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CHARACTERIZATION OF CERTAIN STARCHES AND THEIR AMYLOSES[1]

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In pursuing further the study of starches of various genera, we have followed two main channels of investigation. The one has had for its purpose the characterization of a starch as a function of its constituent amyloses; the other, a search for those chemical properties that would give us more rigid definitions of the amyloses themselves. All the work in this Laboratory is based primarily on the assumption that the organized starches are heterogeneous materials, the two principal components being designated as α- and β-amylose. This nomenclature corresponds approximately to that of Arthur Meyer.[2] From this point of view the amyloses are not to be considered as degradation or scission products and do not correspond to the types of amyloses obtained by Pringsheim through the action of *B. Macerans*, to those obtained by Pictet through the action of heat or to those obtained by Ling and Nanji through the action of enzymic agents.

Part I. Ratio of Amyloses in Wheat and Tapioca Starches

This consideration that starch granules are composed of different chemical substances rather than one homogeneous material was first definitely advanced by Raspail[3] in 1825. In his report a soluble and an insoluble fraction exhibited different colorations with iodine. The conception of differing chemical individuals was immediately challenged by some experimenters and defended by others. Since that time fully a hundred major investigations have been devoted to the problem of separating and characterizing these components. Specific reference need not be made here to the various observations and hypotheses which have been communicated, as a complete review of this literature has been published recently.[4]

The most obvious feature to be noted in a review of these earlier communications is the marked divergence in the reported ratios of the two or more materials. This might naturally be expected in that there are no precise definitions for these components and in all methods of separa-

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[1] The work covered in this paper is taken from a dissertation presented by R. P. Walton to the Faculty of Pure Science of Columbia University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] A. Meyer, "Untersuchungen über die Stärkekorner," Jena, 1895.

[3] Raspail, *Annales des sciences naturelles*, 6, 224–239, 384–427 (1825).

[4] Walton, "A Comprehensive Survey of Starch Chemistry," Chemical Catalog Co., New York, 1928.

tion it is necessary first to get these materials out of the granule, an end not easily accomplished by the ordinary hot water treatment most generally used.[5]

In the last few years, however, the ratios of the amyloses in a given starch reported by various workers have been more generally consistent. The method of separation used in this Laboratory[6] has been so chosen because it is believed that interference due to unbroken or partially broken granules is eliminated, because fairly concordant results may be obtained easily and because the products have reasonably definite characteristics. The previous separations in this Laboratory gave ratios for the amyloses in corn, rice and potato starches. In this work separation studies have been carried out on wheat and tapioca starches because of certain specially significant characteristics of these common starches.

Experimental

The preliminary treatment with boiling alcoholic hydrochloric acid, as prescribed by Taylor and Iddles, was found to be too drastic for the starches used in this investigation. Treatments with alcoholic hydrochloric acid at 40–50° for one-half to one hour were usually sufficient to furnish starches which gave a relatively fluid paste upon subsequent thiocyanate gelatinization. The original purpose of this treatment was the removal of nitrogenous impurities, although it also serves to lower the viscosity of the resulting gelatinization mixture.[5] The granules remain intact, as shown by microscopic examination, although there are evidences of incipient rupture. When all starches thus far investigated are so treated and later gelatinized with hot water, a relatively thick paste is obtained, except in the case of wheat starch.

Wheat Starch.—Two hundred and fifty grams of air-dried starch was made into a paste with 170 cc. of alcohol, mixed with 150 cc. of alcoholic hydrochloric acid and mechanically stirred for one hour at 50°. The concentration of alcoholic hydrochloric acid corresponded to 0.08 g. of hydrochloric acid per cc. of alcohol, as determined by titration. The alcoholic hydrochloric acid was removed by suction filtration and the starch washed free of hydrochloric acid by successive additions of fresh alcohol. The starch thus treated was introduced slowly into a large mortar containing 90 g. of ammonium thiocyanate in a solution of 450 cc. of water and 120 cc. of alcohol. After grinding for one hour at 45–50°, the solution became fluid and translucent. Microscopic examination indicated complete disruption of the granules. The gelatinized starch was thereupon precipitated from solution by alcohol and dehydrated by vigorous and prolonged grinding with absolute alcohol. After washing with ether and drying in air, 178.2 g. (dry weight) was mixed with 300 cc. of alcohol, introduced into 4 liters of water at about 55° with vigorous stirring and the resulting suspension placed in the usual apparatus[6] with a d. c. potential of 220 volts across the electrodes. Unless the gelatinized starch is introduced as an alcohol paste, bulky lumps will form which are difficult to break

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[5] Taylor and Beckmann, This Journal, **51**, 294 (1929).

[6] Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713–717 (1926).

down. In the course of one or two weeks the combined effects of gravity and anodic migration caused a sharp separation of solid at the bottom of the jar, leaving an upper clear layer. The upper layer containing the soluble fraction (β-amylose) was siphoned off, fresh distilled water added and the procedure repeated until the upper layer no longer gave an iodine coloration. Toward the end of the operation the fresh distilled water was first heated to a temperature of 50–60° and the suspension stirred vigorously. The β-amylose was obtained from the clear solution by precipitation with alcohol. The insoluble fraction (α-amylose) was collected on a suction filter and dehydrated with a current of air at 100° for about five days.

Tapioca Starch.—The same technique was used as before. The behavior is different from that of wheat starch in that the first downward movement of the boundary layer is somewhat slower. Toward the end of the procedure, however, the effect of the electrical potential is very marked. If the electrodes are reversed a sharp upward movement of the boundary layer may be noted. Also, the physical consistency of the alcohol precipitate differs from that obtained with wheat starch. With the latter the precipitate was mealy and easily ground with alcohol, although with tapioca starch the product was stiff and more difficult to dehydrate with alcohol.

Corn Starch.—Separations of the amyloses from corn starch were made in order to obtain products for the subsequent nitrations. Dehydration was effected by heating on a steam-bath with an excess of benzene and absolute alcohol.[5] The alcohol precipitate from the gelatinization mixture had the same consistency as tapioca starch.

While not of any unique significance, the record of the electrophoretic treatments is given in Table I. It serves to show that with the potential employed the separation is not fast and that repeated washing is necessary in order to accomplish complete separation.

TABLE I

RECORD OF ELECTROPHORETIC SEPARATION

Starch	Tapioca	Tapioca	Tapioca	Wheat	Wheat
Sample no.	1	2	3	1	2
Dry gelat. starch in 4 liters of water, g.	299	138	133.1	178.2	167.4
Electrodialysis, days	48	24	28	46	36
Changes of dialysis water	14	10	11	17	10

In Table II the amount of α-amylose as separated by this method is given for two starches not heretofore investigated.

TABLE II

α-AMYLOSE IN TAPIOCA AND WHEAT STARCHES

Starch	Tapioca	Tapioca	Tapioca	Wheat	Wheat
Sample no.	1	2	3	1	2
α-Amylose, g.	52.4	22.5	21.9	42.5	39.5
α-Amylose, %	17.5	16.3	16.4	23.8	23.5

The specific rotations of the soluble or β-amyloses from these starches are given in Table III.

TABLE III

OPTICAL ROTATIONS OF β-AMYLOSES AT 25°

Sample	Wheat No. 1	Wheat No. 2	Tapioca No. 2
Concn., g./20 cc. water	0.2032	0.4776	0.3728
Angular rotation, 2-dm. tube	4.51°	10.67°	8.14°
Sp. rot. λ = 5461 Å.	221.2°	223.4°	218.3°
Sp. rot. λ = 5892.5 Å.	186.9°	188.7°	184.4°

The factor (0.845) for converting readings with the green mercury line (5461 Å.) to the D sodium line (5892.5 Å.) was obtained by direct comparisons, using a concentrated aqueous solution of β-amylose. The lines were obtained from a continuous spectrum, patches of which having passed the spectroscope were chosen so that the optical center of each path had the desired wave length. The optical centers were determined by means of a quartz test plate calibrated by the Bureau of Standards. The procedure has been described by Levene and Bencowitz.[7] All β-amyloses gave the usual deep blue colorations with iodine–iodide test solutions.

From the results in Table II it is seen that the ratio of α- to β-amylose in the case of tapioca starch is approximately 17:83, the β-amylose being calculated by difference. Taylor and Iddles obtained practically the same ratio for corn and rice starches. The ratio of α- to β-amylose in the case of wheat starch is approximately 24:76 and indicates a higher content of α-amylose than any of the starches previously investigated by this method.

Part II. Characterization by Determination of Associated Non-Carbohydrate Materials

In the foregoing section it was stated that the particular method of separation used was preferred because of certain advantages in manipulative technique and because the separated products exhibited distinctively different characteristics which did not seem to be dependent merely upon the method of separation.

From the standpoint of physical behavior it has been found that β-amylose is fairly soluble and α-amylose relatively insoluble. β-Amylose passes through collodion membranes of a definite degree of permeability, whereas α-amylose is retained by the same membranes. β-Amylose does not migrate under the electrical potential imposed, whereas α-amylose exhibits a definite electro-negative behavior. As found previously for corn, rice and potato starches, these physical characteristics were likewise noted here in the case of wheat and tapioca starches.

The distribution of associated non-carbohydrate material, notably

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[7] Levene and Bencowitz, *J. Biol. Chem.*, **72**, 627–634 (1927).

fatty acid residues and phosphate residues, has also been found previously to constitute a further basis of definition of the amyloses. This distribution accordingly was determined in these cases also and is reported in the following.

Experimental

Fatty acids were determined according to the method of Taylor and Nelson,[8] all analyses being run in duplicate. The results agreed within 2% for "fatty acids by hydrolysis," although determinations of "extraneous fat" were considerably less accurate and the extraneous fat extracted from some samples is negligible.

The acid number for the ether-soluble, fatty material liberated by hydrolysis was determined by titration with 0.05 *N* alkali, after dissolving the fat in hot alcohol previously neutralized to phenolphthalein. In the case of corn starch, Taylor and Lehrman[9] have shown that these fatty acids consist exclusively of palmitic, oleic and linolic acids, the combination giving an acid number of 186.

The results of the inquiry into the content of non-carbohydrates in these two newly investigated starches is given in Tables IV and V.

TABLE IV

DISTRIBUTION OF ETHER-SOLUBLE MATERIAL IN WHEAT AND TAPIOCA STARCHES

Sample	Wheat starch	Gelat. wheat	Wheat α-amylose		Tapioca starch	Tapioca α-amylose
No.	....	....	1	2	....	2
Extraction time, hours	3	7	6	6	5	10
Ether sol. mat., prelim. ext., %	0.031	0.063	Neg.	Neg.	0.048	0.015
Ether-sol. mat. after hyd., %	0.58	0.38	0.92	1.18	0.10	0.16
Acid no. of ether-sol. material	178.2	...	...	172.7	....	....

Phosphorus determinations were carried out according to the microanalytical method described by Pregl.[10] This is based on the previously developed method of von Lorenz[11] and consists in successive oxidation, acid digestion and precipitation as ammonium phosphomolybdate.

TABLE V

PHOSPHORUS DISTRIBUTION IN AMYLOSES

Sample (wheat)	No.	P expressed as P_2O_5, %	Sample (tapioca)	No.	P expressed as P_2O_5, %
Starch (untreated)		0.270	Starch (untreated)		0.137
Starch (gelatinized)		.224	α-Amylose	1	.068
α-Amylose	1	.353	α-Amylose	2	.059
α-Amylose	2	.265	β-Amylose	2	.041
β-Amylose	2	.041			

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[8] Taylor and Nelson, THIS JOURNAL, **42,** 1726–1738 (1920).

[9] Taylor and Lehrman, *ibid.*, **48,** 1739–1743 (1926).

[10] Pregl-Fyleman, "Quantitative Organic Microanalysis," **1924.**

[11] Von Lorenz, "Landwirtschaftlichen Versuchs-Stationen," **51,** 183 (1910); Neubauer and Lücker, *Z. anal. Chem.*, **51,** 161 (1912).

The phosphorus may be considered as non-dialyzable phosphate for the case of the α-amyloses, in that they have been in contact with several changes of water over a period of about two months. This is not the case, however, for the whole starches or the β-amyloses.

From the fatty acid determinations given in Table IV it is evident that wheat starch is closely similar to those other cereal starches, corn and rice starch, characterized by Taylor and Iddles. A large proportion of the "fatty acids by hydrolysis" is retained by the starch even after complete disruption of the granule. Practically all the fatty acids of the whole starch are accounted for in the insoluble α-amylose fraction, separated by electrodialysis.

Tapioca starch, a tuber or root starch, likewise contains a definite amount of ether-soluble material, although in considerably smaller quantity. The insoluble α-amylose fractions in all cases contain an appreciably greater amount of fatty acid residues than the whole starch.

In the case of wheat starch it is evident that practically all of the combined phosphorus of the whole starch is contained in the α-amylose fraction. With tapioca starch the unusually low content of phosphorus makes such a conclusion somewhat hazardous, although it is reasonably safe to assume that here also practically all the combined phosphorus is contained in the α-amyloses.

The unusually low content of phosphorus and of fatty acids in tapioca starch may possibly be connected with its reported superiority in certain industrial processes. This starch has been considered by some operators as being the most suitable source material for the manufacture of alkali-starch adhesives, nitro starches and certain types of dextrin.

Part III. Characterization by Means of Derivatives

In Part II physical differences of starch components were described and differences in amount of associated non-carbohydrate materials were determined. In this section nitrate derivatives were prepared and characterized in a further attempt to define the amyloses. These nitrate esters were investigated as a continuation of the work of Taylor and Werntz,[12] in which the chemical behavior of the amyloses was studied in methylation and acetylation reactions. Also, the rather recent industrial prominence of starch nitrates as explosive materials lent interest to the study of these derivatives.

Nitration.—A number of investigators consider that starch is not subject to extensive degradation during the usual nitration processes. Brown and Millar[13] treated a starch nitrate with ammonium sulfide and hydrogen sulfide and demonstrated that the regenerated starch had the

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[12] J. H. Werntz, "Studies of the Corn Amyloses," Columbia Dissertation, **1926.**

[13] Brown and Millar, *J. Chem. Soc.*, **75**, 308–315 (1899).

same rotatory power as soluble starch. Béchamp[14] treated various starch nitrates with ferrous salts and also showed that the rotation of the regenerated starch was identical with that of soluble starch. Okada[15] regenerated with alcoholic ammonium hydrosulfide and demonstrated a low content of reducing material in the recovered product. All regenerated starches exhibited the characteristic deep blue iodine coloration. Commercial nitrations with "mixed acids" leave the starch granules practically unchanged in physical appearance.

On the premise that nitration does not involve profound degradation it was considered that a comparison of the optical rotations of the amylose nitrates might have a bearing on certain of the definitions given for amylose and amylopectin[16] by Samec and Mayer.[17] According to their definition the molecular weights of amylose and amylopectin from potato starch are approximately 80,000 and 113,000 as determined by osmotic pressure methods. Also they report the optical rotation of amylopectin or rather its immediately solubilized succedent as being about 7° higher than that of amylose.

Should there be a relation of 2:3 in the molecular complexity of these components in common starches, it might be expected that uniformly prepared nitrate derivatives would also exhibit significant differences in rotatory power. In the following work, however, a fairly close agreement was obtained with the nitrates of the different amyloses from three starch varieties.

With respect to further possible chemical distinctions in the derivatives of the starch fractions, the recent communication of Friese, Smith and Hess[18] is of interest. They report that the solubilities of the triacetates of "amylopectin" and "amylose" from wheat starch differ qualitatively in the solvents chloroform, acetone, benzol and ethyl acetate, the amylopectin derivative being insoluble and the amylose derivative being fairly soluble. These fractions were separated by successive treatments of starch with hot water. The reported insolubility of the acetate, however has not been confirmed by the recent findings of Brigl and Schinle.[19]

In the following the uniform nitrate derivatives of the two fractions obtained from three different starches indicated no qualitative differences in solubility. This was noted with acetone, in which both are readily soluble, and in hot and cold alcohol, in which the solubility is comparatively slight.

[14] Béchamp, *Ann. chim. phys.*, [3] **64**, 311–344 (1862).

[15] Okada, *Cellulose Industry* (Tokyo), **3**, 3–14 (1927).

[16] "Amylose" and "Amylopectin" may be considered as corresponding approximately to the "β-amylose" and "α-amylose" of this Laboratory.

[17] Samec and Mayer, *Kolloidchem. Beihefte*, **13**, 272–288 (1921).

[18] Friese, Smith and Hess, *Ber.*, **61**, 1975 (1928).

[19] Brigl and Schinle, *ibid.*, **62**, 99–103 (1929).

Experimental

The method of Will and Lenze[20] was selected as being best adapted to the preparation of stable nitrates of fairly high nitrogen content and of sufficient uniformity. According to this procedure, oven-dried starch is dissolved in 10 parts (by weight) of cold fuming nitric acid (sp. gr. 1.52) and the nitrate precipitated by the addition of 20 parts of cold, concentrated sulfuric acid. The nitrating acids are removed by washing repeatedly with cold water and the lower nitrate derivatives by fractional extraction with hot alcohol. Further impurities are removed by dissolving the resulting nitrate in a mixture of acetone and alcohol and precipitating by evaporation of the acetone. The product is stabilized by boiling an aqueous suspension for at least twenty-four hours.

This latter treatment with boiling water is essential to the stability of the product, although the duration of the procedure may be shortened if a stabilizing treatment such as that recently proposed by Snelling[21] is used. This method involves suspending the nitrate in a boiling aqueous solution of sulfur dioxide and subsequently neutralizing with a small amount of ammonia. Unstable nitrates are supposedly reduced without affecting the more stable nitrates. Fairly good results were obtained by this method. The addition of stabilizing agents such as those used in industrial practice[22] was not resorted to because of the probability that such materials would affect the constants subsequently determined. A typical product prepared in these experiments showed a heat stability of twenty minutes at 135° with methyl violet test paper[23] and an explosion temperature of 182° when a 0.25-g. sample was heated in a test-tube at the rate of 2° per minute.

Starch nitrates are invariably decomposed on drying unless they have been subjected to some stabilizing treatment; even drying *in vacuo* at 50° initiates a steady decomposition involving the loss of nitrogen. This marked instability of starch nitrates has, until the last few years, prevented their successful application as commercial explosives, despite a number of very serious attempts in this direction.

In order to obtain strictly comparable nitration conditions, the temperature of the nitrating acid was maintained at 2–6° and the time of nitration made exactly two hours. This was sufficient to obtain practically complete solution of the materials nitrated. Occasional small lumps of undissolved starch nitrate were removed by filtering through glass wool. The method of Will and Lenze prescribes a twenty-four-hour treatment, which may explain the higher nitrogen content of their products. Introduction of the starch as a fine dust by means of an air jet was found to give good results, although this procedure was not used consistently.

Besides tapioca starch and potato starch, specially purified corn starch has been found suitable for large scale nitrations, Sadtler's preliminary purification treatment[24] being particularly satisfactory. This special treatment consists essentially in successively treating the starch with 2% aqueous sodium hydroxide and saturated bleaching powder solutions. Removal of fatty material is probably one of the principal purposes of this operation. That this material may be an interfering element is evidenced by the difficulty encountered when nitrating corn α-amyloses

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[20] Will and Lenze, *Ber.*, **31**, 68–90 (1898).

[21] Snelling, U. S. Patent 1,504,986 (1924).

[22] U. S. Patents 779,421, 875,913–875,928, 1,386,438, 1,462,074, 1,473,257.

[23] Scott, "Standard Methods of Chemical Analysis," D. Van Nostrand Co., New York, **1925**, Vol. II, p. 1403.

[24] Sadtler, U. S. Patent 1,211,761 (1917).

in these experiments, a preliminary treatment with dilute alkali being necessary for successful nitration. Wheat α-amylose, however, was nitrated without the necessity of such a treatment, although clear solutions in acetone were obtained only when the material had been first washed with dilute alkali before the nitration.

Nitrogen was determined by decomposing the nitrate with alkali and alcohol, reducing with Devarda's alloy in alkaline solution and distilling the resultant ammonia into standardized acid as described by Kesseler.[25] The alcohol acts as a highly effective catalyst in the alkaline saponification of the nitrate ester. Analyses were run in duplicate, the results agreeing within 2%. The method was found to agree well with the micro-Dumas combustion method. Identical samples, for example, analyzed by this method and the micro-Dumas gave 12.22 and 12.12% of nitrogen, respectively. As noted by Kesseler and several other experimenters, determinations with the Lunge nitrometer were generally unsatisfactory because of the insolubility of starch nitrate in sulfuric acid.

A commercial starch nitrate prepared with "mixed acids" and still retaining the characteristic granular form of starch was included in the

TABLE VI

SPECIFIC ROTATIONS AND NITROGEN CONTENT OF NITRATED STARCHES AND STARCH COMPONENTS AT 25°

Material	N, %	Concn., g./50 cc. of acetone	Angular rot., 2-dm. tube	Specific rotation	
				λ = 5461Å. Green Hg	λ = 5892.5Å. Yellow Na
Tapioca β-Amylose	12.0	0.5916	3.34	141.0	119.8
		.5032	2.86	142.1	120.8
Tapioca α-Amylose	12.2	.3764	2.10	139.5	118.6
		.2652	1.49	140.4	119.4
Tapioca starch	12.1	.3877	2.20	141.8	120.5
Corn No. 1 β-amylose	12.2	.3781	2.16	142.8	121.4
		.3568	2.05	143.6	122.0
Corn No. 2 β-amylose		.4242	2.44	143.7	122.1
		.5475	3.12	142.4	121.2
Corn α-amylose	12.0	.3245	1.86	143.6	122.1
		.3062	1.75	142.8	121.4
Corn starch (Sadtler treated)	11.5	.2263	1.29	142.5	121.1
		.4091	2.33	142.9	121.5
Wheat β-amylose	12.5	.3644	2.04	140.0	119.0
		.3259	1.84	141.1	119.9
Wheat α-amylose	11.8	.3064	1.68	141.7	120.4
		.3462	1.95	140.8	119.7
Commercial "Nitro-starch"	12.8	.6728	3.73	138.5	117.7
		.4860	2.70	139.05	118.2
		.6897	3.82	138.8	118.0

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[25] Kesseler, Röhm and Lutz, *Z. angew. Chem.*, **35**, 145 (1922).

determinations. The sample was subjected to preliminary washings with carbon tetrachloride and water.

Rotations with the green mercury line were converted to those of the yellow sodium line by means of the factor 0.849, determined with a spectroscope and calibrated quartz plate in the way mentioned previously for β-amyloses. Corrections were not made for ash content of the nitrates, which was ordinarily less than 0.20%.

The influence of degree of nitration on the corresponding rotatory power has been investigated by Béchamp, whose work constitutes the only previous report on the optical rotation of starch nitrates. Calculating the approximate relation of the wave length of light used in his experiments to the D sodium light, a rotation of $\alpha_D = 142.0°$ is found to correspond to a mono-nitrate (6.7% of nitrogen), while a rotation of $\alpha_D = 120.0°$ corresponds to a di-nitrate (11.1% of nitrogen). Accordingly for each variation of 1% in the nitrogen content of the whole there is an approximate decrement of 5° rotation. It is apparent that the nitrogen content of the products in the above table was of sufficient uniformity for the purposes of this experiment.

Conclusion.—From the close correspondence of solubilities and optical rotations of the nitrate derivatives of several different amyloses obtained in these experiments, it might be concluded that they are closely similar in molecular complexity. The observations obtained with these nitrate derivatives, however, do not help to delineate the chemical nature of the amyloses.

Acknowledgment.—Mr. Kaufman of the Stein-Hall Company and Mr. W. A. Nivling of the Huron Milling Company supplied the samples of starches used. Dr. L. W. Bass, of the Rockefeller Institute for Medical Research, assisted in the determination of relative rotatory dispersions. Dr. A. Elek, of the same institution, carried out the micro-analytical determinations of nitrogen and phosphorus. These favors have been gratefully appreciated.

Summary

1. The α- and β-amylose ratio, according to the method of Taylor and Iddles, was determined for wheat and tapioca starches.

2. Relative rotatory dispersions of β-amylose and starch nitrate were determined for the green mercury line and the yellow sodium line.

3. The distribution of the non-carbohydrate constituents, fatty acids and phosphate residues was determined for wheat and tapioca starches.

4. Physical characteristics of the nitrate derivatives of starch components and of whole starches were determined.

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