[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

Potato Starch[1]

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In the cereal starches such as corn, wheat and rice, there is a certain amount of material which cannot be dissociated and subsequently dispersed to give a clear solution in any amount of water. This is called α amylose[2] or amylopectin and carries with it combined fatty acids.[3] Whether potato starch which has no appreciable amount of combined fatty material contains any of this insoluble α amylose or is all soluble beta amylose is a moot question.

Potato starch does carry, however, combined with it phosphate radicals, but where they are and how they contribute to the properties of the potato starch is also an open question despite considerable work. Some think the phosphorus is attached to one amylose and not to others[4] and some think that it is distributed throughout.[5]

In line with the attempts which have been made in the laboratory to define more rigorously the starches and their amyloses, potato starch has been carefully studied because many modern interpretations of starch structure are dependent upon data of this kind. This is a report of such a study.

It scarcely need be recalled that all attempts to gain information concerning the amyloses, involves some sort of disorganization of the starch granules. This break-up should be brought about without chemical change but it has been difficult in the past to do other than express the hope that no change had taken place during dissociation, since there have existed no sufficiently sensitive tests by which to detect chemical alteration.

Previous investigations[6] on potato starch have placed considerable significance on the phosphorus content of various alleged amyloses. It will be shown, however, that it is almost impossible to rely on these findings in attempting to delineate any distinct potato starch amyloses.

In this work various methods of disorganization of the granule have been studied and correlated with phosphorus content of the recovered amyloid material, ease of retrogradation[7] and chemical alteration as measured by the

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(1) An abstract of a dissertation presented by T. J. Schoch to the Faculty of Pure Science of Columbia University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Meyer, "Die Starkekörne," Jena, 1895.

(3) (a) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); (b) Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

(4) (a) Samec, *Kolloidchem. Beihefte*, **4**, 132 (1912); **5**, 141 (1913); **6**, 23 (1914); **8**, 33 (1916); (b) Baldwin, THIS JOURNAL, **52**, 2907 (1930).

(5) Karrer, *Helv. Chim. Acta*, **12**, 1144 (1929); **15**, 48 (1932).

(6) (a) Malfitano and Catoire, *Compt. rend.*, **177**, 1309 (1923); (b) see **4** (b); (c) Sherman and Baker, THIS JOURNAL, **38**, 1885 (1916).

(7) Walton, "A Comprehensive Survey of Starch Chemistry," The Chemical Catalog Co., New York, 1923, p. 135.

recent alkali labile method of Taylor and Salzmann[8] all with a view toward finding and defining the amylose or amyloses in potato starch.

I. A repetition of the ammonium thiocyanate gelatinization and electrophoretic separation technique of Taylor and Iddles with slight modifications, gave an insoluble migratable residue of approximately 3.5% containing 0.036% phosphorus and a soluble fraction containing 0.060% phosphorus. The original potato starch phosphorus content was 0.069%.

Since any insoluble material resulting from an attempted fractionation is commonly designated amylopectin (α amylose), potato starch would be considered to have about 3.6% of this fraction. Contrary to Samec's[4a] findings and the results of the work of Baldwin,[4b] the phosphorus is mainly in the soluble portion under these conditions, the insoluble material which migrates here having only half the original phosphorus content. Later, it will be shown that under other conditions insoluble residues may be found that are high in phosphorus. No inorganic phosphate is likely to be the cause of this discrepancy since the experiment is carried out under conditions that bring about a forced dialysis of electrolyte away from the amyloid material. Experiments on additions of known amounts of secondary potassium phosphate to the cells during the course of the separation showed that it was quantitatively removed to the level of the original organic (non-dialyzable) phosphorus. So the distribution of the phosphorus in what appears to be amylose fractions in the above experiments is not due to a partitioning of extraneous phosphate ions. Since the presence of thiocyanate ions prevents retrogradation, this insoluble residue probably comes from organized starchy material. Furthermore, the electrical mobility of the insoluble fraction turns out to be due apparently to the presence of adsorbed thiocyanate ion for when this is removed by long extraction of the sample with alcohol the same amount of suspension containing identically the same amount of phosphorus as in the previous case does not migrate even under a difference of potential of 1200 volts over long periods of time.

II. Instead of disorganization by gelatinization with a swelling agent, samples of potato starch were dry ground in a pebble mill[9] and then dispersed in boiling water. As has been noticed frequently, previously clear dispersions of potato starch rapidly become cloudy and deposit on standing the bulk of their solid material in an insoluble form. This is "retrogradation or reversion"[7] and the material so deposited cannot be redispersed successfully. In this instance, the clear 15% dispersions made in boiling water reverted on standing unless the pre-grinding of the starch had approximated five hundred hours when it was possible to examine the solutions without development of excessive cloudiness even during relatively long periods of time.

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(8) Taylor and Salzmann, THIS JOURNAL, **55**, 264 (1933).

(9) (a) Alsberg and Perry, *J. Biol. Chem,*, **63**, 66 (1925); (b) Taylor and Beckmann, THIS JOURNAL, **51**, 294 (1929).

In the electrophoretic cell[3b] there was no migration of the cloudy material from incipient retrogradation over a period of four months under 1200 volts d. c., the solution being protected from molds by toluene.

Suction filtration of another sample of this 5% dispersion through especially prepared collodion membranes (see Experimental section) on a modified Buchner funnel gave no residue other than a little silica. The clear filtrate was electro-dialyzed[10] against running water for several days, and the solids precipitated from it by the addition of alcohol. Practically the entire amount of starch could be accounted for in this filtrate and substantially all the phosphorus of the original starch was present as combined phosphate in the solid precipitated from the clear filtrate by alcohol. (Original starch P% 0.070, 0.077 and 0.069. Alcohol precipitated amylose from filtrate P% 0.078, 0.076 and 0.071.) These findings are contrary again to those of Samec[4] and others, who find little or no phosphorus in the soluble material from potato starch.

If the dry starch is ground for shorter periods of time, the dispersion in hot water reverts so quickly that large quantities of potentially soluble material become insoluble and the fraction of alleged amylopectin is increased. For example, a 5% paste made from potato starch ground for one hundred twenty hours will in the course of a few days deposit substantially all of its previously soluble material as an insoluble mass. The insoluble material deposited during retrogradation contains the same amount of phosphorus as the original starch from which it came. This may explain Samec's observations that the insoluble material (false amylopectin[11]) contains such a large amount of phosphorus.

Unless the solubilizing effect of grinding is accompanied by no chemical changes, the results are, of course, open to doubt. One criterion is the determination of the alkali labile[8] portion or its complement, the alkali stable portion, of a starch after a given treatment. Such a determination shows that in a raw potato starch of good quality 98% is stable to the effect of hot aqueous alkali, while after one hundred twenty hours grinding 78% is unchanged and after five hundred hours 55% is unchanged. Since some change is indicated, in the samples used here, the disorganization by the pebble mill is not above suspicion.

III. Mechanical disintegration of swollen granules in a water paste in a homogenizer[9b] was not successful in the past in completely disorganizing the starch but it was tried again here in a totally different type of mill[12] but was still not completely successful. While something was accomplished in reducing the paste viscosity,[9b] which is a rough measure of the progress of disorganization, there were still many micromolecular clots

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[10] A cell as used by Taylor and Iddles (see Ref. 3 (b)) may be used when provision is made for passing tap water through the two outside electrode compartments.

[11] Reychler, *Bull. soc. chim. Belg. Grand*, **29**, 309 (1920).

[12] See Experimental section.

present. This paste was nevertheless placed in the electrophoretic cell and the potential applied. The gross insoluble material migrated to the anode leaving a clear supernatant liquid containing only about 20% of the weight of the original starch. Soluble material was precipitated with alcohol and analyzed. It contained 0.003% phosphorus (dry basis) and 93% of starch material was stable to alkali.

After several trials with a more viscous suspending medium, including mineral oil and several concentrations of glycerin, a 5% potato starch paste was made in 85% glycerin by repeatedly passing the cooled mixture through the colloid mill. From the paste the solids were precipitated by the addition of an excess of alcohol, giving a material which will *completely* redisperse but which retrogrades readily. A newly prepared solution will pass in its entirety through a collodion ultrafilter. The filtrate after forced dialysis against water gave a residue on precipitation with alcohol, 93% of which was stable toward alkali[8] and which contained 0.065% phosphorus.

It is evident that the amyloid material in potato starch may be completely dispersed to give clear solutions *without alteration* as measured by the action of aqueous alkali and that, despite its solubility, it contains substantially all the organic phosphate radicals.

IV. Contrasted with the foregoing methods are those in which a starch paste is made by partial gelatinization at 63 and at 85°, and the swollen material frozen, then thawed and any surviving soluble material recovered by filtration. Repeating this old technique as modified by Baldwin,[4b] 17% of the original starch was recovered, containing 0.019% phosphorus. The material, it would seem, represents the small fraction of the total ultimately available soluble material in potato starch, released from a few granules that have disintegrated or partially leached from others; which soluble material fortuitously has not undergone retrogradation. If the residue from the filtration is gently ground with sand, a further amount of material becomes soluble. In the cold, any of this soluble material is to a large extent irreversibly precipitated from water solution, that is, it undergoes retrogradation to a fibrous mass.[13]

Hirst, Plant and Wilkinson[14] also have recently reported that by maintaining potato starch pastes at −10 to −15° overnight, soluble amylose may be readily filtered from the insoluble residue. Quoting in part from this report, "The material which remained after long extraction of the fibrous mass with hot water was considered to be amylopectin," *i. e.*, alpha-amylose. In this investigation, freezing temperatures over a period of several days have been shown to result in more than 95% retrogradation of the soluble amylose derived from the original raw starch.

Incidentally, Hirst finds no chemical differences between the soluble and

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[13] Scharling, *Ann.*, **49**, 315 (1844).

[14] Hirst, Plant and Wilkinson, *J. Chem. Soc.*, 2375 (1932).

the insoluble material from potato starch with regard to yield of methylated glucoses and therefore glucose anhydride chain length.[14,15]

Thus far, all evidence points to the presence of a soluble phase, only, in potato starch providing care is exercised to avoid such complicating factors as retrogradation or incomplete disorganization of the granule. It is also obvious that the amount of phosphorus combined with the carbohydrate varies over wide limits in various fractions, no matter how obtained, and irrespective of solubility, a finding in accord with the observations of Karrer.[5]

To explain these observations, there is the hypothesis that chains of glucose anhydrides make up the amylose unit and that aggregations of these make the micelli which are in turn arranged in microscopic geometrical packages called granules. It is possible to use the postulates of Meyer and Mark[16] concerning the presence of more or less completely esterified tribasic phosphoric acid, either with hydroxyl groups on one chain or bridging between two chains of glucose anhydrides. These chains may vary over a wide range with regard to the amount of phosphate held to them, giving rise to a kind of heterogeneity even though all these fractions unlike those from the cereal starches are soluble.

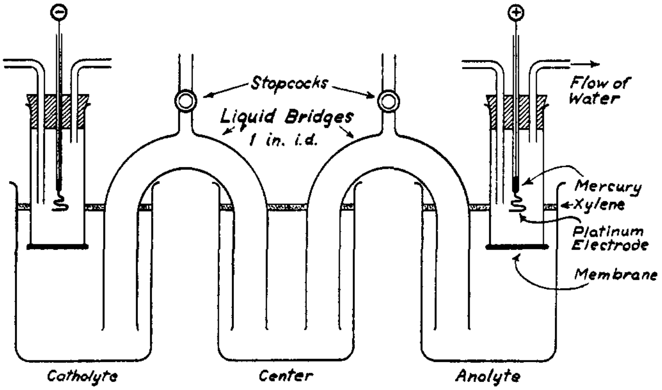

Fig. 1.

To test this experimentally on the supposition that the electrical mobility of certain of the soluble fractions might be different from others due to variation in the ratio of phosphorus to carbohydrate and possibly to the degree of esterification and therefore admit of a qualitative separation, a technique similar to that used for the determination of transport number was employed (see Fig. 1). The results are given in Table I.

Run (1) 4% clear hot-water dispersion of 500-hr. dry ground starch. Time of treatment in cell, five days.

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(15) Haworth and Percival, *J. Chem. Soc.*, 2278–2279 (1932).

(16) Meyer and Mark, "Der Aufbau der hochpolymeren organischen Naturstoffe," Leipzig, 1930.

TABLE I

Results in runs of same number are to be compared

Electrode compartment	Run[a]	Dry wt., %	Phosphorus, %		Alkali labile mg.[8] iodine per 100 mg. sample	
	1	3.8	0.041	0.052	23.8	..
Anode	2	4.0	.081	..	23.6	..
	3	1.5	.079	...	4.4	..
	1	4.0	.043	...	28.0	..
Center	2	4.0	.047	0.058	26.7	..
	3	1.4	.075	...	5.0	..
	1	3.9	.017	...	27.9	27.7
Cathode	2	4.1	.024	0.029	27.4	27.3
	3	0.3	.012	...	5.3	..

[a] It is interesting to note that Fernbach [*Compt. rend.*, **138,** 428 (1904)] observed that the smaller granules of potato starch contained more phosphorus than the larger ones.

Run (2) 4% clear hot-water dispersion of 500-hr. dry ground starch. Time of treatment in cell, twelve days.

Run (3) test run on 1% simple hot water gelatinized cloudy suspension of whole starch. Time of treatment, ten days.

Original starch P% 0.074.

Dry weight was estimated semi-quantitatively by the addition of a large excess of alcohol, followed by filtration, drying and weighing.

Alkali lability was here determined iodimetrically since the alcohol precipitation method is not sufficiently precise to disclose minor deviations. Minimum excesses of iodine and of alkali, as predetermined in each case by rough titration, were employed to avoid over-oxidation.

In each instance there has occurred a migration of the high phosphorus-bearing amylose toward the anode, apparently with a compensating counter-flow of material low in phosphorus It will be noticed that there is a gradation in alkali lability of the various fractions, certainly too uniform to be fortuitous. Since the production of free aldehydic groups is the probable cause of alkali lability, and since the latter reaches a maximum in those fractions where the phosphorus content is lowest, it might be presumed that a portion at least of the phosphate is glycosidically linked in potato amylose. Heterogeneity as regards phosphorus distribution may be tentatively correlated with the length of carbohydrate chains each bearing a glycosidically-linked phosphate. The changes which occur in this series of cells can hardly be attributed to hydrolysis of the amylose-phosphoric acid by hydrogen or hydroxyl ions generated about the electrodes, since the *P*H as determined colorimetrically was substantially the same in the compartment where the combined phosphorus is high as where it is low (*i. e.*, approximately 5.3). Any free phosphate ions are known by test to pass through the membranes and be removed by the water passing through the electrode compartments.

Experimental

The potato starch was a good grade imported sample.

In the experiments described under I, the starch was treated with hot alcoholic hydrogen chloride solution, 0.06 g. per cc., with stirring for ten to twelve minutes and subsequently gelatinized at 65° with the requisite amount of ammonium thiocyanate[3b] and precipitated with alcohol. A 3% paste of this material was made in warm water with stirring and placed in the electrophoretic cell.

In experiments described under II, the membranes were graded commercial variety marketed by Pfaltz and Bauer, New York City. To avoid clogging the more permeable (0.5 to 3.0 μ) membrane was placed on top, separated from the under and less permeable membrane (10 to 50 millimu) by a disk of filter paper. The starch solutions in the special Buchner (Pfaltz and Bauer) funnel above the filtering medium was kept well agitated by a motor-driven stirrer while suction was applied to the filter flask below.

For the mechanical disintegration described under III, a "colloid mill" of the type having disk rotors revolving with little clearance at high speed in opposite directions and surrounded by a water-cooled jacket was used. The machine was made available through the courtesy of Mr. M. P. Hofmann of the U. S. Colloid Mill Corporation of Long Island City, N. Y.

The technique described under IV was exactly that given by Baldwin, whose results were corroborated.

The phosphorus was determined after wet digestion in a Kjeldahl flask according to Neuman[17] in nitric acid to which was added subsequently sulfuric acid and ammonium nitrate. Digestion was carried to the cessation of evolution of brown oxides of nitrogen and the appearance of clear yellow liquid. Fresh additions of acid were often necessary during the digestion.

The digested liquid was made up then to 100 cc. with water, neutralized to methyl red with ammonia, made slightly acid with nitric acid, warmed to 70°, 30 cc. of ammonium molybdate reagent[18] added and the mixtures allowed to stand overnight. The phosphomolybdate precipitate was filtered through a Jena fritted glass funnel and washed free of acid with 1% potassium nitrate solution. The phosphorus was determined alkalimetrically or oxidimetrically. The samples taken usually represented between 2 and 4 mg. of phosphorus. On test runs samples of primary potassium phosphate were analyzed to check the method. The results are given below.

Sample contained 3.02 mg. phosphorus: found 3.24, 3.15 alkalimetrically; and 3.20, 3.14 oxidimetrically.

The alkali labile determinations were made by the method of Taylor and Salzmann.[8]

When the amount of residue unattacked by alkali was desired, it was precipitated by acetone, dried and weighed. For more critical work the amount destroyed was estimated iodimetrically and the results expressed in mg. of iodine consumed per 100 mg. of sample.

Conclusions

1. The absence of any insoluble material in potato starch in the sense in which it is present in cereal starches, is established by several different procedures.

2. Organic phosphorus is shown to be randomly distributed in potato starch, and this circumstance must arise from a diversity of relatively simple and very similar amylose-phosphates.

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(17) Plimmer and Bayliss, *J. Physiol.*, **33**, 439 (1905).

(18) Fales, "Inorganic Quantitative Analysis," The Century Co., New York, 1925, p. 216.

3. Insoluble material is attributable, principally, either to incomplete disorganization of the associated material in the granule or to retrogradation. Both conspire to give the erroneous impression that potato starch contains a non-dispersible fraction comparable to the alpha-amyloses of the cereal starches.

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Alpha Substituted Ethyl *n*-Butyl Ethers[1]

BY HENRY R. HENZE AND JOHN T. MURCHISON

In this Laboratory the alpha chloroalkyl ethers have been studied for the past several years with two major purposes in mind: (1) to extend the series[2] through the synthesis of additional members, as far as seemed practically desirable, securing rather complete physical data relative to the properties of all new compounds; and (2) to investigate the utilization of these alkoxy derivatives in the preparation of new examples of compounds of bifunctional type. In this investigation the preparation, from alpha chloroethyl *n*-butyl ether[2] or alpha cyanoethyl *n*-butyl ether, of representative examples of the more important alkoxy types was effected. The use of the chloro ether in further syntheses was limited because of its rapid hydrolysis and extreme tendency toward complete decomposition upon brief contact with mildly alkaline reagents. However, the cyano derivative is a stable substance which served well as an intermediate for the preparation of other and more important compounds. From the data of the vapor pressures of these new compounds the latent heats of vaporization were calculated. Thermodynamical data have not previously been reported in the literature for compounds of the types of alkoxy ketones, alkoxy esters, etc.

Experimental

Di-*n*-butyl Acetal.—The method of Descudé[3] was adopted, with slight modification, for the synthesis of di-*n*-butyl acetal. Forty-seven grams of alpha chloroethyl *n*-butyl ether was added slowly with shaking and cooling to a solution of twelve grams of sodium in 200 cc. of *n*-butyl alcohol. The mixture was heated on the water-bath at 100° for about fifteen minutes, filtered, washed with distilled water, dried over anhydrous sodium sulfate, and fractionated; b. p. 51° (12 mm.) (corr.); d_4^{20} 0.8296; n_D^{20} 1.4079; M_R calcd., 51.36; M_R found, 51.78.

Anal. Calcd. for $C_{10}H_{22}O_2$: C, 68.89; H, 12.73. Found: C, 69.35; H, 12.71.

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[1] An abstract of the dissertation submitted by John T. Murchison to the Faculty of the University of Texas, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, June, 1933.

[2] Henze and Murchison, THIS JOURNAL, **53**, 4077 (1931).

[3] Descudé, *Bull. soc. chim.*, [3] **27**, 1216 (1902).