[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF IOWA STATE COLLEGE]

Fractionation of Waxy and Ordinary Cornstarch*

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The heterogeneous character of starch is generally acknowledged.[1] There is agreement in recognizing a more soluble and a less soluble fraction,[2] but the technique for separating and characterizing these constituents has led to contradictory concepts. Recent publications from the laboratory of Meyer[3] and of Hess[4] may be cited as specific examples. Meyer reports in cornstarch 10–20% of amylose consisting of unbranched chains of the magnitude of 300 glucose units and 80–90% of amylopectin consisting of branched chains with molecular weights of 50,000 to 1,000,000. Hess has isolated an amylose fraction of similar magnitude (238 glucose units) but cannot agree that the product is unbranched.

Variation in the relative amounts of amylose and amylopectin is presumed to explain the differences in physical properties associated with starches from various sources. Variation in starches from a particular genus is generally small although it has been demonstrated.[5] Exceptions to this close similarity within a genus are the starches from certain varieties of rice, sorghum, millet and maize which give a red-brown color with iodine. On the basis of this iodine color, Weatherwax[6] characterized the carbohydrate from waxy corn as an "erythrodextrin," presumably produced by a genetic mutation intermediate to starchy and sweet varieties of corn. This carbohydrate must, however, be considered a starch rather than a dextrin since it exists in granule form and gives an X-ray diffraction pattern almost identical with ordinary cornstarch.[7] Experience[8] in this Laboratory indicates that ordinary and waxy cornstarches show as much difference in physical properties as do the cereal and tuber starches.

The genetic relationship and the close similarity in X-ray diffraction patterns between waxy and ordinary cornstarch should make them particularly valuable for comparative studies of structure and physical properties. This paper reports a study of factors affecting the degree of separation of amylose and amylopectin by electrodialysis and by freezing. Aging of the pastes was investigated to determine whether this phenomenon has any influence on the separation of the components. The two cornstarches have been digested with beta-amylase, the limit dextrins isolated and compared. The action of the beta-amylase would be expected to be limited to the same linkages in both starches so that any structural variations between them would be concentrated in the residual limit dextrins.

Experimental

Fractionation of Starch Pastes by Electrodialysis.—The starch pastes were prepared by suspending the weighed sample of starch in a small volume of water and pouring it into water held at the desired temperature of gelatinization to give a concentration of 1.25 to 1.33%. Samples heated above 100° were gelatinized by pouring into boiling water, then autoclaving at the desired temperature. The time of heating was thirty minutes in all cases. The homogenized samples were prepared by passing them through a hand homogenizer until microscopic examination showed no granules left intact. Cornstarch pastes heated above 130° contain no unruptured granules. The dialyzer and its operation have been described previously.[9] After dialysis was completed, the amount of amylose was determined in two 50-cc. aliquots of the supernatant liquor by evaporating them at 100° and weighing. The amylopectin was derived by difference. The procedure was checked by actual isolation of the amylopectin after repeated suspension and electrodialysis to remove the soluble amylose.

Figure 1 shows that the yield of amylose from cornstarch depends on the temperature and mechanical treatment of the paste. As the temperature of pasting was increased, longer periods became necessary for electrodialysis, twelve hours or less being sufficient below 120°. The cornstarch paste heated at 152° and the waxy maize paste heated at 100° showed no sign of separation after three and one-half days of electrodialysis.

Reducing values (R_{Cu})[10] of the pastes are also included in Fig. 1. The reducing values increase, although not

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(1) Radley, "Starch and its Derivatives," Chapter III, D. Van Nostrand Company, New York, N. Y., 1940; Schoch, *Cereal Chem.*, **18**, 121 (1941).

(2) In this manuscript the more soluble fraction will be designated as amylose and the less soluble fraction as amylopectin according to the terminology introduced by Maquenne and Roux, *Compt. rend.*, **142**, 1387 (1906).

(3) Meyer, *et al.*, *Helv. Chim. Acta*, **23**, 854 (1940).

(4) Hess, *Ber.*, **73**, 976 (1940).

(5) Reichert, "The Differentiation and Specificity of Starches in Relation to Genera, Species, etc.," *Carnegie Inst. Publ.*, **173**, 1913.

(6) Weatherwax, *Genetics*, **7**, 568 (1922).

(7) Bear and French, THIS JOURNAL **63**, 2298 (1941).

(8) Brimhall and Hixon, *Ind. Eng. Chem., Anal. Ed.*, **11**, 358 (1939).

(9) Hixon and Dawson, *ibid.*, **11**, 395 (1939).

(10) Richardson, Higginbotham and Farrow, *J. Text. Inst.*, **131**, 27 (1936).

consistently, with the temperature of preparing the pastes. This is probably due to hydrolytic cleavage, which is very responsive to *p*H changes at higher temperatures.[11] Buffering of the samples was not desirable for these studies.

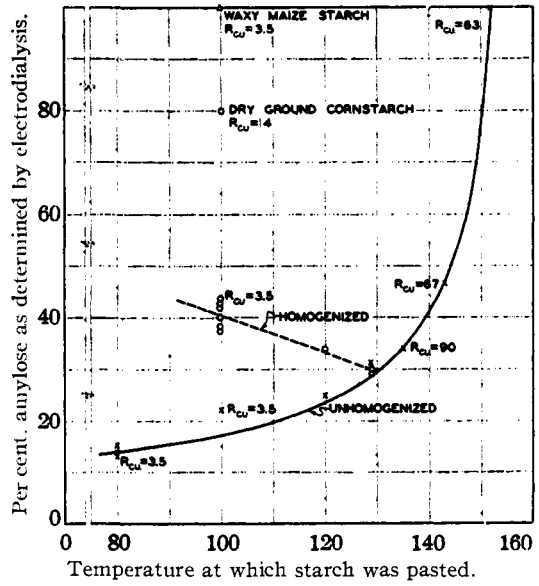

Fig. 1.

Isolation and Properties of Amylose and Amylopectin.—The supernatant liquors from dialysis which contained the amylose were cloudy in all cases. The amylose was isolated by evaporating the liquor to a small volume under reduced pressure, adding 3–4 volumes of alcohol, dehydrating with absolute alcohol and drying in a vacuum oven at 70°. The amylopectin was freed from amylose, coagulated by adding 3–4 volumes of alcohol, dehydrated and dried like the amylose. The amylopectin fraction is easily redispersed in boiling water and is similar in colloidal properties to the original starch, while the amylose cannot be redispersed in water even by prolonged boiling. Both fractions dissolve readily in 2.5% aqueous alkali to give clear solutions. Amylose and amylopectin fractions, separated by electrodialysis of cornstarch pastes prepared at 100° and homogenized, had the following specific rotations (2.5% NaOH, 2 dm., *c* 0.40): amylopectin, $[\alpha]^{25}D$ +205.0°; amylose, $[\alpha]^{25}D$ +141.2°. Reducing values[10] were, for amylopectin 1.3, and for amylose 4.5, as compared to 3.5 for the original starch.

Fractionation of Starch Pastes by Freezing.—Pastes of 2.5% concentration were left for three hours in the evaporator of an electric refrigerator. The frozen paste was melted at room temperature, centrifuged, and the sediment weighed after drying (insoluble fraction). The supernatant liquid, concentrated almost to dryness under reduced pressure, was precipitated with 3–4 volumes of alcohol, dehydrated in absolute alcohol, dried and weighed (soluble fraction). The data for a number of samples are shown in Table I.

From the cornstarch which had been dry-ground 600 hours (R_{cu} = 14), the soluble fraction obtained by freezing had a reducing value of 19.9; the soluble fraction obtained by electrodialysis had a reducing value of 13.8. The insoluble fractions formed from the different starches by freezing and thawing (retrogradation) all gave the same X-ray pattern. Figure 2 compares the amount of amylose produced by the two methods—electrodialysis and freezing.

TABLE I

FRACTIONATION OF STARCH PASTES BY FREEZING

Sample	Treatment before freezing	% insol. fraction	% sol. fraction
Cornstarch	Pasted at 100°; homo-	93.2	5.2
Cornstarch	genized	96.8	2.1
Cornstarch	Pasted at 100°; homo-		
	genized	90.0[a]	9.3[a]
Cornstarch	Pasted at 140°; homo-		
	genized	77.8	22.2
Cornstarch	Pasted at 152°; homo-		
	genized	67.5	32.5
Cornstarch	Dry-ground 600 hrs.;		
	pasted at 100°	60.6	36.1
Amylose	Supernatant liquor		
	from electrodialysis		
	of cornstarch paste	96.7	3.0
Waxy			
cornstarch	Pasted at 100°	0	100.0[b]
Tapioca starch	Pasted at 100°	0	100.0[c]
Potato starch	Pasted at 100°	79.5	20.4

[a] Paste after thawing was heated to boiling before centrifuging. [b] Repeated freezing and thawing will cause separation of an insoluble fraction. [c] There was a slight demarcation but no definite separation of fractions.

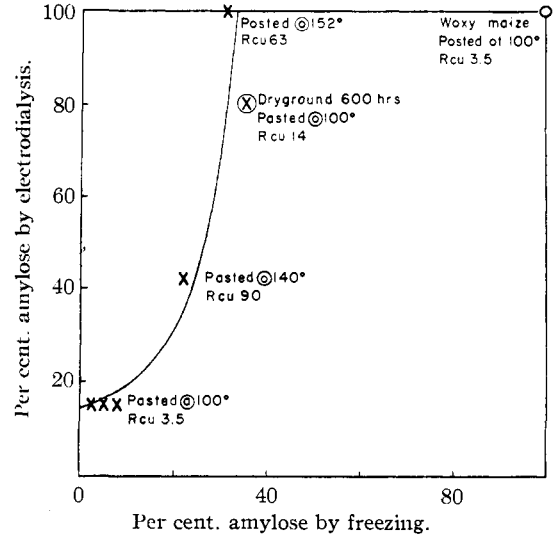

Fig. 2.

Aging of Starch Pastes.—The aging of starch pastes at 7° was followed quantitatively by a modification of the method suggested by Sallinger.[12] In this procedure the paste is digested with saliva to reduce the viscosity, the insoluble residue left by the enzyme digestion is filtered from the mother liquor, washed, dried and weighed. The data must be interpreted as a measure of the rate of crystallization as pointed out in the discussion. The results show

(11) Tychowski and Maisor, *Biochem. Z.*, **1. 291**, 399 (1937).

(12) Sallinger, *Kolloidchem. Beihefte*, **25**, 111 (1919).

that the rate of retrogradation upon aging of ordinary cornstarch is retarded by longer periods of heating, by higher temperatures or by mechanical dispersion, all of which increase the degree of granule disruption. Pastes prepared from 600-hour ground cornstarch and from waxy cornstarch showed no evidence of retrogradation when held at 7° for 214 and 450 hours, respectively.

Preparation of Limit Dextrins with β-Amylase.—β-Amylase concentrates prepared from soybeans,[13] with saccharogenic power varying from 63 to 200, were used. These preparations yield 30–40% limit dextrins from corn, rice, wheat, potato and tapioca starches.[14]

The starch pastes were prepared by stirring a water suspension of the starch into an appropriate volume of water held at the desired temperature, heating for thirty minutes, and cooling to 45°. Enzyme concentrate was then added in sufficient quantity to produce in thirty minutes a weight of maltose equal to that of the starch used as substrate, as calculated from the saccharogenic power. The enzymic digestion was allowed to run twenty-four hours in a thermostat at 40° with a small amount of toluene as a preservative. Small amounts of undigested residue were separated in a Sharples supercentrifuge and the limit dextrins recovered by precipitation with ethyl alcohol. All samples of limit dextrins were boiled in water and redigested with enzyme. Tables II and III compare the yields and the physical properties of limit dextrins from ordinary and from waxy cornstarches.

TABLE II

DIGESTION OF WAXY AND ORDINARY CORNSTARCHES BY SOYBEAN β-AMYLASE

Starting[a] material	Yield in % starting material: Undigested residue	Maltose[b]	Ppt. by 60% alc.	Calcd. recovery
Ordinary cornstarch, pasted 80°	1.4	51.0	22.3 Fr. B	92.9
Fraction A[c]	0.0	15.0	29.0 Fr. D	100
Fraction C[d]	...	11.4	....	...
Fraction B	0.0	8.2	70.0 Fr. E	78.2
Fraction E	...	6.6	....	...
Ordinary cornstarch, pasted 100°	1.3	51.5	31.6 Fr. F	84.4
Fraction F	0.0	11.9	81.7 Fr. G	93.6
Waxy cornstarch, pasted 80°	2.2	42.0	55.6 Fr. H	99.8
Fraction H	1.0	19.9	68.3 Fr. I	88.2
Fraction I	...	5.4	....	...
Waxy cornstarch, pasted 100°	2.2	42.1	44.5 Fr. J	88.8
Fraction J	0.0	10.5	88.7 Fr. K	99.2

[a] All fractions were pasted at 100°. Concentrations of the pastes varied from 2.5–7.0%. [b] Determined by a modification of the Hagedorn and Jenson[15] method for reducing substances and calculated as maltose. [c] Obtained in 18.2% yield by precipitation of the cornstarch digest with 14% alcohol. Microscopic observation indicated that Fraction A consisted of fragments of the original granules. Note the resistance of these fragments to further digestion (Fraction C). [d] Obtained in 56% yield by precipitation of the Fraction A digest with 14% alcohol.

It was found that cornstarch pastes prepared at 152° show the same rate of digestion with β-amylase and are converted by it to the same extent (60% of the theoretical maltose) as pastes prepared at 100°. This is significant in view of the increased reducing value and greater solubility of the 152° pastes.

Methylation of Starch and its Limit Dextrin.—Ordinary cornstarch was methylated according to the method of Freudenberg and Boppel,[16] but due to solubility difficulties this technique was not satisfactory for the limit dextrin. Therefore, acetone solutions of the dextrin acetate were methylated by the Haworth procedure and then given a final treatment in liquid ammonia using sodium and methyl iodide. The methylated dextrin collects on the surface of boiling water as a gummy ball. It can be recovered in 90% yields by skimming it off the boiling water with a spoon, dropping into a second volume of boiling water to remove dissolved salts, and again recovering with a spoon. Filtration on a hot suction filter, as is done with methylated starch, causes considerable loss.

Approximately 10 g. of the methylated products were hydrolyzed and analyzed by the methods of Hassid and Dore[17] and of Haworth.

Anal. Calcd. for trimethyl starch and trimethyl limit dextrin, $C_9H_{16}O_5$: 45.4% OCH_3. Found for trimethyl starch: 45.0, 45.0%; for trimethyl limit dextrin: 44.9, 45.0%.

The amount of dimethyl glucose in the chain was estimated at less than 0.93% for methylated starch and 0.67% for the methylated limit dextrin. Further data on these products are included in Table IV. No attempt was made to prepare the methylated limit dextrin from waxy cornstarch.

Acetylation of Limit Dextrins.—Limit dextrins from ordinary and from waxy cornstarches (Fractions F and H, Table II) were acetylated using pyridine and acetic anhydride. The acetates were obtained in 75–80% yields, and acetyl content determined by the micro method of Friedrich and Rapoport.[18] Since the results were none too satisfactory, the procedure was modified in that distillation of acetic acid from the *p*-toluenesulfonic acid solution was carried just to dryness each time rather than heating for the prescribed five minutes.

Anal. Calcd. for limit dextrin triacetate, $C_{12}H_{18}O_8$: 44.75% CH_3CO. Found for acetate of dextrin from ordinary starch: 42.7, 42.7%; for acetate of dextrin from waxy starch: 43.8, 44.2%.

Table IV shows the specific rotation of these acetates and their molecular weights determined by several methods.

Discussion

The data on fractionation by electrodialysis and by freezing clearly show the difference in the physical properties of ordinary and waxy corn-

(13) Newton and Naylor, *Cereal Chem.*, **16**, 71 (1939).
(14) Martin, Naylor and Hixon, *ibid.*, **16**, 565 (1939).
(15) Gore and Steel, *Ind. Eng. Chem., Anal. Ed.*, **7**, 324 (1935).
(16) Freudenberg and Boppel, *Ber.*, **71**, 2505 (1938).
(17) Hassid and Dore, THIS JOURNAL, **59**, 1503 (1937).
(18) Friedrich and Rapoport, *Biochem. Z.*, **251**, 432 (1932).

TABLE III

PROPERTIES OF LIMIT DEXTRINS FROM ORDINARY AND WAXY CORNSTARCHES

Limit dextrin[a]	% of orig. starch	Soly. in water	Iodine[b] color	R_{Cu}[c]	$[\alpha]^{25}D$ in 2.5% NaOH
Fraction C	10.0	Insol. after long boiling	Blue	49.6	+126.8°
Fraction D	5.2	Sol. cold H_2O	Red	26.2	144.0
Fraction E	15.9	Sol. on boiling (cloudy)	Blue	30.9	154.6
Fraction G[d]	25.9	Sol. on boiling (cloudy)	Blue	17.8	156.6
Fraction I	38.4	Sol. cold H_2O	Red	31.1	156.2 (172 in H_2O)
Fraction K	39.5	Sol. cold H_2O	Red	25.5	150.0 (179 in H_2O)

[a] As designated in Table II. Fractions C, D, E, and G are limit dextrins from ordinary cornstarch; Fractions I and K are from waxy cornstarch. [b] Iodine colors observed using 100 cc. of 0.3% solutions of dextrins. The red-coloring samples required five times as much 0.1 *N* iodine solution (about 1 cc.) to give plainly visible color as did the blue-coloring samples. [c] R_{Cu}[10] is defined as the milligrams of copper per gram of starch or dextrin used: value for starch, 3.5; for maltose, 2055. [d] Fraction G was further fractionated both by freezing (to give 78% of a soluble portion coloring red with iodine) and by electrodialysis (to give 92% of a soluble portion coloring first blue and then red with iodine).

TABLE IV

COMPARISON OF LIMIT DEXTRINS FROM WAXY AND ORDINARY CORNSTARCH

	Limit dextrin from Ordinary starch	Waxy starch
Yield of dextrin (as % original starch)	30–31%	38–39%
$[\alpha]^{25}D$ of dextrin (2 dm., *c* 0.4, 2.5% NaOH)	+154.6°	+156.2°
$[\alpha]^{25}D$ of dextrin acetate (2 dm., *c* 0.8, $CHCl_3$)	+159.0°	+157.2°
$[\alpha]^{25}D$ of methylated dextrin[a] (2 dm., *c* 0.4, $CHCl_3$)	+203.8°	....
Iodine color	Blue-red[b]	Red
Chain length in glucose units as calculated from:		
Viscosity of acetates[c]	263	350
Reducing value of dextrin[d]	133	132
Freezing-point depression of acetate[e]	14.9	13.5
Ratio of trimethyl to tetramethyl glucose[f]	8.6	....

[a] For methylated starch, $[\alpha]^{25}D$ +225.5°. [b] This can be fractionated by electrodialysis or freezing to give a soluble fraction with a red iodine color. [c] By Staudinger's viscosity method using 0.2 g. of dextrin acetate in 25 cc. *m*-cresol at 30°, $K = 1 \times 10^{-4}$. [d] R_{Cu} according to Richardson, Higginbotham and Farrow.[10] [e] Using Rast camphor method with 0.2 g. of dextrin acetate in 0.5 g. of camphor (m. p. 176°). [f] Methylated starch by this method gives a value of about 54 glucose units.

starch. Haworth, Hirst and Woolgar[19] consider waxy cornstarch to be an amylose, the repeating chemical molecule consisting of 26–30 glucose units as in the other starches. They explain the difference in solubility between amylose and amylopectin as due to the extent of aggregation of these molecules. According to Meyer[3] the terms amylose and amylopectin would indicate straight-chain and branched structures, respectively. By the latter interpretation, waxy cornstarch, because of its resistance to retrogradation, would be classed as an amylopectin. The contradiction in the classification of waxy maize starch as above depends upon the relative emphasis placed on solubility in the first case and resistance to retrogradation in the second case.

The vagueness which must be associated with the use of the terms amylose and amylopectin as defined by solubility is borne out in Fig. 1. In pastes prepared at 100°, simple mechanical disruption of the granules is sufficient to increase the amylose content from 20% in untreated cornstarch pastes to 40% in the homogenized pastes and 80% in ball-milled samples. At 130° the disorganization due to temperature alone passes the limit of disruption that can be obtained with the hand homogenizer used in these experiments. The rather unexpected slope of the curve (Fig. 1) for the homogenized samples is probably due to mechanical dispersion of insoluble fragments of the original granule which are then held in suspension by hydrated portions. The number and size of these fragments would decrease with increased pasting temperature. An experimental confirmation of this explanation was obtained by pasting a sample at 100°, passing it through the homogenizer, heating the homogenized sample at 130° and then electrodialyzing. The yield of 30% amylose agreed very well with the value of 31.1% obtained from the sample which was merely pasted at 130°.

The increased reducing power of pastes prepared at higher temperatures (Figs. 1 and 2) is indicative of a small amount of hydrolytic cleavage producing free aldehyde groups. Such changes cannot be interpreted in molecular dimensions until all granule fragments are completely disorganized.

(19) Haworth, Hirst and Woolgar, *J. Chem. Soc.*, 177 (1935).

The amount of amylose obtained by freezing may be plotted as a regular function of the amount of amylose obtained by electrodialysis, independent of the method of preparing the pastes (Fig. 2). The indefiniteness of solubility as a criterion for fractionation of starch can be emphasized by two examples: (a) the soluble fraction obtained by electrodialysis can be retrograded by freezing until only 3% remains soluble at room temperature; (b) waxy cornstarch which resists separation by both electrodialysis and freezing can be made to retrograde by alternate freezing and holding at 4° for long periods of time.

The data confirm frequent statements in the literature to the effect that the fractionation of starch pastes (by electrodialysis and by freezing) depends upon the previous history of the sample. The various soluble fractions are not identical as indicated by differences in their reducing power, resistance to retrogradation and alcohol precipitation. Until laboratory techniques are available for more exact characterization of such fractions, it is not possible to decide whether such differences are due to the methods of peptization, to the methods of fractionation, to chemical differences or to physical differences in the starch.

X-Ray diffraction measurements[7] have shown that all retrograded starch fractions so far investigated, quite independent of their source, are crystalline and have about the same unit cell dimensions as the original starch. It is apparent that such products would be inaccessible to enzyme action just as would the crystal structure in the original granule. The Sallinger procedure for measuring the aging of pastes must then be interpreted as a measurement of the rate at which such crystallization takes place. The conclusion is in harmony with the interpretation by Samec and Katz[20] of their data on the aging of modified starches using malt. In their experiments the resistance to malt digestion increased with time for all samples but the rate and degree of increase differed. They explain the change as due to aggregation into complexes sufficiently large to resist enzyme action but not necessarily large enough to flocculate out of the solution.

(20) Samec and Katz, *Kolloid-Beihefte* **49**, 455 (1939).

Digestion of starch by β-amylase has been assumed to depend upon progressive hydrolysis of maltose units from the non-reducing end of the molecule until some structural irregularity impedes the progress of the enzyme. Structural differences between starches, such as variation in the extent of branching of the chain, would be expected to be found concentrated in the residual dextrins. When this method of fractionation was applied to waxy and ordinary cornstarch, the anticipated differences between the limit dextrins were not observed.

The similarity in physical constants shown in Table IV does not necessarily prove that the two dextrins are structurally alike. However, it is impossible to escape the conclusion that if branching exists in ordinary cornstarch such branch linkages are hydrolyzed as rapidly as the ordinary chain linkages. This conclusion rests on the fact that the dimethyl glucose content (0.67%) of the limit dextrin is not greatly different from that of the parent starch (0.93%).

Summary

1. The ratio of soluble to insoluble components separated from ordinary cornstarch pastes by electrodialysis or by freezing depends upon the extent of peptization.

2. Retrogradation or aging of starch pastes is a crystallization process as evidenced by the definite X-ray patterns. The Sallinger procedure measures the rate at which such crystallization takes place.

3. A comparison of the physical properties of the limit dextrins obtained from waxy and ordinary cornstarch by digestion with β-amylase emphasizes a close similarity rather than a chemical difference between them.

4. The quantity of dimethyl glucose obtained by hydrolysis of methylated cornstarch (0.93%) does not differ greatly from the quantity obtained by hydrolysis of the methylated limit dextrin (0.67%). If branching exists in the original starch, such linkages are hydrolyzed by the β-amylase.

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