the 1→4 linkage between the two xylose units of the mono-*O*-methylaldotriouronic acid (V) is of the β- type and consequently would have the structure 4-*O*-methyl-α-D-GpA-(1→2)-β-D-Xylp-(1→4)-D-Xyl. Using Hudson's optical superposition rules and specific rotations (in water) of +150° for methyl 4-*O*-methyl-α-D-glucosiduronamide (*21*, *26*), −50° for methyl 4-*O*-methyl-β-D-glucosiduronamide (*21*, *26*), +154° for methyl α-D-xylopyranoside, −66° for methyl β-D-xylopyranoside, +94° for α-D-xylose, and −30° for β-D-xylose (*3*), specific rotations were calculated for the four mono-*O*-methylaldotriuronic acids. The calculated specific rotations of +134° and +105° for 4-*O*-methyl-α-D-GpA-(1→2)-α-D-Xylp-(1→4)-α-D-Xyl and 4-*O*-methyl-α-D-GpA-(1→2)-α-D-Xylp-(1→4)-β-D-Xyl, respectively, are much higher than that determined experimentally (+45°). The calculated specific rotations of +65° and +33°, respectively, for 4-*O*-methyl-α-D-GpA-(1→2)-β-D-Xylp-(1→4)-α-D-Xyl (Va) and 4-*O*-methyl-α-D-GpA-(1→2)-β-D-Xylp-(1→4)-β-D-Xyl (Vb) are in fair agreement with the observed value of +45°, indicating that the linkage between the two D-xylose residues is of the β- type. The fact that the mono-*O*-methylaldotriouronic acid displays an upward mutarotation (+45°→+52°) requires that the terminal reducing D-xylose unit exist in the β- form. From these data the mono-*O*-methylaldotriouronic acid is tentatively designated *O*-4-*O*-methyl-α-D-glucosyl-uronic acid-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylose (Vb) (*23*).

Acknowledgment

The authors thank J. K. Hamilton for a sample of the crystalline mono-*O*-methylaldotriouronic acid (Vb) and T. E. Timell for a specimen of the acetate (IV). They also thank M. O. Schultze for his interest in this work.

Literature Cited

(1) Abdel-Akher, M., Smith, F., *Nature* **166,** 1037 (1950).
(2) Aspinall, G. O., Hirst, E. L., Matheson, N. K., *J. Chem. Soc.* **1956,** 989.
(3) Bates, F. J., associates, "Polarimetry, Saccharimetry and the Sugars," Natl. Bur. Standards, Circ. **C440,** 433 (1942).
(4) Briggs, D. R., Garner, E. F., Montgomery, R., Smith, F., *Anal. Chem.* **28,** 333 (1956).
(5) Derungs, R., Deuel, H., *Helv. Chim. Acta* **37,** 657 (1954).
(6) Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., Smith, F., *Nature* **168,** 167 (1951); *Anal. Chem.* **28,** 350 (1956).
(7) Erlich, F., *Ber.* **65,** 352 (1932).
(8) Hamilton, J. K., Thompson, N. S., *J. Am. Chem. Soc.* **79,** 6464 (1957).
(9) Hirst, E. L., Mackenzie, D. J., Wylam, C. B., *J. Sci. Food Agr.* **10,** 19 (1959).
(10) Huffman, G. W., Smith, F., *J. Am. Chem. Soc.* **77,** 3141 (1955).
(11) James, S. P., Smith, F., *J. Chem. Soc.* **1945,** 739.
(12) Jones, J. K. N., Wise, L. E., *Ibid.*, **1952,** 3389.
(13) Kuhn, R., Trischmann, H., Löw, I., *Angew. Chem.* **67,** 32 (1955).
(14) Link, K. P., Dickson, A. D., *J. Biol. Chem.* **86,** 491 (1930).
(15) Lythgoe, B., Trippett, S., *J. Chem. Soc.* **1950,** 1983.
(16) Montgomery, R., Smith, F., Srivastava, H. C., *J. Am. Chem. Soc.* **78,** 2837 (1956).
(17) Myhre, D. V., Smith, F., unpublished work.
(18) Phillips, M., Davis, B. L., *J. Agr. Research* **60,** 775 (1940).
(19) Schinle, R., *Ber.* **65,** 315 (1932).
(20) Smith, F., *J. Chem. Soc.* **1939,** 1724.
(21) *Ibid.*, **1951,** 2646.
(22) Srivastava, H. C., Adams, G. A., *J. Am. Chem. Soc.* **81,** 2409 (1959).
(23) Srivastava, H. C., Bishop, C. T., Adams, G. A., Abstracts, 136th Meeting, ACS, Atlantic City, N. J., 1959, p. 5D.
(24) Timell, T. E., *Can. J. Chem.* **37,** 827 (1959).
(25) Weissman, B., Meyer, K., Sampson, P., Linker, A., *J. Biol. Chem.* **208,** 417 (1954).
(26) White, E. V., *J. Am. Chem. Soc.* **69,** 2264 (1947).
(27) Wise, L. E., Murphy, M., D'Addieco, A. A., *Paper Trade J.* **122** [2], 35 (1946).
(28) Zemplén, G., *Ber.* **59,** 1258 (1926).

Received for review October 2, 1959. Accepted April 27, 1960. Paper 4222, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul. Work supported in part by funds from the U. S. Department of Agriculture, Regional Project NC-25.

RICE AMYLOSES

Molecular Weights of Crystalline Amyloses from Certain Rice Varieties

HSIU YING TSAI, ALLEN T. PHILLIPS, and VIRGINIA R. WILLIAMS

Department of Agricultural Chemistry and Biochemistry, Louisiana State University, Baton Rouge, La.

ALTHOUGH the composition of rice with regard to its major constituents has been studied extensively, the processing characteristics of any variety cannot be predicted on the basis of present compositional information. Certain helpful generalizations can be made regarding amylose-amylopectin ratios and probable cooking quality, but exceptions can always be found (*19*). Consideration of grain type is also useful, but not infallible. Fundamental research is needed in all phases of rice composition to facilitate a more rational interpretation of existing information.

Because the principal chemical constituent of rice is starch, any differences in the chemical and physical properties innately pertaining to the different types of starch present should be reflected in the properties of the rice itself. For this reason, it was desirable to study the physical properties of the amyloses of rices of widely different processing characteristics. The present report describes a study of the crystallization and molecular weight determination of amyloses from four distinctly different varieties: Rexoro, Zenith, Century Patna, and Caloro.

Experimental

Samples of Zenith, Century Patna, and Rexoro rices were obtained from Crowley,

The molecular weights of amyloses crystallized from several varieties of rice were determined to obtain basic compositional information which might be related to known processing behavior. The amylose molecular weights as determined by periodate oxidation were found to be: Century Patna 231, 29,000; Rexoro, 41,000; Zenith, 41,000; and Caloro, 48,000. These findings show a possible relationship to water absorption and alkali dispersibility of the varieties.

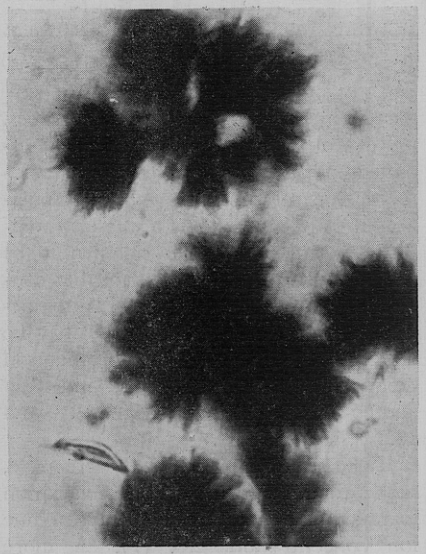

Figure 1. Rice amylose crystals from Rexoro variety

Precipitated with butyl and isoamyl alcohols and stained with iodine. Magnification, 1000 diameters

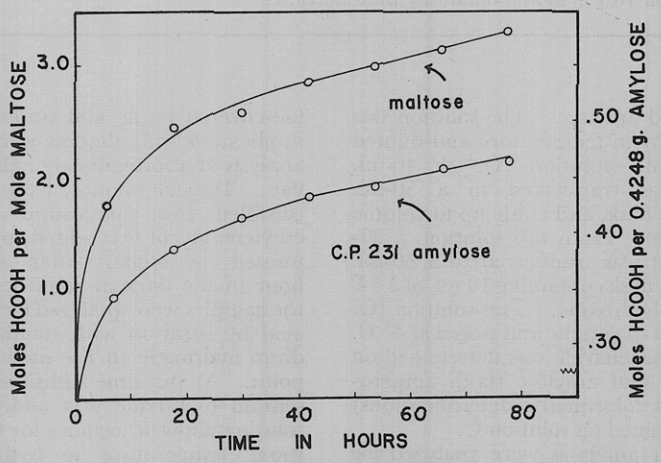

Figure 2. Total formic acid produced by periodate oxidation of maltose and Century Patna 231 amylose

La. The Caloro sample was obtained from Biggs Station, Calif. The rice was 1957 crop and the experiments were conducted during 1957–59.

Preparation and Purification of Crystalline Amylose. Well purified rice starch was prepared by the method of Hogan (*10*). The starch was fractionated by the method of Wilson, Schoch, and Hudson (*20*), modified to extend the cooling period. Approximately 48 hours in a Dewar flask was required for the solution to cool below 50° C. At this time a small amount of precipitated fraction was withdrawn, placed on a microscope slide, stained with iodine solution, and examined under the microscope. If amylose crystals were not present, the mixture was allowed to stand for a longer time. Crystals obtained at this stage were in the shape of rosettes or spherules and stained an intense blue with iodine (Figure 1).

The following method was found the most satisfactory for recrystallizing the rice amylose. The crude moist amylose fraction was dissolved in boiling water with vigorous stirring to give an approximately 0.2% solution. The hot solution was centrifuged as rapidly as possible to avoid cooling; the supernatant solution was decanted carefully and reheated to 100° C. Excess butyl alcohol was added and the solution vigorously shaken for 5 minutes, then shaken frequently for the next 2 hours. After standing overnight, the solution contained well-formed hairlike needles of crystalline amylose. The advantage of this method over the usual procedure of dissolving amylose in water previously saturated with alcohol lies in the ease of solution of the amylose in boiling water, as compared with alcohol-saturated water. No retrogradation occurs in the hot solution, so that the presence of butyl alcohol is not necessary during the dissolving process.

Drying amylose presents further problems. When rice amylose is dissolved in pure water or is dried without the removal of all moisture, it undergoes retrogradation very rapidly. Extreme care must be taken to replace the water content of the amylose by butyl alcohol. Room temperature or below is preferred for drying, because at high temperature the amylose may dissolve in the adhering solvent. Under the latter condition the final product will be a solid cake instead of a fine powder.

PROCEDURE. The amylose which had been stored in water saturated with butyl alcohol was collected by centrifugation. To the precipitate approximately 2 volumes of butyl alcohol was added and the suspension stirred vigorously with a magnetic stirrer for about 1 hour. The supernatant alcohol was decanted, another 2 volumes of butyl alcohol was added, and the suspension was stirred again. This treatment was repeated until the amylose became finely divided. During the alcohol treatment (fourth or fifth operation) the amylose stuck together in a gummy mass. The stirring and alcohol treatment were continued until the mass was dispersed and a very finely divided product obtained. During stirring, care had to be taken to keep all the amylose under the surface of the butyl alcohol. If the amylose dried on the walls of the beaker before its water content had been replaced by butyl alcohol, retrograded amylose was obtained. At the end of the drying treatment, the finely divided amylose was collected on a suction filter and dried in a vacuum desiccator over concentrated sulfuric acid.

Determination of Total Polysaccharide and of Amylose. Two possible sources of error existed in the periodate oxidation of the recrystallized amylose: the presence of insoluble retrograded amylose and traces of amylopectin. Because the amylose samples could not safely be assumed to be free of these contaminants, all samples, except the Caloro check sample, were analyzed for total soluble polysaccharide and amylose. A procedure was devised for preparation of one solution, from which aliquots were taken for total soluble polysaccharide, amylose, and molecular weight determination. All determinations were carried out in duplicate.

An amylose sample containing approximately 9 grams of hot water–soluble amylose was dissolved by heating in 700 ml. of 3.0% sodium chloride solution. When solution appeared to be as complete as possible, the solution was centrifuged while hot to remove

Table I. Amylose Analysis of Hot Water–Soluble Polysaccharide Solutions Prepared from Dried Crystalline Amylose

	Colorimetric Method		Amperometric Method			
Variety	Klett reading[a]	Amylose, %	Wt. of sample, mg.	Av. ml. 0.005N KIO_3	Iodine bound, %	Amylose,[b] %
Caloro	129	82.2	5.18	1.22	15.0	82.9
Rexoro	146	93.0	5.18	1.33	16.3	90.1
Zenith	146	93.0	5.25	1.42	17.2	95.0
Century Patna	157	100.0	5.30	1.51	18.1	100.0

[a] For soln. containing 1.0 mg./100 ml.
[b] Based on 18.1% iodine bound by pure amylose.

Table II. Determination of Molecular Weights of Crystalline Amyloses

Variety	Periodate Oxidation	Viscometry
Century Patna	29,000	...
Zenith	41,000	...
Rexoro	41,000	...
Caloro	48,000	...
Caloro check sample	56,000	44,000

retrograded amylose. The solution was cooled to room temperature and diluted to 720 ml. (solution A). A 40-ml. aliquot was transferred to a 50-ml. volumetric flask, and made up to volume (solution B). From this solution, a 30-ml. aliquot was transferred to a 50-ml. volumetric flask containing 10 ml. of 2.5*N* potassium hydroxide. This solution (C) was diluted to volume and stored at 4° C. Total polysaccharide was determined on solution B and amylose (both amperometric and colorimetric determinations) was determined on solution C.

The four amyloses were analyzed for their hot water–soluble polysaccharide content by the method of Fuller, Lampitt, and Coton (*6*).

Preliminary experiments with the potentiometric determination of amylose by the method of Bates, French, and Rundle (*4*) revealed that the end point was not sharp and that the method was very time-consuming. For these reasons the amperometric method of Larson, Gilles, and Jenness (*13*) was used and an additional check was made by colorimetric analysis, using the method previously published by this laboratory (*19*). For comparison both sets of data are given in Table I. The calculation of purity was based on the Century Patna 231 sample, which bound 18.1% iodine by amperometric titration. This value agrees with the report of Rao, Murthy, and Subrahmanya (*18*) that pure amylose binds 18.2% iodine.

Periodate Oxidation. The periodate oxidation methods described by Potter and Hassid (*16*) and Potter *et al.* (*17*) were tested in preliminary experiments. When the latter method was used, the rice amylose solution precipitated before the oxidation was complete. The concentration of the sodium metaperiodate was then adjusted so that only 2 or 3 days' oxidation was required.

Both free and total formic acid were determined as described by Wolff *et al.* (*22*). Pfanstiehl maltose monohydrate was used as a standard. Other chemicals were reagent grade.

The 680 ml. of solution A remaining after removal of sample for amylose and total polysaccharide analysis was combined with 170 ml. of sodium metaperiodate solution (8.0 grams per 100 ml.) after both solutions had been cooled to 6° C. The solution was placed in a foil-covered bottle and stored at 6° C.; duplicate 50-ml. aliquots were taken for analysis at approximately half-day intervals. To each aliquot, 1 ml. of purified (distilled from potassium hydroxide) ethylene glycol was added to react with unused periodate. After standing 1 hour in the dark at room temperature, the samples were analyzed for free formic acid by titration with standardized sodium hydroxide to the methyl red end point. At this time additional standard sodium hydroxide was added and the solution allowed to stand for 0.5 hour at room temperature to hydrolyze the formyl ester. The solution was then back-titrated with standard sulfuric acid. The reaction was assumed to be complete when 3 moles of formic acid had been produced per mole of maltose.

Oxidation curves for the maltose standard and the Century Patna 231 amylose are given in Figure 2.

Viscometry. The intrinsic viscosity was determined with an Ostwald-Cannon-Fenske viscometer according to the method of Wolff, Gundrum, and Rist (*21*). Separate solutions in 1.0*N* potassium hydroxide were prepared.

Results and Discussion

Total Polysaccharide and Amylose Determinations. Preliminary analyses of the four dried amylose preparations for total hot water–soluble polysaccharide gave 91.4, 98.5, 86.1, and 86.5% for Zenith, Rexoro, Caloro, and Century Patna 231, respectively. The insoluble material was judged to be retrograded amylose. Sample sizes were then adjusted to give solutions of approximately the same concentration of soluble polysaccharide. Total polysaccharide was then redetermined on the final solutions, which were free of insoluble material. With the exception of the second study on Caloro amylose, both amperometric titration and colorimetric analyses were used in determining amylose on the hot water-soluble fraction. Table I shows that the purity of the amyloses increased in the order: Caloro, Rexoro, Zenith, and Century Patna 231. The impurity was treated as amylopectin, for reasons stated below. Because of the low purity of the Caloro amylose, a check sample was prepared which was recrystallized three times.

Regardless of whether amylose is determined by the amperometric, potentiometric, or colorimetric method, calculations are always based on a standard well-purified sample which is assumed to be free of amylopectin. The fact that considerable variation is reported in the iodine-binding capacity of rice amylose may be significant. For example, the Anderson group (*1*), MacMasters (*15*), and Rao and coworkers (*18*) report 19.2, 19.8, and 18.2%, respectively.

If the samples in question were pure, such differences could arise from variation in pretreatment, pH, temperature, iodine ion concentration, etc. (*7*). A more disturbing explanation would be that such differences reflect true varietal differences in iodine binding. Inoue and Onodera (*12*) have reported that the intensity of the iodine coloration of amylose and amylopectin differs for each variety of rice, and therefore, the iodometric determination of amylose in a specific starch should be accompanied by a determination of the intensity of coloration of amylose and amylopectin samples initially separated from this same variety of rice. Bates, French, and Rundle (*4*) also reported that the iodine-combining capacity of amylose varies with the length of the starch chain. If these reports are correct, a completely accurate amylose determination by either the amperometric or colorimetric method would necessitate preparation of separate amyloses, absolutely pure, for each variety. As the varietal differences would probably be no greater than 5% (this being the range of variation in iodine binding for the different cereal amyloses), such a refinement in method would be ill advised for most routine work. In the study reported here, the iodine-binding differences reported for the samples in Table I had to be interpreted as varietal differences or purity differences. The latter explanation was chosen somewhat arbitrarily and the Century Patna 231 sample taken as standard, as it is customary to base analyses on one crystalline standard, the variety being disregarded. Whether or not significant varietal differences exist for the common American varieties

needs to be conclusively established, and until the matter is settled, such a possibility must be kept in mind in interpreting studies of the type reported here. The molecular weight determinations are reported in Table II after correction for amylopectin impurity on the basis of the amylose analyses given in Table I. The impurity present in the recrystallized amyloses was assumed to be amylopectin, because it is the only other hot water-soluble polysaccharide that could be present.

Periodate Oxidations. The periodate oxidation values were corrected for formic acid produced from such amounts of amylopectin, using the data of Halsall, Hirst, and Jones (*9*) as a basis for the calculation. The use of their data gave the correction: 3.125×10^{-4} mole of formic acid per gram of amylopectin.

The molecular weights of the amyloses were calculated by the formula:

$$\text{Corrected molecular weight} = \frac{A}{B - (C \times D/100 \times E)} \times C/100 \times F$$

where

- A = millimoles of formic acid per mole of maltose at 53 hours—i.e., 3000
- B = millimoles of formic acid per sample of amylose at 53 hours
- C = weight of sample, grams
- D = percentage of amylopectin impurity
- E = millimoles of formic acid per gram of amylopectin—i.e., 0.3125
- F = percentage of amylose in recrystallized preparation

The calculations were based on the colorimetric analyses, as they were in good agreement with the amperometric data and no advantage was to be gained from averaging the two.

The range of values reported in Table II exceeds the 10% error inherent in the periodate oxidation method. Errors involved in the other analytical methods employed are much smaller. In view of the preceding discussion on possible varietal differences in iodine absorption, the data of Table II indicate, in the light of existing information, that there are varietal differences in the molecular weights of the crystalline amyloses studied here.

Caloro Check Sample. Because the Caloro amylose contained almost 20% impurity, the formic acid correction was large, and a second batch of Caloro amylose was prepared. This sample was carefully recrystallized three times and was approximately 100% pure. Molecular weight was determined by periodate oxidation exactly as described for the above samples and also by viscometry. From the intrinsic viscosity the molecular weight was calculated by the modified Staudinger equation, using the newly evaluated constants for amylose in potassium hydroxide solution reported by Everett and Foster (*5*). This gives a viscosity-average molecular weight which shows surprisingly good agreement with the first periodate oxidation value. As viscosity measurements give a type of molecular weight that is always nearer to a weight-average molecular weight than a number-average (as obtained by periodate oxidation), however, it is felt that 56,000 is more nearly correct than 44,000 for Caloro.

Relationship to Other Varietal Studies. Assuming for the present that these corrected values represent real varietal differences in amylose molecular weight, it has been of interest to relate these findings to studies on varietal differences in response to chemical treatment. Unfortunately, the scarcity of such studies makes possible only a few comparisons. A more serious difficulty arises from the compositional differences in the starch of these four varieties. Amylose analyses (*19*) of typical samples gave the following results for white milled rice: Rexoro, 23.6%; Zenith, 14.9%; Caloro, 14.3%; and Century Patna 231, 12.9%. These will vary slightly from sample to sample, but are representative. Because the amylose content of the Rexoro variety is so much higher than the other three, any difference in response of this variety to chemical treatment as opposed to the other varieties is probably due to its starch composition rather than to molecular weight differences in the amylose, if all factors except starch could be safely excluded.

With this point in mind, only Caloro, Zenith, and Century Patna 231 will be compared. Batcher, Helmintoller, and Dawson (*3*) made quantitative measurements of some physical and chemical characteristics of different varieties of rice and found that the amount of estimated starch in the residual cooking liquid increased in the order: Century Patna 231 < Zenith < Caloro. Batcher, Deary, and Dawson (*2*) conducted further studies on 26 varieties of milled white rice and obtained similar results for these three varieties. These workers also measured the water uptake ratio (the weight of the cooked rice divided by the weight of the raw rice) of different varieties and reported the order Caloro < Zenith < Century Patna 231. These differences are fairly large and may be a reflection of amylose molecular weight differences. The cohesiveness scores reported for cooked rice by Batcher, Deary, and Dawson place the varieties in the following order of increasing cohesiveness: Caloro < Zenith < Century Patna 231. The same order is also observed in the alkali test as reported by Little, Hilder, and Dawson (*14*). When scored for clearing and spreading in 1.7% potassium hydroxide, the varieties rank as follows: Caloro > Zenith > Century Patna 231.

Recently Hogan and Planck (*11*) studied the water absorption and amount of undissolved solids and dissolved material of cooked rice. Their study differed from those of Dawson in technique and in that the mechanical loss of rice due to cooking was taken into consideration. The percentage absorption was calculated as the weight of moisture in cooked rice divided by the weight for dry material in cooked rice. The varietal differences in the water absorption were reported in the order of Caloro > Zenith > Century Patna 231 when measured at 70° C. At 100°, results showed Caloro > Century Patna 231 (no data for Zenith). The water absorption of rices below their gelatinization temperatures has been related to the gelatinization characteristics of the rice by Halick and Kelly (*8*). The nature of the compositional factors which control the gelatinization temperature of a variety has never been delineated. Halick and Kelly have shown that it is unrelated to amylose content. Their data suggest that it is also unrelated to amylose molecular weight: Caloro, 67.5°; Zenith, 65.0°; Century Patna 231, 79.5°; and Rexoro, 73.5° C. Their water uptake numbers as measured above gelatinization temperatures, however, give the same order as the other studies cited: Caloro > Zenith > Century Patna. The signs are reversed from those in Dawson's study because of the method of calculation of values and possibly because of technique.

If the varietal differences reported above result from the behavior of the rice starch and not other components, it is possible that they are a reflection of amylose molecular weight differences, or distribution, as Caloro, Zenith, and Century Patna 231 varieties have approximately the same proportions of amylose to amylopectin. Of course, if differences exist in the molecular weights of the amylopectins, such speculation is futile. This is obviously a fertile area for continued research.

Acknowledgment

The authors thank Lester Hulett for his assistance with the amperometric titrations.

Literature Cited

(1) Anderson, D. M. W., Greenwood, C. T., Hirst, E. L., *J. Chem. Soc.* **1955,** 225.
(2) Batcher, O. M., Deary, P. A., Dawson, E. H., *Cereal Chem.* **34,** 277 (1957).
(3) Batcher, O. M., Helmintoller, K. F., Dawson, E. H., *Rice Journal* **59** (13), 4 (1956).
(4) Bates, F. L., French, D., Rundle, R. E., *J. Am. Chem. Soc.* **65,** 142 (1943).

(5) Everett, W. W., Foster, J. F., *Ibid.*, **81,** 3464 (1959).
(6) Fuller, C. H. F., Lampitt, L. H., Coton, L., *J. Sci. Food Agr.* **6,** 656 (1955).
(7) Greenwood, C. T., *Advances in Carbohydrate Chem.* **11,** 335 (1956).
(8) Halick, J. V., Kelly, V. J., *Cereal Chem.* **36,** 91 (1959).
(9) Halsall, T. G., Hirst, E. L., Jones, J. K. N., *J. Chem. Soc.* **1947,** 1427.
(10) Hogan, J. T., Southern Utilization Research and Development Division, U. S. Dept. Agr., private communication.
(11) Hogan, J. T., Planck, R. W., *Cereal Chem.* **35,** 469 (1958).
(12) Inoue, Y., Onodera, K., *J. Agr. Chem. Soc. Japan* **25,** 135 (1951–52).
(13) Larson, B. L., Gilles, K. A., Jenness, R., *Anal. Chem.* **25,** 802 (1953).
(14) Little, R. R., Hilder, G. B., Dawson, E. H., *Cereal Chem.* **35,** 111 (1958).
(15) MacMasters, M., Northern Utilization Research and Development Division, U. S. Dept. Agr., private communication.
(16) Potter, A. L., Hassid, W. Z., *J. Am. Chem. Soc.* **70,** 3488 (1948).
(17) Potter, A. L., Silveira, V., McCready, R. M., Owens, H. S., *Ibid.*, **75,** 1335 (1953).
(18) Rao, B. S., Murthy, A. R. V., Subrahmanya, R. S., *Proc. Indian Acad. Sci.* **36B,** 70 (1952).
(19) Williams, V. R., Wu, W. T., Tsai, H. Y., Bates, H. G., J. AGR. FOOD CHEM. **6,** 47 (1958).
(20) Wilson, E. J., Jr., Schoch, T. J., Hudson, C. S., *J. Am. Chem. Soc.* **65,** 1380 (1943).
(21) Wolff, I. A., Gundrum, L. J., Rist, C. E., *Ibid.*, **72,** 5188 (1950).
(22) Wolff, I. A., Hofreiter, B. T., Watson, P. R., Deatherage, W. L., MacMasters, M. M., *Ibid.*, **77,** 1654 (1955).

Received for review October 12, 1959. Accepted March 14, 1960. Research supported by a grant from General Foods Corp., Hoboken, N. J.

STARCH SOURCES

Barley Flour Composition and Use for Starch Production

K. J. GOERING and JOHN D. IMSANDE[1]

Chemistry Department, Montana State College, Bozeman, Mont.

Barley is a very inexpensive source of carbohydrate in certain areas in the West and thus a potential source of starch. The alkali process can be used on barley flour to produce a starch of good quality. Barley protein is readily dispersed in alkali and can be recovered in yields of 77 to 86% after removal of the starch by adjustment of the pH. Of the six varieties tested, Compana appears most promising as a starch source. Analysis indicated that the amino acids in two varieties of barley protein were different. The behavior on dispersion in alkali also indicated differences in the nature of the protein. The existence of a new "pectinlike" polysaccharide in barley flour is reported.

BARLEY is rapidly becoming to the Intermountain area what corn is to the Corn Belt—the cheapest source of carbohydrate. This immediately suggests the possibility of using this cereal for starch production.

Very little information has been published on the production of starch from barley. The wet milling technique was found unsatisfactory (*15*). The other logical method is the adaptation of the alkali process developed by Dimler *et al.* (*9*) for use with wheat and tried by him on one variety of barley. This process requires flour as the starting material. The present study was initiated to examine the composition of barley flour and to see if starch could be satisfactorily recovered from it by the alkali process.

Materials and Methods

Six varieties of barley with a known history, which were well adapted to Montana, were used for this study. Flours were prepared in an experimental Buhler flour mill equipped with 10 XX bolting silk. In one case the barley was pearled before milling.

Protein in the various products was determined by a modified Kjeldahl method (*2*), using 6.25 as the conversion factor to convert per cent nitrogen to protein. Starch was determined polarimetrically by the procedure of Clendenning (*6, 7*). However, on the "tailings fraction" it was necessary to use a modified procedure (*10*). Crude fiber and ash were determined by the usual method (*3*). To determine moisture, the samples were dried 16 hours at 110° C. under a vacuum of 28 inches of mercury. Fat in the flour samples was determined by ether extraction (*4*). Fat in the purified starches was determined by acid hydrolysis and extraction from the hydrolyzate, as suggested by Taylor and Nelson (*18*). Magnesium, potassium, and sodium were determined by a modification of the method of Pro and Mathers (*17*). Calcium was determined by the flame photometric procedure suggested by Cooley (*8*), and silica was determined photometrically by the procedure of Kerr and Trubell (*11*). Phosphorus was determined photometrically by the procedure of Allen (*1*). The amino acids were determined by the procedure described by Moore and Stein (*16*). The pentosans were measured by the AOAC procedure (*5*), the distillate being redistilled and the furfural precipitated with thiobarbituric acid. The "pectin" was determined by the evolution of carbon dioxide using the method of McCready, Swenson, and Maclay (*14*) on material isolated with an ammonium oxalate extraction and purified by the procedure described by Kertesz (*12*). The fermentable sugars were determined by suspending 10 grams of flour, which had been autoclaved in a dry state to inactivate the β-amylase, in 100 ml. of water containing 5 ml. of an active distiller's yeast. The loss in weight after 24 hours was used to calculate sugars after making corrections for the fermentable material present in the inoculum.

The alkali process (*9*) developed for the production of starch from wheat was used in this study. This consists of dispersion of the flour protein in a dilute aqueous alkaline solution and removal

[1] Present address, Department of Biochemistry, Duke University, Durham, N. C.