[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE NEW JERSEY COLLEGE FOR WOMEN, RUTGERS UNIVERSITY]

SEPARATION AND PROPERTIES OF THE TWO MAIN COMPONENTS OF POTATO STARCH[1]

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Since starch became recognized as granules composed of two distinct parts, an outer envelope and an inner more soluble portion, various methods for the separation of these components and certain of their distinguishing properties have been recorded.

Gatin-Gruzewska[2] effected a separation by treating potato starch with dilute alkali and, after neutralization and sedimentation, decanting the dissolved fraction or "amylose" from the residue or "amylopectin." The "amylose" represented 40–45% of the original starch, stained blue with iodine, and had a specific rotation of +182.4° when measured in a 0.643% solution. The "amylopectin" represented 55–60% of the starch, stained red-violet with iodine, and had a specific rotation of +221° when measured in a 0.178% solution.

Tanret[3] by treatment of potato starch with hot water, sedimentation and decantation, estimated 27% of "amylose."

Sherman and Baker[4] subjected a thin paste of potato starch, prepared at 85°, to centrifugal force and obtained about 15% in the more soluble or "β-amylose" fraction.

Samec and Mayer[5] electrodialyzed a dispersion of potato starch prepared at 120°. The less soluble or "amylopectin" fraction represented 83% of the starch, contained 0.77% of phosphorus,[6] and had a specific rotation of +195–196° in solutions concentrated by distillation. The "amylose" fraction represented 17% of the starch, contained practically no phosphorus and had a specific rotation of + 189°.

Ling and Nanji[7] fractionated potato starch by freezing a paste and, after melting the frozen mass, separating the solution of "amylose" from the undissolved residue of "amylopectin" by centrifuging. The "amylopectin" after being more completely freed of "amylose" by enzymic action had a specific rotation of +221°.

[1] Some of the data included in this paper are taken from a dissertation, "On a 'Phosphorus-Free' Amylose from Potato Starch," by M. E. Baldwin, Columbia University, 1928.

[2] Gatin-Gruzewska, *Compt. rend.*, **146,** 540 (1908); **152,** 785 (1911).

[3] Tanret, *Bull. soc. chim.*, [4] **17,** 83 (1915).

[4] Sherman and Baker, THIS JOURNAL, **38,** 1885 (1916).

[5] Samec and Mayer, *Kolloid-Chem. Beihefte,* **13,** 272 (1921).

[6] Samec, Minaeff and Ronzln, *ibid.*, **19,** 203 (1924).

[7] Ling and Nanji, *J. Chem. Soc.*, **123,** 2666 (1923).

Taylor and Iddles[8] disintegrated starch grains, previously treated with hydrogen chloride in alcohol, with ammonium thiocyanate solution, then precipitated with alcohol and subjected the precipitate to ultrafiltration or electrodialysis. They obtained 97–98% of potato starch in the dissolved fraction ("β-amylose") which had a specific rotation of 181.6 calculated as $[\alpha]_D^{25}$ from the mercury vapor line by a factor obtained from work on sucrose.

A comparison of the results of these different methods reveals discrepancies among the values reported for specific optical rotation and shows that the ratio of the two fractions obtained varies with the solvent used, the effect of strong chemicals being to increase the percentage of starch in the more soluble fraction.

The purpose of this investigation has been (1) to develop a method for the fractionation of potato starch which will make a sharp separation of the material of the outer envelope from that which makes up the interior of the grain, without subjecting the starch to strong reagents or high temperatures, also, (2) to define more rigidly and compare the products.

Experimental

The method of separation which has resulted from this work is based upon the difference in water-solubility between the two parts of the starch grain. In general, it consists of gelatinizing the grains and subjecting them alternately to a freezing process and a series of extractions, each step in the separation being carried out under such conditions as were found by experiment to be most favorable to the separation. In developing the method, series of test separations were made in which conditions of time, temperature and sequence of processes were systematically varied. The influence of each change on the separation was measured by making a comparative study of the properties of the products. Since it was known from the previous work of Gatin-Gruzewska,[2] Tanret[3] and Samec and his co-workers[5,6] that the two main components of starch differ in specific rotation, phosphorus content and color-with-iodine, these properties were used as criteria of progress. Such conditions as caused the greatest divergence in these properties were selected and trial separations continued until the properties became, for each fraction, constant. These trial separations gave experimental bases for selecting the details of the final form of the method.

The products obtained have been defined by determining the following properties: (1) specific optical rotation, (2) temperature coefficient of specific optical rotation, (3) phosphorus content, (4) color-with-iodine, (5) precipitability with alcohol, (6) precipitability with iodine in potassium iodide solution, (7) retrogradation.

[8] Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

The more soluble component has been designated as β-amylose and the less soluble as α-amylose.

The starch used was obtained from mature potatoes and purified by repeated sedimentation in distilled water. The air-dry product contained 13.6% of moisture. Its gelatinization temperature was 63°. Some of the grains became swollen at 60° but in order to have every grain swollen, a temperature of 63° for two minutes was necessary.

Measurement of the Properties Used as Criteria of Progress in Developing the Method

Specific Optical Rotation.—The accuracy of the polariscope used was verified by determinations of the specific rotation of 5% solutions of pure glucose in water. These values checked the recent equilibrium value of 52.5° reported by Nelson and Beegle.[9]

Clear dispersions of starch and of α-amylose were prepared for use in the polariscope by heating 0.5% dispersions at 120° for one to two hours then concentrating to 2–3% by distillation under reduced pressure. Solutions of β-amylose were also concentrated to 2–3% in this way.

Optical rotations were read to 0.05° and estimated to 0.025°. The exact concentration of each dispersion was calculated from the weight of the material left after an aliquot was evaporated to dryness. α-Amylose was dried at 110°, starch and β-amylose at 100°.

Phosphorus Content.—The percentage of phosphorus in starch and in α-amylose was determined by the magnesium ammonium phosphate method[10] and the strychnine phosphomolybdate method;[11] the percentage in β-amylose by the strychnine phosphomolybdate method.

All reagents were tested for phosphorus and purified when necessary.

Samples to be analyzed were ignited with magnesium oxide according to the method used by Vozarik[12] and by Hibbard.[13] The residue was dissolved in hydrochloric acid and neutralized with ammonium hydroxide.

Manipulation of the magnesium ammonium phosphate method was checked by determinations upon Na_2HPO_4 which had been purified and dried.

The strychnine phosphomolybdate method makes use of the cloud formed when strychnine phosphomolybdate is precipitated in acid solution. According to the directions of Pouget and Chouchak[11] a known phosphate and the unknown are compared in Nessler tubes and the depth of the one which equals the whole of the other is measured. This manipulation was varied somewhat to increase the delicacy of the method. A series of aliquots of the standard was set up for comparison with a similar series of the unknown and after formation of the precipitate these two series were dovetailed. In this way two columns of percentages were obtained of which one column contained values too high and the other too low. By making the increments of the series sufficiently small, the percentage of phosphorus was determined with accuracy to the third decimal place. The amount of phosphorus used in the known series ranged from 0.00131 to 0.00393 mg.

Color-with-Iodine.—The method of Tanret[3] was used in analyzing amylose solu-

[9] Nelson and Beegle, THIS JOURNAL, **41**, 559 (1919).

[10] Fales, "Inorganic Quantitative Analysis," The Century Co., New York, **1925.**

[11] Pouget and Chouchak, *Bull. soc. chim.*, **5**, 104 (1909); **9**, 649 (1911).

[12] Vozarik, *Z. physiol. Chem.*, **76**, 426 (1912).

[13] Hibbard, *J. Ind. Eng. Chem.*, **5**, 998 (1913).

tions for blue-staining and violet-staining material. This method is based upon the property of cellulose to adsorb from water solution β-amylose but not α-amylose. Cellulose was placed in the amylose solutions and allowed to stand. At intervals of thirty minutes a portion of the liquid was removed by filtration and both the cellulose and the filtrate were tested with iodine in dilute potassium iodide solution. These tests were repeated until the color of the filtrate ceased to change. This method makes possible the detection of small amounts of either of the amyloses in solutions of the other.

The Method of Separation

Only the final form of the method is given here: two variations are included, one of which has been especially adapted to the preparation of α-amylose and the other to β-amylose.

Variation I. Separation of β-Amylose.—The details of the method with conditions so defined as to give the best preparations of β-amylose are as follows. One liter of 1.5% paste[14] is made up by pouring 15 g. of starch mixed with 100 cc. of distilled water into 900 cc. of water at 63°. The temperature of the mixture is raised to 63° with a 70°-water-bath and held there for two minutes. After standing for two hours it is frozen in thin layers in aluminum pans. Freezing is completed in ten minutes. The frozen mass is melted over a 40° water-bath and filtered through washed filter paper to give Filtrate I and a residue which is mixed with the original volume of water, heated to 55–60°, toluene added and the mixture placed in an oven at 55–60° for sixteen hours. It is then filtered and the extraction process repeated. The residue is again frozen and the extraction repeated three times or until the filtrate is no longer stained with iodine. This method yields a series of six extracts containing β-amylose and a residue consisting of the grain envelopes or α-amylose. β-Amylose in the solid form is obtained from the extracts by precipitation with alcohol or by retrogradation.

Variation II. Separation of α-Amylose.—The details of the method with conditions so defined as to give the best preparations of α-amylose are as follows. Four portions of 1.5% paste, 300 cc. each, are made up by mixing, for each portion, 4.5 g. of starch with 30 cc. of distilled water and pouring the mixture into 270 cc. of water at 85°. The temperature of the paste is raised to 85° over a boiling water-bath. The paste is allowed to stand for two hours and is completely frozen in ten minutes. The frozen mass is melted and filtered. The residue is suspended in 8 liters of distilled water at room temperature and allowed to settle for twenty hours. The extract is removed by decantation. This process is repeated with water at room temperature, then at 85° and three times at room temperature or until the filtrate no longer stains with iodine. The grain envelopes, α-amylose, may be suspended in water and kept under toluene or dried and ground to a fine powder.

Quantitative Measurements of the Fractions Obtained by the Two Variations of the Method

Separations for the purpose of measuring quantitatively the fractions obtained were carried out parallel with separations used for preparing material for analyses. The data obtained are given in Tables I and II.

Some Details from the Experimental Development of the Method.—Except for the more significant observations and conclusions, the data from the individual series of test separations which led to the above method have been omitted.

[14] The word *paste* is used to mean gelatinized grains suspended in water.

TABLE I

VARIATION I FOR PREPARATION OF β-AMYLOSE

Filtrate number	Process by which filtrate was obtained	Separation No. I 400 cc. 1.5% paste		Separation No. II 200 cc. 1.5% paste		Separation No. III 200 cc. 1.5% paste	
		Milligrams of amylose	Amylose, percentage of starch	Milligrams of amylose	Amylose, percentage of starch	Milligrams of amylose	Amylose, percentage of starch
1	Freezing 63° paste	683.2	11.39	330.1	11.00	284.4	9.48
2	Extracting at 55–60°	150.0	2.50	83.3	2.77	113.0	3.77
3	Extracting at 55–60°	27.2	0.45	22.6	0.75	30.7	1.02
4	Freezing residue	13.6	.23	10.3	.34	8.7	0.29
5	Extracting at 55–60°	21.7	.36	10.5	.35	12.7	.42
6	Extracting at 55–60°	12.8	.21	7.2	.24	9.4	.31
7	Extracting at 55–60°	12.2	.20	5.5	.18	6.8	.23
8	Extracting at 55–60°	9.7	.16	2.5	.08	3.0	.10
		930.4	15.50	472.0	15.71	468.7	15.62

TABLE II

VARIATION II FOR PREPARATION OF α-AMYLOSE

Filtrate number	Process by which filtrate was obtained	Separation No. I 100 cc. 1.5% paste		Separation No. II 100 cc. 1.5% paste	
		Milligrams of amylose	Amylose, percentage of starch	Milligrams of amylose	Amylose, percentage of starch
1	Freezing 85° paste	193.7	12.91	156.1	10.41
2	Washing at room temperature	54.1	3.61	87.0	5.80
3	Washing at room temperature	8.6	0.57	9.7	0.65
4	Washing at 85°	45.7	3.05	39.5	2.63
5	Washing at room temperature	19.3	1.30	18.1	1.21
		321.4	21.44	310.4	20.70

Microscopic Study.—The gelatinized grains of potato starch when first stained with iodine were violet. After a few seconds the color deepened and changed to blue. If the grains were crushed under a cover glass when first stained they appeared as burst sacs, stained violet, from which a blue-stained liquid had poured forth. If swollen, unbroken grains were frozen, then melted, they appeared as long fibers or collapsed sacs, floating in a liquid. The sacs stained violet; the liquid, blue. The expansion of the water with change of state during freezing exerted a pressure upon the grains which forced out the inner liquid. The collapsed sacs, when placed in warm water, became swollen for a second time and if they were ruptured by crushing, a liquid poured forth. This reswelling of the grains, crushed by freezing, to give a round sac containing a liquid showed that freezing did not rupture the sac. It was not necessary to rupture the sac in order to obtain from the grains the β-amylose fraction.

If gelatinized grains were allowed to stand in water, β-amylose passed slowly from the interior of the grain into the surrounding liquid. The grain sacs behaved as dialyzing membranes through which the β-amylose

passed. β-Amylose could thus be obtained by soaking the grains after gelatinization although freezing hastened and helped complete its removal.

Influence of Change of Conditions.—Trial separations showed that in order to make a sharp separation it was necessary to control: (1) the temperature of gelatinizing the grains, (2) the time required for freezing the paste, (3) the temperature of extracting the grains.

Temperature of Gelatinizing the Grains.—An illustration of the trial separations used to develop the method is given in Table III, where the temperature to which the grains were heated in making the paste was the condition varied.

TABLE III

INFLUENCE OF TEMPERATURE OF GELATINIZING THE GRAINS

Temp., °C.	Properties of material extracted: Phosphorus content	$[\alpha]_D^{20}$	Color-with-iodine[a]
63	0.038% of extract	188.8	Blue, no violet detected
	0.0009% of precipitate[b]	189.1	Blue, no violet detected
		189.0	
75			Blue, trace of violet
85	0.049% of extract	191.6	Blue, violet present
		190.8	
		191.3	
100			Blue, violet in large amount

[a] Colors were analyzed by Tanret's method. [b] The solid separating from solution on standing contained 0.0009% of phosphorus; the filtrate from this solid contained free phosphate.

From the data in this table it was learned that the material extracted at 63° had a blue color with iodine, which according to Tanret's method of analysis contained no violet-staining material, was practically phosphorus-free, and had an $[\alpha]_D^{20}$ of +189°. If higher temperatures were used in gelatinizing the grains, the phosphorus content and specific rotation increased, while violet-staining material was found present.

Time Required for Freezing the Paste.—Microscopical observations indicated that during the freezing, retrogradation[15] took place and that the longer the time required for freezing the larger the amount of material which retrograded. This conclusion was verified by quantitative experiments. The precipitate which formed during the more rapid freezing consisted of finer particles which appeared as perfect spheres when magnified, while that obtained with slower freezing was made up of coarser particles which appeared as larger spheres and conglomerates of spheres.

The ease with which retrograded amylose was dissolved and completely extracted from the grain sacs decreases as the size of the particles increased. When only ten to twenty minutes had been required for freez-

[15] The term *retrogradation* is used to mean the separation of the material from solution as a solid, on standing.

ing, the precipitate passed into solution as soon as the temperature was raised to 55°. In cases where freezing required several hours, the precipitate was so coarse that several days at 55–60° were insufficient for its solution.

From the experiments on retrogradation during freezing and upon the character and solubility of the precipitate, it was concluded that shortening the time interval during which β-amylose was held at a low temperature greatly facilitated the complete removal of β-amylose from the grain sacs. Therefore, the practice of freezing the paste completely in ten minutes and melting it immediately over warm water was adopted. The material was thus subjected to a low temperature for not more than twenty minutes.

The Temperature of Extracting β-Amylose from the Grains.—A detailed study of extracts made at different temperatures led to the following conclusions: at temperatures from 20 to 30° retrogradation of β-amylose took place within the grain sac and made its removal incomplete; at temperatures above 75° the solvent action of water on the α-amylose became appreciable. A temperature from 55–60° was found to be most favorable to the separation.

Properties of the Products, α- and β-Amylose

Specific Optical Rotation in Aqueous Solution. Starch.—The temperature at which different investigators prepared clear dispersions of starch in water has been reported variously from 120 to 150°. A study of this variable upon the specific rotation of starch was considered necessary as a basis for establishing conditions for further work. In preparing dispersions for this purpose it was found that the greater the concentration, the higher the temperature and longer the time required to form dispersions clear enough for use in the polariscope. To obtain clear dispersions at a relatively low temperature and in a short period of heating, they were made up containing 0.5% starch and after heating in the autoclave, were concentrated to 2 to 3% by distillation under reduced pressure. The specific rotations of aqueous starch dispersions prepared at temperatures from 120 to 135° are given in Table IV.

TABLE IV

SPECIFIC OPTICAL ROTATION OF STARCH

Temperature and duration of heating dispersion	$[\alpha]_D^{\text{room temp.}}$ Determination		$[\alpha]_D^{20}$ Determination	
	No. I	No. II	No. I	No. II
0.5 hour at 120°			193.8	194.1
1 hour at 120°	194.0		194.2	194.3
1 hour at 125°	194.2		193.9	194.3
1 hour at 130°			192.1	192.8
1 hour at 135°	189.5	189.5		

The data in Table IV show that heating the dispersion at a temperature of 130° or higher caused a decrease in specific rotation; heating at 120 or 125° for one hour caused no decrease. Therefore, the temperature of 120° was selected for later determinations. The $[\alpha]_D^{20}$ values for starch ranged from +193.8 to +194.3° with an average of +194.1°.

α-Amylose.—This fraction was prepared for the autoclave by boiling 0.5% suspensions with vigorous stirring until the grain sacs were broken into fine particles that settled very slowly. The suspensions were heated for one to two hours at 120° and concentrated to 2–3% by distillation under reduced pressure. Determinations upon different preparations are given in Table V. The $[\alpha]_D^{20}$ values for α-amylose (prepared by Variation II of the method) ranged from +195.1 to +195.7° with an average of +195.5°.

β-Amylose.—The solutions of β-amylose used for rotation reading were the first filtrates (Filtrate I) obtained in the separation. Filtrates from later steps in the separation were so dilute that during the time required to concentrate them, retrogradation took place. The first filtrates contained 0.1 to 0.4% of β-amylose. They were concentrated to 2–3% before direct readings were taken. Determinations upon different preparations are given in Table V. The $[\alpha]_D^{20}$ values for β-amylose (prepared by Variation I of the method) ranged from +188.8 to +189.6°, with an average of +189.3°. The specific rotation of β-amylose was found to be unaltered by previous heating for one hour at 120°.

TABLE V

SPECIFIC OPTICAL ROTATION OF THE AMYLOSES

α-Amylose				β-Amylose			
Prepared by Variation I		Prepared by Variation II		Prepared by Variation I		Prepared by Variation II	
B5	195.6	C1	195.6	B1	189.6	C3	190.8
B5	195.1	C3	195.4	B2	189.1	C4	191.6
B6	195.5	C4	195.2	B3	189.6	C5	191.3
B7	195.7			B4	188.8		
				B8	189.3		
				B9	189.2		

TABLE VI

EFFECT OF TEMPERATURE UPON SPECIFIC OPTICAL ROTATION

Starch			β-Amylose			α-Amylose		
Dispersion no.	Temp. of direct reading, °C.	$[\alpha]_D$	Prepn. no.	Temp. of direct reading, °C.	$[\alpha]_D$	Prepn. no.	Temp. of direct reading, °C.	$[\alpha]_D$
11	17.5	197.3	B8	20.0	189.3	B5	20.0	195.6
12	20.0	194.3	B9	20.0	189.2	B10	20.0	195.7
16	20.0	194.3	B9	21.25	188.9	C3	20.0	195.4
12	21.0	193.3	B10	21.50	189.4	B5	21.0	194.2
16	21.5	193.0	B8	22.50	189.0	B5	21.5	193.5
11	24.5	189.4	B8	25.00	188.7	B5	22.0	193.1
11	28.5	185.8	B9	25.50	188.3	C3	23.0	191.8
11	32.75	181.3	B8	27.50	188.3	B5	23.5	191.5
			B9	33.50	187.3	B10	26.5	187.5
						B5	27.75	185.6
						B10	30.00	183.6

Effect of Temperature upon Specific Optical Rotation.—Determinations of specific rotation were made at different temperatures from 20 to 30° and the data are given in Table VI.

From these data in Table VI it may be seen that when the direct rotation readings are made at different temperatures the $[\alpha]_D$ values for starch and its components decrease as the temperature increases. Expressed as formulas these data become

$$[\alpha]^t_D \text{ of starch} = 214.2 - 1.00t \ (t = 20\text{–}30°)$$
$$[\alpha]^t_D \text{ of } \beta\text{-amylose} = 191.9 - 0.13t \ (t = 20\text{–}30°)$$
$$[\alpha]^t_D \text{ of } \alpha\text{-amylose} = 220.1 - 1.23t \ (t = 20\text{–}30°)$$

The variation per degree in the $[\alpha]_D$ value is about ten times as great for α-amylose as for β-amylose. This difference between the amyloses is expressed graphically in Fig. 1.

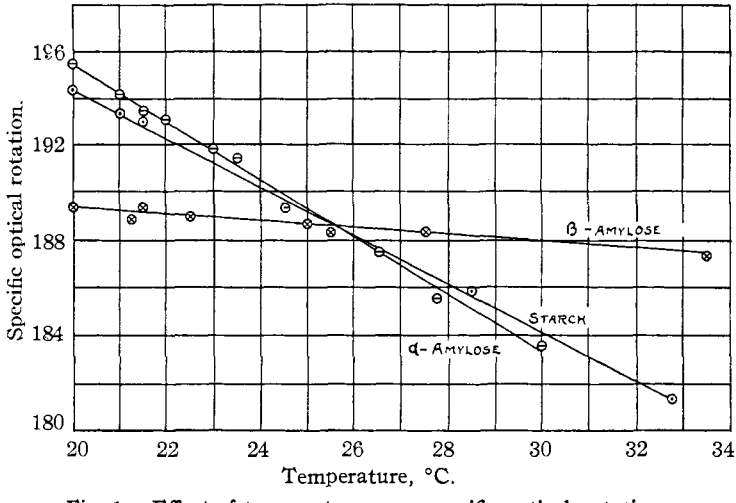

Fig. 1.—Effect of temperature upon specific optical rotation.

Phosphorus Content. Starch.—Determinations of the phosphorus in this sample of potato starch, by the magnesium ammonium phosphate method, gave 0.0691, 0.0696 and 0.0694%; by the strychnine phosphomolybdate method, 0.069% ± 0.0005.

α-Amylose.—Determinations of the phosphorus in α-amylose by the magnesium ammonium phosphate method gave 0.077 and 0.075%. Determinations on several samples by the strychnine phosphomolybdate method each gave 0.076% ± 0.0005.

β-Amylose.—Determinations of phosphorus in β-amylose were made by the strychnine phosphomolybdate method after it had been checked against the magnesium ammonium phosphate method. Samples of β-amylose were obtained in the solid form by precipitation with alcohol and

by retrogradation. Samples of precipitated β-amylose contained 0.19% phosphorus, of retrograded amylose 0.006 to 0.0009%, the percentage decreasing with the speed of retrogradation, which increased with decrease of temperature.

The phosphorus in the two fractions, α- and β-amylose, does not represent the total phosphorus of the original starch; the remainder was found as free phosphate after precipitation or retrogradation of β-amylose.

Color-with-Iodine.—The color which α-amylose solutions developed on addition of iodine in potassium iodide solution varied with the amount of iodine, as had been noted previously by Samec and Mayer.[5] Small amounts of iodine, added either to the hydrated grain sacs or their clear dispersions, produced a light blue color which changed on further addition of iodine through violet to red-violet. In order to see whether the blue color which first develops could be detected in the presence of the red-violet, the region of the complementary absorption bands was determined. When the dispersions were placed in the spectroscope and successive additions of iodine made, the absorption band passed from left to right, through the red and orange region to the yellow, yellow-green. From the results it may be concluded that the blue color which first developed disappeared and was not covered up by the deeper red-violet formed with increased amounts of iodine.

β-Amylose gave with iodine a blue color in all concentrations of the amylose down to 0.0004%, and with all proportions of iodine until the yellow color of the iodine was so great as to give with the blue a green color.

Precipitability with Alcohol. α-Amylose.—Dispersions for use in precipitation experiments were made by heating 0.5 to 2.0% suspensions of α-amylose in water at 120°. When alcohol was added to these dispersions, until the final concentration of alcohol reached 85% by volume, no precipitation took place even after long standing. The addition of ether to the 85% alcohol mixture to give a final concentration of 34% ether by volume caused practically complete (98–99%) precipitation of the α-amylose. The addition of sodium chloride also caused precipitation.

β-Amylose.—Filtrates obtained by Variation I of the method were used in precipitation experiments. When alcohol was added to these solutions, which contained from 0.1 to 0.4% β-amylose, until the final concentration of alcohol reached 65% by volume, precipitation of β-amylose was practically complete (98–99%).

Precipitability with Iodine.—Addition of iodine in potassium iodide solution to 4% dispersions of α-amylose did not cause precipitation. β-Amylose, in concentration of 0.1% or greater, was precipitated by iodine to give a dark blue precipitate in a clear liquid, colored faintly yellow by excess iodine. More than 99% of the β-amylose was thus precipitated.

Retrogradation.—β-Amylose retrograded slowly at low temperatures.

Filtrates from Variation I were found to yield 90–95% of the dissolved material as retrograded amylose in two weeks, although precipitation was probably incomplete at this point. Previous heating of the solution increased the speed of retrogradation.

The properties of the amyloses are summarized in Table VII.

TABLE VII

PROPERTIES OF α- AND β-AMYLOSE

Properties	Starch, gelatinized at 63° B β-Amylose	A α-Amylose
$[\alpha]_D^{20}$	189°	195.5°
Temperature coefficient of $[\alpha]_D^{20}$	0.13	1.23
Phosphorus content	0.0009%	0.076%
Color-with-iodine	Blue	Red-violet
Precipitability with alcohol	Precipitates, 98–99%	Does not precipitate[a]
Precipitability with iodine	Precipitates, 99%	Does not precipitate
Retrogradation[b]	Retrogrades, 90–95%	Does not retrograde

[a] In absence of electrolyte. [b] The separation as a solid from solution on standing.

Discussion of the Separation

Since β-amylose is more rapidly dissolved than α-amylose and since it was obtained in a series of extracts, it was expected that if the extracts contained α-amylose as an impurity, the amount would increase progressively in the series and the properties would alter progressively toward those of α-amylose, listed under A, Table VII. In so far as the methods of measuring the properties could be made applicable to increasingly dilute extracts, the properties were found to be identical and the β-amylose uniform in character throughout the series.

Removal of β-amylose by repeatedly freezing and extracting grains gelatinized at 63° did not rupture the grain sacs. Sixteen per cent. of the starch was the total amount that could be obtained by these processes below the gelatinization temperature. When the residual unbroken sacs were broken into fine particles by vigorous agitation, allowed to stand in water, then filtered, the filtrate contained no dissolved material, from which it was concluded that no more material could be obtained by rupture of the sacs and that 16% was the total amount that could be obtained in solution at 63° or below.

When by gelatinizing and extracting the original starch at a higher temperature a larger amount of the starch was obtained in solution, the properties of the dissolved material diverged toward those of α-amylose, listed under A, Table VII.

The α-amylose preparations passed slowly into solution at the boiling point. By filtering before the whole sample had dissolved there were obtained a dissolved portion and an undissolved residue. The properties of these two portions were determined separately. It was expected that

if the α-amylose preparations contained β-amylose as an impurity, the properties of the dissolved portion would diverge toward those of β-amylose listed under B, Table VII. The properties of both portions were identical, and the same as those listed under A, Table VII. The identity of the dissolved and the undissolved portion was evidence that the α-amylose preparations were free from β-amylose.

The temperature coefficients of specific rotation serve to explain most of the discrepancies among the values given in the literature for this analytical constant.

Comparison of the Products.—Of the six properties which were studied in order to define the amyloses, some differ numerically, others are antithetical.

While the $[\alpha]_D^{20}$ values of the amyloses differ by 6.5 degrees, a great difference between the amyloses is shown by the changes which this analytical constant undergoes with temperature. Between 20 and 30° the $[\alpha]_D^t$ for β-amylose is $191.9 - 0.13t$, that for α-amylose is $220.1 - 1.23t$. Thus the temperature coefficient of α-amylose is about ten times that of β-amylose.

α-Amylose is a phosphorus-bearing compound while β-amylose is "phosphorus-free."

In their behavior toward alcohol and iodine as precipitants and in regard to retrogradation, the amyloses are antithetical.

Summary

This method for the fractionation of potato starch effects the separation of the outer envelope (α-amylose) from the inner more soluble portion (β-amylose) without subjecting the material to contact with strong reagents or high temperatures. It is based upon their difference in water-solubility and consists of gelatinizing the grains, then subjecting them alternately to a freezing process and a series of extractions, each step being carried out under such conditions as were found by experiment to be most favorable to the separation. During the separation the sacs act as dialyzing membranes toward β-amylose and are not ruptured by freezing.

In developing the method, series of trial separations were carried out under systematically varied conditions and the effect of each change on the properties of the products was measured. Data from these experiments showed that the trial separations were continued until the properties, which diverged under increasingly more favorable conditions, finally ceased to diverge and became constant for each fraction. A sharp separation of the amyloses was thus indicated.

The method separates a phosphorus-bearing (α-amylose) from a "phosphorus-free" (β-) amylose which contrast strongly in their temperature coefficients of specific optical rotation and are antithetical with regard to retrogradation and toward alcohol and iodine as precipitants. A compari-

son of the properties of the products thus supplies additional evidence that the method effects a sharp separation of the amyloses.

β-Amylose has an $[\alpha]_D$ value at 20° of 189° which varies with the temperature according to the formula $[\alpha]_D^t = 191.9 - 0.13t$. It is practically free from phosphorus, gives a blue color with iodine which according to Tanret's method of analysis is free from violet, is precipitated by alcohol and by iodine, and retrogrades. The speed of retrogradation is increased by previous heating of the solution. β-Amylose represents 16 ± 1% of the original starch.

α-Amylose has a $[\alpha]_D^t$ value at 20° of 195.5° which varies with temperature according to the formula $[\alpha]_D^t = 220.1 - 1.23t$. It contains 0.076% of phosphorus, gives a red-violet color with iodine which according to Tanret's method of analysis is free from blue, it is not precipitated by alcohol in absence of electrolyte, or by iodine; it does not retrograde. It represents 84 ± 1% of the original starch.

The specific optical rotation of potato starch is expressed by the formula $[\alpha]_D^t = 214.2 - 1.00t$. Heating starch dispersions at 130° or above causes a decrease in the $[\alpha]_D$ value.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MARYLAND AND THE HYGIENIC LABORATORY, UNITED STATES PUBLIC HEALTH SERVICE][1]

THE CHEMISTRY OF ORGANIC GOLD COMPOUNDS. I. AUROUS CHLORIDE CARBONYL AND A METHOD OF LINKING CARBON TO CARBON

BY M. S. KHARASCH AND H. S. ISBELL[2]

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In the course of our systematic investigation of organic gold carbon compounds, we became interested in the theoretical aspects of the type of organic radicals which might form stable compounds with monovalent gold. In view of the fact that at that time our only method of preparing gold carbon compounds was by the Grignard reagent, we were naturally led to a search for some monovalent gold compounds which might be soluble in organic solvents. The literature revealed none. A method of attacking this problem was suggested by the fact that the carbonyl of platinous chloride dissolves in organic solvents. Accordingly, we decided to investigate the action of carbon monoxide with gold salts.

Previous Work.—The action of carbon monoxide on a water suspension of aurous chloride leads to the formation of a colloidal gold solution.[3]

[1] Published by permission of the Surgeon General.

[2] This work was done in 1925 and submitted to the Graduate School of the University of Maryland in partial fulfilment of the requirements for the doctorate degree in 1926.

[3] Donau, *Monatsh.*, **26**, 525 (1905); *ibid.*, **27**, 71 (1906).