# Hemicellulose Components of Rice

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In the first systematic study of hemicelluloses of rice bran, rice polish, and polished rice, these fractions were quantitatively determined and the isolated polysaccharides were examined by paper chromatography. Rice grain hemicelluloses are composed chiefly of arabinose and xylose in a ratio of approximately 1 to 1, and contain small quantities of galactose, mannose, and uronic acid. These data provide basic information for use in the production, processing, and utilization of rice.

THE POLYSACCHARIDE FRACTIONS of the cereal grains, considered to be hemicelluloses, have recently been given increased attention, with the exception of the rice grain (*9*, *21*, *23*, *24*, *30*). The chemical composition of rice has been extensively investigated (*14*), but published data on the hemicellulose components are limited and, because this group of polysaccharides has not been clearly defined (*11*, *12*, *26*), the data and the terminology have varied with the experimental procedure. The data submitted herein were obtained with the polysaccharide material that was solubilized by aqueous potassium hydroxide and removed from the acidified solution by precipitation with ethyl alcohol.

## Materials and Methods

Brown rice (California Pearl) of the 1953 crop was experimentally milled, using a standard McGill milling machine. The first pass through the machine (10-pound load, 30 seconds) yielded a bran fraction of 6.3%; the second pass (6-pound load, 30 seconds) yielded a polish fraction of 1.7%. The bran, polish, and polished rice were stored at 4° C. until needed for the experiments. The general composition of the three fractions, determined by standard procedures (*2*), is given in Table I.

Each of the three fractions was extracted 40 hours in a Soxhlet apparatus with a mixture of ethyl alcohol–benzene (32 to 68 v./v.), followed by a 24-hour extraction with 80% ethyl alcohol. With forceps and magnifying lens, rice fragments too large to pass a 20-mesh screen were removed as completely as possible from the bran and the polish fractions. All fractions were ground in a Wiley mill to pass a 40-mesh screen and treated according to the procedure indicated in Figure 1.

**Table I. General Composition of Rice Fractions Used for Polysaccharide Studies[a]**

| | Bran, % | Polish, % | Polished Rice, % |
|---|---|---|---|
| Protein (N × 5.95) | 14.10 | 10.77 | 6.66 |
| Crude fat (ether extract) | 18.81 | 9.73 | 0.35 |
| Crude fiber | 5.74 | 1.12 | 0.23 |
| Ash | 11.25 | 5.77 | 0.47 |
| Reducing sugars[b] | 0.32 | 0.16 | 0.05 |
| Total sugars[b] | | | |
| Invertase hydrolysis | 7.55 | 3.20 | 0.22 |
| Hydrochloric acid hydrolysis | 7.73 | 3.20 | 0.20 |

[a] Moisture-free basis.
[b] As glucose.

The extractive-free residues (free from benzene- and ethyl alcohol–solubles) were suspended in distilled water, heated to gelatinize the starch, and cooled. A few drops of chloroform were added and the mixtures were continuously stirred for 24 hours at room temperature (25° C.). The suspensions were centrifuged and washed thoroughly with distilled water. The slightly turbid water extracts were filtered through a mat of Celite to give a sparkling clear solution. The extracts were eventually examined for water-soluble polysaccharides.

Preparatory to amylase treatment of the three residues from the water-extraction treatment, the three fractions were again suspended in distilled water, boiled to "sterilize" the mixtures, and cooled to room temperature. Filtered, freshly prepared salivary amylase and a few drops of chloroform were added and the mixtures were incubated at 30° C. for 24 hours with constant agitation. Additional treatments of the three residues with salivary amylase together with periodic examinations of the hydrolyzates by paper chromatography indicated that a 24-hour period was not sufficient for complete solubiliza-

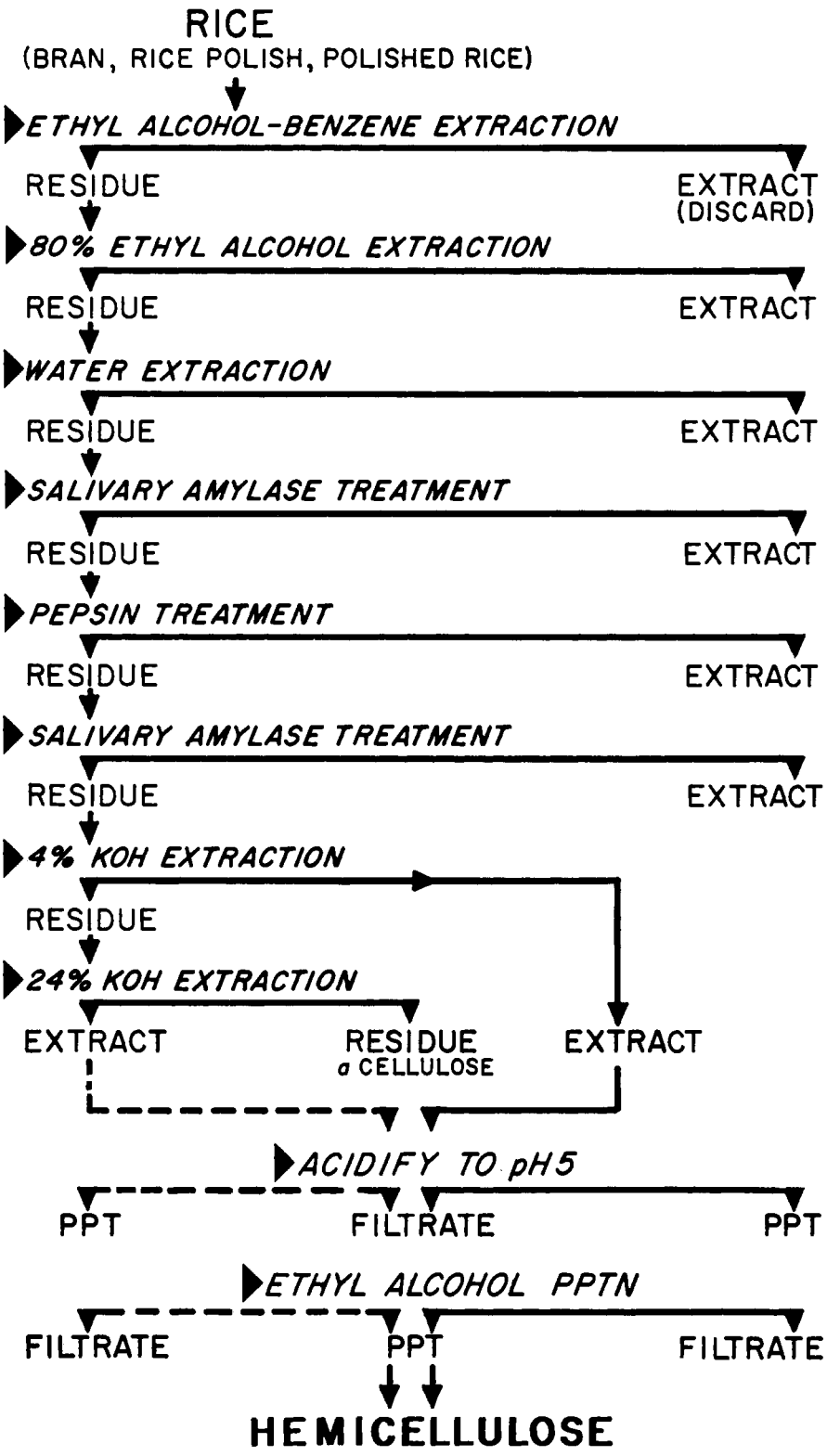


Figure 1. Method of preparing hemicelluloses

tion of the starch. Approximately 400 hours were required to remove the starch from the polished-rice fraction. At 24-hour intervals the mixture was centrifuged and the residue resuspended in distilled water containing freshly prepared salivary amylase. The bran and polish fractions were treated 72 hours with salivary amylase. Between the fourth and fifth amylase treatments of the polished rice and between the second and last treatments of the bran and the polish, each residue of the three fractions was suspended in a 0.06*N* hydrochloric acid solution. An excess of crystalline pepsin was added and the solutions were agitated for 3 hours at 25° C. The residues were removed by centrifugation and washed thoroughly with distilled water, and the treatment with salivary amylase was repeated as described above. In a typical experiment, the amounts of solids extracted by each procedure are shown in Table II.

The residues from the final amylase treatment were suspended in 4% potassium hydroxide solutions and agitated under nitrogen for 24 hours. The suspensions were centrifuged, and the residues were washed with water, then with aqueous 2% acetic acid solution, and again with water until the washings were neutral. The residues were suspended in 24% potassium hydroxide solutions and again agitated under nitrogen for 24 hours, followed by centrifuging and thorough washing of the final residues, which were considered to be α-cellulose (*29*).

The 4 and 24% potassium hydroxide extracts were adjusted to pH 5 with 50% acetic acid and allowed to stand for 24 hours at room temperature. The precipitates formed were removed by centrifugation and washed thoroughly with water, then with aqueous acetic acid solution, water, and finally absolute ethyl alcohol. The slightly turbid supernatant liquids were filtered through mats of Celite to give crystal-clear solutions which were added slowly with constant agitation to sufficient absolute ethyl alcohol to give a final concentration of 80% ethyl alcohol. After standing at room temperature for 24 hours, the precipitates were centrifuged and washed thoroughly with 80%, 95%, and absolute ethyl alcohol, respectively. The supernatant liquids were filtered through mats of Celite and the alcohol was removed by evaporation on a steam bath. The remaining aqueous solutions were passed through columns of ion exchange resins Amberlite IR-120 and Duolite A-4, respectively. The deionized solutions were brought to dryness on a steam bath and later examined for any polysaccharides that may not have been

**Table II. Solids Extracted by Various Treatments of Rice Fractions[a]**

| Treatment[b] | Bran, % | Polish, % | Polished Rice, % |
|---|---|---|---|
| Benzene–ethyl alcohol extraction | 21 | 14 | 1.5 |
| 80% ethyl alcohol extraction | 9 | 2 | 0.2 |
| Rice fragments > 20 mesh, mechanically removed | 10 | 26 | ... |
| Salivary amylase hydrolysis | 33 | 43 | 91.0 |
| Pepsin digestion | 7 | 5 | 4.0 |

[a] All fractions on moisture-free basis.
[b] Listed in order in which they were accomplished.

removed by the acetic acid and ethyl alcohol treatments.

The acetic acid and ethyl alcohol precipitates, the α-cellulose fractions, and the solids obtained from the water, pepsin, salivary amylase, and alcohol filtrates were treated for 2 hours with 1*N* hydrochloric acid in sealed tubes in a boiling-water bath. Additional acid hydrolyses on the fractions were made for 4 and 6 hours with 1*N* hydrochloric acid. Aliquots of the hemicellulose fractions were also treated with 38% hydrochloric acid for 16 hours at 0° C. (*26*) prior to diluting to 1*N* and heating for 2 hours. In addition, portions of the solids from the water extracts were treated with salivary amylase for 24 hours at 30° C.

The acid and enzyme hydrolyzates were examined for sugars by paper chromatography (*8*, *28*). For approximations of the amounts of the component sugars on the paper chromatograms the aniline–trichloroacetic acid spray reagent was used (*28*); the sprayed paper was heated for 2 minutes at 105° C. and immediately thereafter the developed spots were read with the Photovolt reflectance unit with a light of 515 mμ wave length (*4*, *16*). A dip reagent consisting of 4 grams of aniline in 100 ml. of acetone, 4 grams of diphenylamine in 100 ml. of acetone, and 20 ml. of 85% phosphoric acid (*7*, *10*) was also used to examine the glucose polymers obtained from the starch hydrolyzates.

No corrections were made in the analytical data for any endosperm that was unavoidably included in the bran and polish fractions.

### Results and Discussion

Examination of the 80% ethyl alcohol extracts confirmed earlier reports (*17*, *20*, *27*) that raffinose, sucrose, glucose, and fructose were the only free sugars present in polished rice. This same sugar pattern was apparent in the bran and polish fractions, differing only in that the two fractions contained a larger quantity of raffinose and sucrose.

Data on the bran, polish, and polished-rice solids soluble in water are shown in Table III. Soluble solids decreased appreciably from the bran layer to the endosperm, partly as a result of the high phytin content of the bran and polish fractions. Partial compositional data (Table III) show that water-soluble polysaccharides accounted for 30% of the soluble solids in the bran, 75% in the polish, and 93% in the polished rice. These values may be low because of the presence of limit dextrins not readily hydrolyzed. Paper chromatographic examination of the acid-hydrolyzed water extracts indicated that glucose was the principal sugar with trace amounts of arabinose and xylose present in all three fractions. Chromatograms of the amylase-treated water extracts showed glucose, maltose, and a series of glucose polymers larger in molecular weight than maltose. The sugar pattern was the same in all three fractions. The results obtained from the salivary amylase treatment (*3*), together with the specific color reactions produced by the aniline-diphenylamine reagent (*1*) illustrated that the water-soluble polysaccharides were primarily composed of glucose polymers in which the glucose units were combined mainly through 1,4-glucosidic bonds having the α-configuration. Such evidence suggests that this water-soluble glucan is part of the starch component of the rice grain.

At least three components of rice are difficult to remove wholly without seriously denaturing other constituents present—i.e., pectinaceous material, starch, and protein. No attempt was made to remove and evaluate any "true" pectic substances that may have been present in the rice fractions. It would have required methods and reagents which may have degraded or contaminated other components in the grain (*26*). The mildest extraction procedures believed feasible were used for the removal of starch and protein prior to the extraction of the hemicellulose fractions.

As much as 95% of the solids of polished rice may be starch (*15*), and it was realized earlier in the experimental procedure that salivary amylase treatment would not remove so large a quantity within a short period of time (*18*, *26*). The first 24-hour salivary amylase treatment removed 80% of the extractive-free rice solids. After 96 hours of enzyme hydrolysis 92% of the solids had been solubilized, including 0.5% protein and 0.3% ash. Pepsin treatment of the residue removed 5% of the solids or 80% of the original protein.

**Table III. Water-Soluble Components of Rice Fractions[a]**

| | Bran, % | Polish, % | Polished Rice, % |
|---|---|---|---|
| Extractive-free solids | 55 | 57 | 98 |
| Amount of extractive-free solids soluble in water | 11 | 9 | 2 |
| Partial Composition of Extractive-Free Solids Soluble in Water | | | |
| Polysaccharides (glucose polymers[b]) | 30 | 75 | 93 |
| Phytin (P × 4.4) | 40 | 12 | 2 |
| Protein (N × 5.95) | 5 | 2 | 1 |

[a] Moisture-free basis.
[b] Sugars obtained by action of salivary amylase on water-soluble components.

**Table IV. Amount of Hemicelluloses and α-Cellulose in Extractive-Free Rice Fractions[a]**

| | Bran, % | Polish, % | Polished Rice, % |
|---|---|---|---|
| 4% KOH | 5.9 | 1.7 | 0.10 |
| 24% KOH | 2.8 | 0.9 | 0.06 |
| α-Cellulose | 10.4 | 1.4 | 0.17 |

[a] Moisture-free basis.

The additional 300-hour amylase treatment progressively removed an additional 1.5% of rice solids, of which 25% was protein and 6% was ash. The residue remaining after the final amylase treatment amounted to 1% of the extractive-free polished rice solids, and 42% of this residue was protein (N × 5.95). A detailed study by paper chromatography of the extracts obtained by the action of salivary amylase on rice solids showed that the sugars present were those ordinarily derived from the action of α-amylase on starch—i.e., maltose, glucose, and a series of glucose polymers.

The extracts obtained by the treatment of the rice solids with pepsin contained no sugars other than traces of arabinose and xylose.

Examination of the precipitates formed by the acidification, pH 5, of the 4 and 24% potassium hydroxide extracts with acetic acid showed that they were composed of glucose and protein material with but a trace of arabinose and xylose.

The quantity of hemicellulose material obtained from the three extractive-free rice grain fractions are shown in Table IV. The values include small amounts of protein, ash, and some dextrins. Protein was present in amounts of 1 to 4% of the hemicellulose material; ash content ranged from 2 to 10%, in part because of the presence of residual acetate salts. Dextrins formed during the fractionation procedure become increasingly difficult to remove because of limited solubility and increased resistance to hydrolysis (*18*, *25*). The data in Table IV show that 4% potassium hydroxide did not completely remove all of the hemicellulose material from the fractions; that the 24% potassium hydroxide removed an additional amount equal to 50% of that removed by the 4% alkali treatment. The largest amount of hemicellulose material was removed from the bran fraction, with the smallest quantity appearing in the polished rice.

Examination by paper chromatography of the acid-hydrolyzed hemicellulose components of the bran, polish, and polished-rice fractions showed that they had a very similar qualitative sugar pattern, as illustrated in Table V. Galactose, glucose, arabinose, and xylose

were found in the 4% potassium hydroxide extracts of the three fractions. The pattern was the same for the 24% potassium hydroxide extracts with the additional observation that mannose appeared in all fractions. The residue from the three rice fractions, assumed to be α-cellulose, contained very small quantities of galactose, mannose, arabinose, and xylose in addition to glucose (*26*). Polished rice α-cellulose contained about 10% mannose, and galactose could not be detected. In all fractions, except the α-cellulose fraction, the principal sugars were arabinose and xylose, occurring apparently in the ratio of 1 to 1. The amounts of galactose present were small: 1 to 5% of the hemicellulose fraction. Mannose appeared in the order of 0 to 2%, with indications of even a larger quantity in the α-cellulose fractions. Whether the glucose present in the hemicellulose fractions was derived wholly from contaminating dextrins or was in part a component of the hemicelluloses was not proved.

A uronic acid was observed by paper chromatography but not identified in the hemicellulose fractions of the bran, polish, and polished rice.

The solutions remaining after the removal of the ethyl alcohol precipitates contained no sugars other than traces of arabinose and xylose, indicating that the final solutions contained little readily hydrolyzable polysaccharide material.

Increasing the time of acid hydrolysis progressively from 2 to 6 hours did not change the qualitative sugar pattern on the chromatograms; a 2-hour period of hydrolysis proved to be sufficient. The hydrolyzates from the solutions treated longer than 2 hours and the ones treated with 38% hydrochloric acid became progressively darker in color, possibly because of the formation of decomposition products (*19*, *26*). In all fractions, a considerable amount of residue remained after acid hydrolysis, regardless of the time of treatment or the concentration of the acid. Additional periods of acid hydrolysis of such residues failed to produce any significant amounts of additional sugars.

The data show that the hemicelluloses of the rice grain are a complex fraction not readily resolved into polysaccharides of single sugar units and that this complex, which is apparently uniform in composition, is distributed throughout the rice grain.

The problem of proving that a hemicellulose preparation is homogeneous or heterogeneous, or that a "pure" polysaccharide may be composed of several different sugar units, has been the basis for considerable research and discussion (*5*, *11–13*). Before a pure polysaccharide can be isolated from rice and examined structurally, improved methods must be found to remove extraneous materials. To elaborate further on the true nature of the hemicellulose complex of rice will require additional studies (*6*, *22*, *24*), beyond the scope of this paper.


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**Table V. Sugars Present in Hemicellulose and α-Cellulose Fractions of the Bran, Polish, and Polished Rice**

| Fraction | Galactose | Glucose | Mannose | Arabinose | Xylose |
|---|---|---|---|---|---|
| Bran | | | | | |
| 4% KOH | + | + | − | + | + |
| 24% KOH | + | + | + | + | + |
| α-Cellulose | + | + | + | + | + |
| Polish | | | | | |
| 4% KOH | + | + | − | + | + |
| 24% KOH | + | + | + | + | + |
| α-Cellulose | + | + | + | + | + |
| Polished rice | | | | | |
| 4% KOH | + | + | − | + | + |
| 24% KOH | + | + | + | + | + |
| α-Cellulose | − | + | + | Trace | Trace |


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