Cl−

Pectin 0.544 g

Soil. 600 ml
  acidified to pH 5 with AcOH
  treated with amylase (1 reaction neg. in 24 hrs.)
  made to 860 ml
  450 ml Fehling soln. added
  450 ml Me₂CO added

Cu salt (white)
  removed from Cu++; removed from Cl−

Purified hemicellulose (IV) 7.65 g

Fig. 1. The procedure of isolating the hot-water-soluble hemicellulose from the soybean protein-extraction residue.
After filtration the residue was treated with another batch of 0.2% sodium hydroxide (28 l) for one hour. The residue, washed twice with water and dried under the sun, gave 402 g of protein-extraction residue (with 8.22% moisture). The treatment with 0.2% sodium hydroxide is effective in solubilizing most of the protein and all of the sugars present\textsuperscript{1,8}. The yield of the protein-extraction residue amounted to 14.5% of the defatted meal.

2. Isolation of hot-water-soluble hemicellulose.

The protein-extraction residue was extracted with hot water (repeated six times), and the hot-water-soluble extract was evaporated under reduced pressure. Two volumes of 95% ethanol were added to obtain a precipitate, which was dissolved in hot water, precipitated as the copper salt with Fehling's solution and acetone, and freed from the copper ion. The procedure is shown in Fig. 1.

3. Qualitative and quantitative tests on the hot-water-soluble hemicellulose.

The polysaccharide III was contaminated with a substance giving a positive reaction (blue) to the starch-iodine test. The purified sample IV was free from it. The result of the tests on III and IV is summarized in Table I.

4. Reducing power of the hydrolyzate of hot-water-soluble hemicellulose.

The Somogyi method\textsuperscript{17} was applied. (a) Preliminary experiments with pure sugars showed that 1 ml of 0.005 N sodium thiosulfate corresponded with 0.133 mg glucose, 0.139 mg arabinose, 0.157 mg galactose. (b) In the first experiments 5.9 mg of the hot-water-soluble hemicellulose IV was placed into 5 tubes containing 5 ml N hydrochloric acid and the mixture was kept at 100° for 2, 4, 6, 8 and 10 hours. The hydrolyzate after neutralization was analyzed for reducing sugar. The result expressed as galactose is shown by curve I of Fig. 2. (c) In the second experiments hydrolysis of 6.15 mg hemicellulose IV with 5 ml N hydrochloric acid was carried out in the same way as above for 1, 2, 3, 4 and 5 hours. The result is shown by curve II of Fig. 2.

5. Determination of uronic acid.

The method of Dickson et al.\textsuperscript{13} was applied with success. The result is shown in Table II.


The Tollens distillation method was applied. The experimental data are shown in Table III.

7. Determination of total "galactose."

The method of Van der Haar\textsuperscript{19} was applied. Preliminary experiments with pure galactose showed that the recovery was 89.9%. When 0.3874 g of sample-

Table II
Determination of uronic acid.

<table>
<thead>
<tr>
<th>Sample, g</th>
<th>Titration, ml</th>
<th>Uronolactone, g</th>
<th>%</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4737</td>
<td>10.86</td>
<td>0.09164</td>
<td>19.34</td>
<td>19.2 %</td>
</tr>
<tr>
<td>0.4386</td>
<td>9.96</td>
<td>0.08338</td>
<td>19.01</td>
<td></td>
</tr>
</tbody>
</table>

Note 1: The sample was dried in vacuo over phosphorus pentoxide for 16 hours.
Note 2: Uronolactone (g) = 0.0022 × titration (ml) × 0.9589 × 4, where 0.9589 is the factor of HCl soln.

Table III
Determination of furfural yield.

<table>
<thead>
<tr>
<th>Sample</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Furfural phloroglucide</td>
<td>0.3387 g</td>
</tr>
<tr>
<td>Furfural phloroglucide to be formed from galacturonolactone4</td>
<td>0.0272 g</td>
</tr>
<tr>
<td>Furfural phloroglucide to be formed from pentose</td>
<td>0.0680 g</td>
</tr>
<tr>
<td>Pentosan</td>
<td>0.0603 g or 17.8 %</td>
</tr>
</tbody>
</table>

Note 1: 0.3387 g × 0.1918 (see Table II)/2.39 = 0.027188, where 2.39 is the factor for converting phloroglucide to galacturonolactone51.

IV dried with the Abderhalden drier for 5 hours was added with 60 ml nitric acid (d = 1.15) and the mixture was concentrated to 20 g, a little dark green precipitate was formed. (This precipitate removed and dried weighed 0.018 g or amounted to 0.46% of IV; its nature was not examined.) After filtration of this minute amount of precipitate, the filtrate and washing (with water) were combined and concentrated to 20 g. Mucic acid (0.5101 g) was added and the mixture was allowed to stand for 48 hours with intermittent stirring and rubbing of the internal wall of the beaker. The crystals of mucic acid weighed 0.6870 g after filtration, washing, and drying. Thus the "galactose" content was (0.6870 - 0.5101)/0.3874 × 100/89.9 = 70.6%.

8. Determination of pentose and galactose.

Albaum and Umbreit20 reported the method to determine separately various pentoses and pentose phosphates based upon different degrees of the coloration in the orcinol pentose reaction. Drury21 extended this method to determine colorimetrically pentoses and glucose. Under certain conditions glucose and pentose behaved

Table IV
Colorimetry of the hydrolyzed sample by the Drury method.

<table>
<thead>
<tr>
<th>Sample, γ</th>
<th>R</th>
<th>G</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41.1</td>
<td>51.4</td>
<td>82.3</td>
</tr>
<tr>
<td>Reading R</td>
<td>0.069</td>
<td>0.098</td>
<td>0.173</td>
</tr>
<tr>
<td>Reading G</td>
<td>0.039</td>
<td>0.052</td>
<td>0.088</td>
</tr>
<tr>
<td>Arabinose, %</td>
<td>7.88</td>
<td>11.1</td>
<td>14.2</td>
</tr>
<tr>
<td>Galactose, %</td>
<td>70.2</td>
<td>72.5</td>
<td>74.5</td>
</tr>
</tbody>
</table>

Note: R and G are the readings of the mixture of the sample and the reagents with red (660 mμ) and green (540 mμ) filters, respectively. For calculation, see the reference62.)

— 73 —
differently in the orcinol reaction, and
the colorimetric readings at intervals
gave the pentose content within a 2% error after suitable mathematical treat-
ment. We have determined arabinose
and galactose by the Drury method
with the result as shown in Table IV.
The details of this method are describ-
ed elsewhere25.

9. Detection of component sugars
by paper chromatography.

9.1. Detection of xylose, arabi-
nose, and galactose. About 8 ml of
the solution (containing 12.34 mg of
sample hemicellulose IV per 10 ml)
was heated with 10 ml N hydrochloric
acid in a boiling-water bath for 5 hours.
The acid hydrolyzate was neutralized
by mixing with the resin Amberlite
IRA 400 (previously activated with 0.5N
sodium hydroxide). The filtrate at pH
7.0 was concentrated in vacuo to about
1 ml. This was kept in a test tube
with absolute alcohol. The concentra-
tion of sugar was about 0.96%. The
result of chromatography is shown in
Table 5. The technique used was the
one-dimensional ascending method.
The developing reagent used was aniline
hydrogen phthalate. The spot of ga-
lactose was brown, while the spots of
xylose and arabinose were pink. The
spot of the sample corresponding to
galactose was always more strongly
colored than the pentose spots.

9.2. Detection of uronic acid as
well as the three sugars. About
0.01 g of the sample hemicellulose IV
was heated in sealed tubes with 0.2 ml
4% sulfuric acid. After 1, 6, 12 and
24 hours the acid hydrolyzate was
directly chromatographed on paper.
The one-dimensional ascending tech-
nique was used with anisidine hydro-

---

Table V

<table>
<thead>
<tr>
<th>Solvent</th>
<th>BuOH-AcOH-H₂O (2:4:1)</th>
<th>BuOH-pyridine-H₂O (6:4:5)</th>
<th>PhOH-H₂O (85:15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.  H.</td>
<td>S.  H.</td>
<td>S.  H.</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.152</td>
<td>0.158</td>
<td>0.362</td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.078</td>
<td>0.083</td>
<td>0.282</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.430</td>
<td>0.447</td>
<td>0.473</td>
</tr>
</tbody>
</table>

Note: S.: Rₜ for standard sugars. H.: Rₜ of spots from hemicellulose.

Table VI (a, b, c)

<table>
<thead>
<tr>
<th>Solvent: BuOH-AcOH-H₂O (5:2:3)</th>
<th>Corresponding sugar</th>
<th>Color of spot</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentose</td>
<td>Red</td>
<td>0.41</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>Yellow</td>
<td>0.33</td>
<td>0.295</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uronic acid</td>
<td>Red</td>
<td>0.28¹</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Aldobiuronic acid (?)</td>
<td>Faint</td>
<td></td>
<td></td>
<td></td>
<td>0.145²</td>
</tr>
</tbody>
</table>

Note 1: The spot appeared later; the duration of hydrolysis and the degree of the color intensity were, resp., 1 hr., —; 6 hrs., +; 12 hrs., ++; 24 hrs., +++; while the other 2 spots always gave a higher coloration (+++).

Note 2: This spot appeared faintly only after 24 hrs.
chloride as the developing reagent. The chromatogram is shown in Table 6 (a). The rest of the hydrolyzate was combined. After neutralization with barium carbonate (b), 4 parts of ethanol were added to obtain the precipitate (c) or acid sugar fraction and the soluble part (d) or neutral sugar fraction. The chromatograms of (b) (neutralized hydrolyzate), (c), and (d) are shown in Table VI (b, c, d). Thus the hydrolyzed sample contained aldobionic acid (?) \((R_f 0.145 \text{ with butanol-acetic acid-water (5 : 2 : 3)})\) (cf. Mino\(^{22}\)), uronic acid, galactose, arabinose, and xylose.

### Table VI (d)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>BuOH–AcOH–H(_2)O (4 : 1 : 5)</th>
<th>PhOH–H(_2)O (5 : 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose (yellow)</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Arabinose (red)</td>
<td>0.288</td>
<td>0.31–0.32</td>
</tr>
<tr>
<td>Xylose (red)</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** S.: \(R_f\) for standard sugars. H.: \(R_f\) of spots from hemicellulose.

The chief difference between the samples III and IV concerns the contamination with starch or a similar polysaccharide giving a blue coloration with iodine. The starch present in a minute amount in soybeans\(^{23}\) does not give a positive iodine reaction when tested directly on soybeans or defatted soybeans. However, the crude polysaccharide III gave a starch-iodine reaction, evidently because starch was completely freed from the interfering substance such as fatty acids.

The hot-water-soluble hemicellulose had no reducing power; it contained pentoses (arabinose and xylose), galactose, and galacturonic acid, but no ketose or methylpentose (Tables I, V, and VI). The detection of xylose in the hydrolyzed sample was not expected and it is the first time that xylose was found as a constituent of soybean carbohydrates.

Concerning the condition of hydrolysis, this hemicellulose gave maximum reducing power when heated with N hydrochloric acid for 5 hours (see curve II of Fig. 2). Curve I of Fig. 2 shows that it lost some of its reducing power with N hydrochloric acid upon heating longer than 5 hours. This may be due to the presence of pentoses and uronic acid, which are liable to decomposition.

The analyses showed that this hemicellulose consisted of pentoses (arabi-
Calculation of true galactan content.

<table>
<thead>
<tr>
<th></th>
<th>As detd.</th>
<th>Equiv. mucic acid calc'd.</th>
<th>Calcd.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total &quot;galactose&quot;</td>
<td>70.6</td>
<td>52.2&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>21.1&lt;sup&gt;4&lt;/sup&gt;</td>
<td>17.3&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>True galactose</td>
<td></td>
<td>34.9&lt;sup&gt;3&lt;/sup&gt;</td>
<td>46.5&lt;sup&gt;4&lt;/sup&gt; as galactose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>41.5&lt;sup&gt;4&lt;/sup&gt; as galactan</td>
</tr>
</tbody>
</table>

Note 1: 70.6/1.33 = 52.2.
Note 2: 19.2 (as detd.) x 194 (M.W. of C\(_6\)H\(_{10}\)O\(_5\)/176 (M.W. of C\(_6\)H\(_8\)O\(_6\)) = 21.1.
Note 3: 21.1 < sup>1</sup> of 194 (M.W. of C\(_6\)H\(_{10}\)O\(_5\)/194 (M.W. of C\(_6\)H\(_{10}\)O\(_5\)/176; since only 76% of theory can be recovered as mucic acid from galacturonic acid.<sup>24</sup>)
Note 4: 34.9 x 1.33 = 46.5.
Note 5: 46.5 x 0.90 = 41.9.

Note 1: 70.6/1.33 = 52.2.
Note 2: 19.2 (as detd.) x 194 (M.W. of C\(_6\)H\(_{10}\)O\(_5\)/176 (M.W. of C\(_6\)H\(_8\)O\(_6\)) = 21.1.
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Note 4: 34.9 x 1.33 = 46.5.
Note 5: 46.5 x 0.90 = 41.9.

The true galactose or galactan content can be calculated from the value of "galactose" which is determined by oxidation to mucic acid (70.6%, paragraph 7) and the value of galacturonolactone (19.2%, Table I). This correction is necessary, since not only galactose but also galacturonic acid gives rise to mucic acid by oxidation. As shown in Table VII, the true galactan content is 41.9%.

The result of colorimetry by the Drury method (11.4% pentose and 73.9% galactose) (Table IV) is not reliable, since the hydrolyzed hemicellulose contains not only pentoses and hexose but also hexuronic acid.

In conclusion this hemicellulose consists of 17.8% pentoses (as pentosan), 19.2% galacturonic acid (as lactone), and 41.9% galactose (as galactan), the pentoses being arabinose and xylose.

References

6) S. Kawamura, (Part II), Ibid., 5, 1 (1953).
10) S. Kawamura, ibid., 78.
15) This derfated soybean flake was donated by Ajinomoto Co., through the courtesy of Mr. Mitio Goto of the Yokohama Factory.
16) F. Ehrlich, Ber., 65, 552 (1952).

Kagawa Agricultural College,
Kagawa-ken;
and Faculty of Agriculture,
University of Tokyo,
Tokyo.

[Received, Oct. 14, 1954]