defects inherent in the dinitrophenylation technique employed for the determination of lysine in intact proteins. These drawbacks have been discussed in detail by Greenstein and Winitz (*2*).

Literature Cited

(1) Block, R. J., Weiss, K. W., "Amino Acid Handbook," p. 341, Charles C Thomas, Springfield, Ill., 1956.
(2) Greenstein, J. P., Winitz, M., "Chemistry of the Amino Acids," Vol. **2**, p. 1351, Wiley, New York, 1961.
(3) Keller, S., Block, R. J., *Arch. Biochem. Biophys.* **85,** 366 (1959).
(4) Kimmel, J. R., Smith, E. L., *Bull. Soc. Chim. Biol.* **40,** 2049 (1958).
(5) Kott, Y., Lichtenstein, N., *Anal. Chim. Acta* **22,** 401 (1960).
(6) Long, G., "Biochemist's Handbook," p. 994, E. and F. N. Spon, London, W. C. 2, 1961.
(7) Porter, R. R., Sanger, F., *Biochem. J.* **42,** 287 (1948).
(8) Sanger, F., *Ibid.*, **39,** 507 (1945).
(9) *Ibid.*, **45,** 563 (1949).
(10) Spier, H. W., Pascher, G., *Z. Physiol. Chem.* **296,** 147 (1954).
(11) Spies, J. R., Chambers, D. C., *J. Biol. Chem.* **191,** 787 (1951).

Received for review January 21, 1965. Accepted July 19, 1965.

GENETIC VARIATION

Varietal Differences in Physicochemical Properties of Rice Starch and Its Fractions

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Granular starches were isolated from milled rice of waxy and nonwaxy varieties which differed in gelatinization temperature. The starches were fractionated into their amylose (Fraction A) and amylopectin (Fraction B) components. The resultant components were characterized and their properties compared. These physicochemical properties generally differed among the varieties and were not correlated with gelatinization temperature.

Rice varieties of different cooking and eating qualities differ in starch composition (amylose and amylopectin content) and starch gelatinization temperature (*13, 15*). Some high-amylose varieties from Thailand have low gelatinization temperatures (*14*). The United States variety Century Patna 231 has a high gelatinization temperature but a low amylose content (*11, 13*). Since these two properties are not linearly correlated (*13, 14*), differences in cooking properties of rice samples of similar amylose and protein contents must result from differences in physicochemical properties of the starch granule other than composition. Stansel and coworkers (*32*) found waxy rice starches of different gelatinization temperatures to differ in molecular weights.

Gelatinization is a complex phenomenon which may depend on granule size, amylose content, molecular weight of its components, and the micellar organization of the granule (*9, 31*). This study was undertaken to obtain data on the physicochemical properties of rice starch and starch fractions from varieties with different gelatinization temperatures.

Materials and Methods

Rough rice samples of the varieties Malagkit Sungsong Puti, Kung-Shan Wu-Hsiang-Keng, Early Prolific, Century Patna 231, Taichung 65, Taichung (Native) 1, and Peta were grown at the institute in 1963. The sample of the Bengawan variety was obtained from Indonesia, and those of Niaw Sanpatong, Khao Dawk Mali 105, Nahng Mon S-4, and Leuang Yai 34, from Thailand. Milled rice samples of the waxy × nonwaxy hybrids, Waxy Century Patna 231 and Crowley 2585-6-1, were obtained from Beaumont, Tex. All samples used were nonwaxy, except Malagkit Sungsong Puti, Niaw Sanpatong, Waxy Century Patna 231, and Crowley 2585-6-1.

Rough rice samples (1 kg.) were dehulled with a McGill sheller and milled and polished with a McGill miller No. 3 (*35*). Contaminant translucent kernels were sorted by hand from Malagkit Sungsong Puti milled rice. The milled rice was soaked in water, disintegrated in a Waring Blendor at medium speed for 5 minutes, and passed through a 180-mesh sieve. Rice starch was prepared from this homogenate by extracting contaminant protein by shaking it five times with a 3% aqueous solution of Santomerse No. 1 (sodium dodecyl benzene sulfonate, 40% active ingredient) which had 0.12% sodium sulfite (*12*). The purified starch was washed free of detergent by being suspended in distilled water, collected in a Sharples supercentrifuge at 20,000 r.p.m., and washed thoroughly with distilled water. The washed starch was air-dried at 35° C., ground to a fine powder with a mortar and pestle, and defatted for 24 hours with refluxing 95% ethanol in a Soxhlet extractor (*39*). The average recovery was 85% of total starch. Protein content was calculated from Kjeldahl nitrogen (*2*) determinations using the factor 5.95. Each starch gave 99% of glucose on hydrolysis with Taka-diastase and hydrochloric acid (*2*) and after correcting for protein content.

The purified starches were fractionated by the method of Wilson and coworkers (*39*) as employed by Tsai *et al.* (*34*). However, Pentasol 27 (mixed amyl alcohols, from the Pennsalt Chemicals Corp.) was used instead of isoamyl alcohol. Amylopectin (Fraction B) was isolated from the mother liquor. Amylose (Fraction A) was recrystallized once in boiling water saturated with 1-butanol following the procedure of Tsai and coworkers (*34*) and dried in a vacuum desiccator over phosphorus pentoxide. Amylose recoveries as a percentage of total amylose were: 43% for Taichung (Native) 1; 54 to 64% for Peta, Nahng Mon S-4, and Leuang Yai; and 75 to 84% for the rest. Amylopectin was precipitated by pouring the mother liquor from amylose crystallization into 5 volumes of 95% ethanol. After 2 days at room temperature, the precipitate was finely divided with a spatula, washed thoroughly with methanol, and dried in a vacuum desiccator over phosphorus pentoxide. Recoveries as a percentage of total amylopectin were 60 to 78% for the nonwaxy samples and 95% for the waxy samples.

The moisture content of starch and starch fractions was determined from the loss in weight of 50-mg. samples at 56° C. over phosphorus pentoxide for 18 hours in vacuo in a vacuum desiccator or an Abderhalden tube.

Phosphorus content was determined by wet oxidation with hydrogen peroxide,

Table I. Physicochemical Properties of Purified Defatted Rice Starch Granules of Nonwaxy and Waxy Varieties

Variety	Granule Size, Microns: Range	Granule Size, Microns: Mean	Protein[a] (N × 5.95), %	Gelatinization Temp., ° C.	IBC,[b] %	Apparent Amylose,[a,c] %	Density	k,[d] Days^{-1} × 10	Phosphorus,[a] Mg. %
Waxy									
Malagkit Sungsong Puti	1.9–7.6	4.6	0.60	59.5–61	0.17	0.9	1.490	3.52	2.7
Niaw Sanpatong	2.7–8.1	5.6	0.37	56 –64.5	0.24	1.3	1.496	4.13	
Crowley 2585-6-1	1.7–6.9	4.1	0.86	67 –75	0.15	0.8	1.480	1.34	
Waxy Century Patna 231	1.7–8.0	5.0	0.81	68 –75.5	0.17	0.9	1.487	1.49	
Nonwaxy									
Leuang Yai 34	2.7–8.7	5.7	1.01	52.5–59	4.98	25.8	1.500	2.04	33
Nahng Mon S-4	2.6–7.8	5.0	0.71	53 –59.5	4.94	28.4	1.500	2.21	32
Taichung (Native) 1	2.2–7.1	4.7	1.18	55 –63	6.96	37.2	1.496	2.43	45
Taichung 65	2.2–7.6	4.3	0.58	59.5–65	3.70	18.5	1.504	2.55	16
Khao Dawk Mali 105	2.6–7.9	4.8	1.81	58.5–66	2.88	16.6	1.498	2.74	
Bengawan	1.8–8.2	4.9	0.94	55 –66.5	4.25	21.0	1.510	1.58	31
Kung-Shan Wu-Hsiang-Keng	1.8–7.7	4.3	0.24	61.5–69.5	2.40	13.3	1.511	2.15	
Peta	1.9–6.7	4.5	1.15	64 –71	5.52	28.6	1.508	1.51	24
Century Patna 231	1.9–5.8	3.8	0.62	66 –74.5	2.86	15.5	1.510	1.34	12
Early Prolific	1.6–8.1	4.6	1.93	66.5–74.5	2.36	13.1	1.489	1.44	

[a] Dry weight basis. [b] Iodine-binding capacity. [c] Calculated from (IBC of starch/IBC of amylose) × 100. For waxy starches, mean IBC of amyloses of 18.7% was used. [d] Corrosion rate constant in 2.2*N* hydrochloric acid at 35° C.

followed by colorimetric estimation of the phosphomolybdate complex as described by Radomski and Smith (*29*).

The relative viscosity, η_{rel}, of amylose and amylopectin was determined at 30.0° ± 0.02° C. on 0.2% solutions in 1.0 ± 0.01*N* potassium hydroxide using No. 50 Cannon-Fenske viscometers as recommended by Leach (*18*). Intrinsic viscosity, $[\eta]$, was calculated using the equation

$$[\eta] = \frac{\ln \eta_{rel}}{c} + k\left[\frac{\ln \eta_{rel}}{c}\right]^2$$

The value of k employed was +0.000380 for amylose and −0.000265 for amylopectin. Following the procedure of Wolff and coworkers (*40*), these k values were derived experimentally using one of the samples from a plot of $\ln \eta_{rel}/c$ against c, where c is in grams per milliliter. The number average degree of polymerization, $\overline{D.P.}_n$, of amylose was approximated by the equation $\overline{D.P.}_n = 7.4\ [\eta]$ (*5*). The k value for amylose was identical to that derived for rice amylose by Phillips and Williams (*26*).

The iodine-binding capacity (IBC) was determined at 30.0° ± 0.02° C. using calcium chloride solution following the method of Colburn and Schoch (*4*). However, only half of the volume of solvent was employed. The samples were 10 mg. for amylose and 40 mg. for starch and amylopectin. Titration values were corrected for the blank titration at 240 mv. The amylose content was calculated from the ratio of IBC of starch and of amylose.

Sizes of starch granules were obtained from photomicrographs enlarged to give a final magnification of about 1000 diameters. The sizes of from 80 to 100 granules were measured to the nearest 0.1 mm. with a vernier caliper. [Least significant difference of the mean (5% level) = 0.4 micron.]

The gelatinization temperature range of the starches was determined with a polarizing microscope with an electrically heated stage as described by Schoch and Maywald (*31*).

The absolute density of the starch granules was determined by xylene displacement following the method of Schoch and Leach (*30*).

Granular susceptibility to attack by 2.2*N* hydrochloric acid was measured at 35.0° ± 0.02° C. for 1, 2, 4, and 8 days (*8*). The granules were dried for 18 hours at 56° C. in vacuo over phosphorus pentoxide. The corrosion rate constant, k, was determined from a plot of $\ln c$ against time in days, where c is the percentage of residual starch.

Blue values of amylose and amylopectin at 680 mμ were determined by the method of McCready and Hassid (*22*) as employed by Gilbert and Spragg (*7*). The amylose obtained from the Nutritional Biochemicals Corp. had a blue value of 0.970.

The β-amylolysis limit was measured for amylose (50 mg.) and amylopectin (60 mg.) using Wallerstein analytical grade β-amylase (25 mg.) dissolved in acetate buffer (pH 4.8) at 30.0° ± 0.02° C. After 20 hours of hydrolysis, reducing sugars were determined by the alkaline ferricyanide method (*1*) using maltose hydrate as the standard. The β-amylolysis limit was calculated as the percentage of the theoretical yield of maltose hydrate. The results obtained for amylopectin were identical to those obtained with crystalline β-amylase from the Nutritional Biochemicals Corp., but those for amylose were 3 to 6% higher.

Amylopectin was oxidized with sodium metaperiodate using the procedure of Potter and Hassid (*27*) as modified by Greenwood and Thomson (*10*). However, the oxidation time used was 30 to 35 hours. The average length of the unit chain was expressed in anhydroglucose units. The outer chain length was calculated from (β-amylolysis limit × unit chain length/100) + 2.5 (*24*).

The reducing power of amylopectin was measured using alkaline 3,5-dinitrosalicylic acid following the procedure of Lansky and coworkers (*17*) and the $\overline{D.P.}_n$ calculated. The method was suitable only with Malagkit Sungsong Puti, Century Patna 231, Taichung 65, and Nahng Mon S-4, as the other amylopectins were insoluble in the reagent.

A differential ultracentrifugation of an aqueous dispersion of Taichung (Native) 1 amylopectin was attempted at 40,000 r.p.m. using the method of Perlin (*25*). The blue values for both supernatant and residue were determined.

Table II. Physicochemical Properties of Recrystallized Rice Amylose

Variety	IBC, %	Blue Value (680 Mμ), Absorbance	$[\eta]$, G./Ml.	Approximate $\overline{D.P.}_n$[a]	β-Amylolysis Limit, %	Phosphorus, Mg. %
Leuang Yai 34	19.3	1.00	144	1100	83	3.0
Nahng Mon S-4	17.4	0.98	177	1300	84	3.3
Taichung (Native) 1	18.7	1.06	151	1100	83	2.4
Taichung 65	20.0	0.96	80.4	600	87	8.4
Khao Dawk Mali 105	17.4	0.80	117	870	86	
Bengawan	20.2	0.99	174	1300	90	4.3
Kung-Shan Wu-Hsiang-Keng	18.0	0.86	154	1100	85	
Peta	19.2	1.02	130	860	83	2.8
Century Patna 231	18.4	1.00	87.9	650	87	3.8
Early Prolific	18.0	0.87	141	1000	86	

[a] $\overline{D.P.}_n = 7.4\ [\eta]$ (*5*).

Results and Discussion

A sodium dodecylbenzene sulfonate solution effectively reduced the protein levels of milled rice to 0.24 to 1.93% after five extractions (Table I). It did not markedly change the gelatinization temperature of the starch. Similarly, Liau (*20*) found no change in the gelatinization temperature of rice starch from protein extraction with dilute alkali.

The purified starches showed a wide range in potentiometric iodine-binding capacity—0.15 to 0.24% for waxy rice and 2.36 to 6.96% for nonwaxy rice. The corresponding amylose values compared with the colorimetric amylose (*38*) data for milled rice. The close correlation between these two methods for samples of United States milled rice had been reported by Williams *et al.* (*38*).

The density of granular starch ranged from 1.480 to 1.496 for waxy rice and from 1.489 to 1.511 for nonwaxy rice. Leach and Schoch (*19*) reported a density of 1.510 for a sample of rich starch. Stansel and coworkers (*32*) similarly noted that waxy rice starch has a lower internal granule density than does nonwaxy starch.

The granular starch samples differed significantly in their mean sizes, which ranged from 3.8 microns for Century Patna to 5.7 microns for Leuang Yai 34. Granule size and gelatinization temperature of the samples were not correlated. The sample of Century Patna 231 starch had the narrowest range of granule size of 1.9 to 5.8 microns, while those of Early Prolific, Bengawan, and Waxy Century Patna 231 had the widest size range.

The ease of corrosion of granular starch in 2.2*N* hydrochloric acid varied significantly with variety. The range constant, *k*, was negatively correlated with the gelatinization temperature for both waxy and nonwaxy starches. Malagkit Sungsong Puti and Niaw Sanpatong were corroded faster than the other waxy starches. For the nonwaxy samples, the varieties resistant to acid corrosion also had a higher density, except for Early Prolific, the density of which was a low 1.489. The waxy starches did not show this relationship between density and gelatinization temperature. Within either nonwaxy or waxy starch, gelatinization temperature and internal granule density measured by mercuric chloride diffusion have been observed to be positively correlated (*32*).

The percentage of recovery of amylose decreased with an increasing amylose content.

Recrystallized amylose had an IBC ranging from 17.4 to 20.2% (Table II). However, their blue values were comparable, except for the low values obtained for the amyloses of Kung-Shan Wu-Hsiang-Keng, Early Prolific, and Khao Dawk Mali 105. The mean IBC value of 18.7% for the 10 amyloses agrees closely with the 18.9% iodine adsorption of five times recrystallized rice amylose (*26*), although the solvents used differed. The wave length range of maximum absorbance of the iodine-amylose complexes was 630 to 660 mμ, which agrees with the 650-mμ maximum reported by Otani and Takahashi (*23*).

The amyloses had significantly different intrinsic viscosities ranging from 80.4 to 177. The approximate $\overline{D.P.}_n$ which was calculated using the linear relationship between $\overline{D.P.}_n$ and $[\eta]$ of Cowie and Greenwood (*5*) seems to reflect true varietal differences in molecular weights, since amyloses were prepared identically. Century Patna 231 amylose similarly prepared from a United States sample had $[\eta]$ of 84 (*26*), whereas a Philippine-grown sample had $[\eta]$ of 87.9.

The β-amylolysis limit range of 83 to 90% for the amyloses was higher than the 68 to 78% range for corn, barley, and wheat amyloses which were fractionated and recrystallized under nitrogen (*10*). The samples were prepared in air and the presence of oxygen during preparation has been shown to reduce the β-limit of amylose (*6*). This implies that rice amyloses pose less of a barrier to the action of β-amylase than the other cereal amyloses. The greater linearity of rice amylose and its low $[\eta]$ may account for the faster rate of retrogradation or gel formation of its starch paste during cooling than wheat, barley, and corn starches. The β-amylolysis limit values were lower than the 96 to 99% reported for rice amyloses by Phillips and Williams (*26*).

Table III. Physicochemical Properties of Purified Rice Amylopectins of Waxy and Nonwaxy Varieties

Variety	IBC, %	$[\eta]$, G./Ml.	β-Amylolysis Limit, %	Unit Chain Length[a]	Outer Chain Length[b]	Phosphorus, Mg. %
Waxy						
Malagkit Sungsong Puti	0.08	46.5	49	20	12	1.1
Niaw Sanpatong	0.09	112				
Crowley 2585-6-1	0.07	153	56	23	15	
Waxy Century Patna 231	0.08	144	56	22	15	
Nonwaxy						
Leuang Yai 34	2.58	164	57	26	17	8.0
Nahng Mon S-4	0.94	106	49	23	14	2.0
Taichung (Native) 1	2.74	168	55	25	16	6.4
Taichung 65	0.51	104	56	23	15	3.3
Khao Dawk Mali 105	0.37	151	57	22	15	
Bengawan	0.93	163	56	27	18	4.3
Kung-Shan Wu-Hsiang-Keng	0.37	163	54	20	13	
Peta	2.02	132	56	24	16	2.1
Century Patna 231	0.34	82.9	54	25	16	1.7
Early Prolific	0.86	153	58	20	14	

[a] From periodate oxidation data.
[b] Calculated from (β-limit × unit chain/100) + 2.5 (*24*).

The purified amylopectins differed in their IBC values (Table III). The amylopectins from high-amylose starches were generally of higher IBC values than those from waxy and low-amylose starches. Blue values for Malagkit Sungsong Puti, Waxy Century Patna 231, Taichung 65, Bengawan, Peta, and Taichung (Native) 1 amylopectins were 0.00, 0.00, 0.04, 0.05, 0.12, and 0.14, respectively, and were consistent with the IBC data. In addition, the wave lengths of maximum absorbance of the iodine-amylopectin complex ranged from 525 mμ for Malagkit Sungsong Puti, 550 mμ for Taichung 65 and Waxy Century Patna 231, 560 mμ for Bengawan, and 565 mμ for Peta, to 590 mμ for Taichung (Native) 1. Otani and Takahashi (*23*) reported absorbance maxima of 520 and 540 to 560 mμ for the iodine complexes of waxy and nonwaxy rice amylopectins, respectively. Taki (*33*) reported 4.8% amylose in amylopectin precipitated from the mother liquor after crystallization of the 1-butanol complex of rice amylose.

The $[\eta]$ of the amylopectins ranged from 46.5 to 153 for the waxy samples and from 82.9 to 168 for the nonwaxy samples. The dinitrosalicylate $\overline{D.P.}_n$ data were: Malagkit Sungsong Puti, 330; Century Patna 231, 570; Nahng Mon S-4, 710; and Taichung 65, 880. These were consistent with the $[\eta]$ data. Malagkit Sungsong Puti, a popular local waxy rice, had the amylopectin of lowest molecular weight, whereas the amylopectin of Niaw Sanpatong and the two high-gelatinization-temperature waxy starches from Texas had higher molecular weights. Waxy Century Patna 231 amylopectin had a higher $[\eta]$ value than that of Century Patna 231.

Waxy starch amylopectin of four United States rice varieties have been reported by Stansel and coworkers (*32*) to have higher molecular weights than amylopectin of nonwaxy starches. Other dinitrosalicylate $\overline{D.P.}_n$ data reported were 850 glucose units for a sample of Japanese rice amylopectin (*16*) and 260, 380, and 490 for three amylopectins of Spanish rices (*28*).

The β-amylolysis limits of the amylopectins differed significantly. They reflected varietal differences in amylopectin structure, since the values were not related with the IBC data of the samples. Amylose-contaminated amylopectin would be expected to have higher β-amylolysis limits. The β-amylolysis limit of Nahng Mon S-4 amylopectin was as low as that of Malagkit Sungsong Puti amylopectin. The two other waxy amylopectins had higher β-limits than Malagkit Sungsong Puti. Greenwood and Thomson (*10*) reported the usual range of β-amylolysis limit of 56 to 58% for the other cereal amylopectins.

The unit chain length from periodate oxidation showed a range of 20 to 23 anhydroglucose units for waxy amylopectins and 20 to 27 for nonwaxy amylopectins. The outer chain lengths calculated from the β-amylolysis limit and periodate oxidation data were 12 to 15 anhydroglucose units for waxy amylopectin and 13 to 18 units for nonwaxy amylopectin. The outer chain length data and the wave length maxima of the iodine complex were consistent with the reported positive correlation between these two properties of amylopectin (*36*). The average length of unit chain of other cereal amylopectins ranged from 19 to 28 (*10*). The comparable unit chains of waxy and nonwaxy amylopectins contrasted with the observation of Stansel and coworkers (*32*) that the amylopectin of waxy rice starch was less branched than nonwaxy amylopectin.

The iodine-blue-complexing property of the amylopectins of Taichung (Native) 1 and Leuang Yai 34 may be due to the presence of an amylopectin fraction of longer chain length or to contaminant degraded amylose. The apparent unit chain lengths of these amylopectins of 25 and 26, respectively, are normal. Degraded amylose was shown to be absent by differential ultracentrifugation of Taichung (Native) 1 amylopectin at 40,000 r.p.m., since the supernatant had almost the same blue value as the starting amylopectin. The residue had a higher $[\eta]$ than the supernatant. Hence, a wide range in the unit chain of these amylopectins which is not evident from the mean unit chain may explain their slight blue color with iodine. In fact, an iodine-blue-complexing fraction has been isolated from corn starches which is less branched than amylopectin and is selectively precipitated from an aqueous mixture with amylopectin (*37*) by 2-nitropropane but not by 1-butanol. Highly branched amylopectin with chain lengths of 13 to 16 glucose units forms 5 to 10% of the starch of potato (*3*) and of wheat (*25*).

Although phosphorus content seemed to be related to starch composition in granular starches (Table I), phosphorus level and β-amylolysis limit were not related in the amyloses (Table II) and amylopectins (Table III). The phosphorus contents in the starch fractions also were lower than those of the starches and were less than 0.01% of the fraction. Such a drop in phosphorus content on fractionation suggests that at least the major portion of the phosphorus contained in the granular starch is not part of the molecule.

A factor affecting gelatinization temperature is the molecular weight of the starch components (*31*). Amylose $[\eta]$ and gelatinization temperature of the granular starch showed no general correlation. With the waxy amylopectins, the two derived from starches of high gelatinization temperature and Niaw Sanpatong had higher molecular weights than Malagkit Sungsong Puti. For the nonwaxy amylopectins, there was no relationship. The amylopectins of high-gelatinization temperature starches, Early Prolific and Century Patna 231, had $[\eta]$ of 153 and 82.9, respectively. In contrast, Stansel and coworkers (*32*) found that within waxy and nonwaxy amylopectins their molecular weight and the gelatinization temperature of the starch are generally negatively correlated. Obviously, the relationship between gelatinization temperature and molecular weight of starch fractions is complex.

Of the other factors affecting gelatinization temperature, granule size may be considered relatively minor, since the samples had similar sizes (Table I). The micellar structure of the molecules in the granule seemed to be the main factor involved in varietal differences in gelatinization temperature. This structure is reflected in its ease of corrosion with acid and the density of the granular starch (Table I). Thus, gelatinization temperature reflects the degree of orderly arrangement of the molecules in the granule and perhaps of the whole endosperm. This would readily explain the slower rate of cooking (*15*) and slower rate of gelatinization in alkali solution (*13, 14*) of the kernels of varieties with starches of high gelatinization temperature. The degree of crystallinity of granular starch and amylose also had been correlated with the gelatinization characteristics of rice flours (*21*).

Acknowledgment

The authors are grateful to H. Siregar, Cereals Research Institute, Bogor, Java, Indonesia, for providing the Bengawan sample; Sala Dasananda, Rice Department, Ministry of Agriculture, Bangkok, Thailand, for the Niaw Sanpatong, Khao Dawk Mali 105, Nahng Mon S-4, and Leuang Yai 34 samples; and James C. Stansel, Rice-Pasture Experiment Station, Beaumont, Tex., for the Waxy Century Patna 231 and Crowley 2585-6-1 samples. They also appreciate the assistance of Remedios Santiago and Lourdes Cruz in making the various determinations.

Literature Cited

(1) American Association of Cereal Chemists, St. Paul, Minn., "Cereal Laboratory Methods," 7th ed., 1962.
(2) Association of Official Agricultural Chemists, Washington, D. C., "Official Methods of Analysis," 9th ed., 1960.
(3) Banks, W., Greenwood, C. T., *J. Chem. Soc.* **1959,** 3436.
(4) Colburn, C. R., Schoch, T. J., *Methods Carbohydrate Chem.* **4,** 161 (1964).
(5) Cowie, J. M. G., Greenwood, C. T., *J. Chem. Soc.* **1957,** 2862.
(6) *Ibid.*, p. 4640.
(7) Gilbert, G. A., Spragg, S. P., *Methods Carbohydrate Chem.* **4,** 168 (1964).
(8) Greenwood, C. T., MacKenzie, S., *Stärke* **15,** 251 (1963).
(9) Greenwood, C. T., Thomson, J., *Biochem. J.* **82,** 156 (1962).
(10) Greenwood, C. T., Thomson, J., *J. Chem. Soc.* **1962,** 222.
(11) Halick, J. V., Kelley, V. J., *Cereal Chem.* **36,** 91 (1959).
(12) Hizukuri, S., Nikuni, Z., "Experimental Methods in High Polymers," Vol. **12,** p. 49, Kyoritsu Publishing Co., Japan, 1958 (English translation, typescript).
(13) Juliano, B. O., Bautista, G. M., Lugay, J. C., Reyes, A. C., J. AGR. FOOD CHEM. **12,** 131 (1964).
(14) Juliano, B. O., Cagampang, G. B., Cruz, L. J., Santiago, R. M., *Cereal Chem.* **41,** 275 (1964).
(15) Juliano, B. O., Oñate, L. U., del Mundo, A. M., *Food Technol.* **19,** 1006 (1965).
(16) Kurasawa, H., Yamamoto, Y., *Nippon Nogeikagaku Kaishi* **31,** 516 (1957) (English translation, typescript).
(17) Lansky, S., Kooi, M., Schoch, T., *J. Am. Chem. Soc.* **71,** 4066 (1949).
(18) Leach, H. W., *Cereal Chem.* **40,** 593 (1963).
(19) Leach, H. W., Schoch, T. J., *Ibid.*, **38,** 34 (1961).
(20) Liau, Y. H., *J. Chinese Chem. Soc. Taiwan* **9,** 147 (1962).
(21) Lugay, J. C., Juliano, B. O., unpublished data.
(22) McCready, R. M., Hassid, W. Z., *J. Am. Chem. Soc.* **65,** 1154 (1943).
(23) Otani, Y., Takahashi, S., *Hakko Kogaku Zasshi* **36,** 448 (1958); *C. A.* **53,** 17415 (1959).
(24) Peat, S., Whelan, W. J., Thomas, G. J., *J. Chem. Soc.* **1952,** 4546.
(25) Perlin, A. S., *Can. J. Chem.* **36,** 810 (1958).

(26) Phillips, A. T., Williams, V. R., *J. Food Sci.* **26,** 573 (1961).
(27) Potter, A. L., Hassid, W. Z., *J. Am. Chem. Soc.* **70,** 3488 (1948).
(28) Primo, E., Casas, A., Barber, S., Barber, C. B., *Rev. Agroquím. Tecnol. Alimentos* **2,** 343 (1962).
(29) Radomski, M. W., Smith, M. D., *Cereal Chem.* **40,** 31 (1963).
(30) Schoch, T. J., Leach, H. W., *Methods Carbohydrate Chem.* **4,** 101 (1964).
(31) Schoch, T. J., Maywald, E., *Anal. Chem.* **28,** 382 (1956).
(32) Stansel, J. W., Whistler, R. L., Kramer, H. H., Proceedings of 9th Meeting Rice Technical Working Group 1960, **ARS, USDA,** p. 16, 1961.
(33) Taki, M., *Nippon Nogeikagaku Kaishi* **33,** 781 (1959).
(34) Tsai, H. Y., Phillips, A. T., Williams, V. R., J. AGR. FOOD CHEM. **8,** 364 (1960).
(35) U. S. Department of Agriculture, "Rice Inspection Manual," AMS GR Instruction No. **918-2** (rev.) (effective April 1, 1962).
(36) Watson, S. A., Whistler, R. L., *Ind. Eng. Chem., Anal. Ed.* **18,** 75 (1946).
(37) Whistler, R. L., Doane, W. M., *Cereal Chem.* **38,** 251 (1961).
(38) Williams, V. R., Wu, W. T., Tsai, H. Y., Bates, H. G., J. AGR. FOOD CHEM. **6,** 47 (1958).
(39) Wilson, E. J., Jr., Schoch, T. J., Hudson, C. S., *J. Am. Chem. Soc.* **65,** 1380 (1943).
(40) Wolff, I. A., Gundrum, L. J., Rist, C. E., *Ibid.*, **72,** 5188 (1950).
Received for review February 18, 1965. Accepted June 14, 1965.

STORAGE EFFECTS IN WHEAT

Changes in Lipid Composition in Wheat during Storage Deterioration

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Titratable acidity was about 7% higher in benzene extracts than in petroleum ether extracts of wheat. Benzene extracts of moistened wheat contained more free fatty acids than did extracts of wheat redried to 11 to 12% moisture. Changes in lipid composition during grain deterioration were followed by qualitative and quantitative thin-layer chromatography (TLC) and fractionation on silicic acid columns. Deterioration of wheat was accompanied by formation of at least four unidentified compounds that showed autofluorescence under ultraviolet light. Grain deterioration was accompanied by lowering of polar lipids and rapid disappearance of at least five ninhydrin- or Dragendorff-reagent positive polar lipids. The breakdown of polar lipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides.

DETERIORATION of grain and of milled products in storage is accompanied by increased acidity. As early as 1914, Besley and Boston (*6*) suggested acidity as a factor in determining soundness of corn. The acids formed include free fatty acids, acid phosphates, and amino acids; but at early stages of deterioration, fat acidity increases at a much greater rate than either of the other two types or all types of acidity combined (*24*). Swanson (*22*), Zeleny and Coleman (*24*), and Bolling (*8*) suggested determining fat acidity as one of the best measures of grain damage.

Zeleny (*23*), Geddes (*11*), Milner and Geddes (*19*), Hutchinson (*15*), and James (*16*) have summarized changes occurring in wheat stored under adverse conditions. While a large volume of information is available on the effects of storage conditions on changes in lipid content and free fatty acid levels, relatively little is known about transformations occurring in the wheat lipids during storage. Free fatty acids may result from the action of seed lipases; but it seems that in deteriorating stored wheat the acids result primarily from fungal lipase activity (*3, 4, 12, 20, 21*).

The purpose of this research was to determine changes in composition during breakdown of lipids by molds.

Experimental

Wheat Samples. Sound, hard red winter wheat of the Comanche variety and soft red winter wheat of the Seneca variety stored for about 6 months after harvest at +4° C. were used. The original moisture of the wheat samples was 12.8 and 13.4%, respectively, and each was moistened to 18 or 22%. The wheat samples were placed in narrow-mouthed glass containers, water was added, the contents were mixed thoroughly, the containers were loosely plugged with cotton, and the wheat was allowed to attain desired moisture levels during storage for 48 hours at +4° C. The conditions of storage would seem to allow for relatively easy gas exchange. However, the possibility of limited carbon dioxide accumulation, especially at advanced stages of deterioration, cannot be excluded. The samples were stored at 49° C. and shaken daily, and subsamples were removed for analyses.

Fat Acidity. Fat acidity was determined by two procedures (*2*), with benzene and petroleum ether as extractants. Fat acidity determinations were made on both moistened samples and samples dried to 11 to 12% moisture in a forced draft oven below 60° C. Additionally, free fatty acids were determined in 35 samples of sound wheat harvested in 1963 and stored up to 6 months at 4° C.

Mold Counts. Mold counts were made by the procedure of Christensen (*9*).

Column Chromatography. Lipids from original wheat, and 22% moisture wheat stored at 49° C., were separated into nonpolar and polar fractions. Wheat was ground to pass a 20-mesh sieve on a micro-Wiley mill, and 15-gram samples were extracted in a Stein mill with 100, 50, and 50 ml. of water-saturated 1-butanol, for 4, 2, and 2 minutes, respectively. The combined extracts were decanted, filtered, and evaporated almost to dryness under vacuum in a glass apparatus at about