[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, COLLEGE OF MEDICINE, UNIVERSITY OF ILLINOIS.]

A METHOD FOR THE PREPARATION OF SOLUBLE STARCH.

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Introduction.

The methods for the preparation of soluble starch may be grouped under three general heads: First, by enzyme action; second, by acid hydrolysis; third, by action of superheated steam. By enzyme action, boiled starch is readily transformed, yielding definite end products; but this method is least suited to the preparation of intermediate hydrolytic products of starch on account of the rapidity of the enzyme action. Various mineral and organic acids with a wide range of percentage dissociation have been used in acid hydrolysis. Both raw starch and starch paste have been subjected to acid hydrolysis. In some methods, a preliminary treatment is applied for the removal of the cellulose from the starch granule and a subsequent treatment is employed for the hydrolysis of the exposed starch.

The Lintner method[1] has been regarded as the standard for the preparation of soluble starch. According to this method raw starch is treated with 7.5% hydrochloric acid (sp. gr. 1.037) at room temperature. The mixture is stirred several times each day, and at the end of 7 days the acid is decanted and the starch washed with large volumes of distilled water until practically free from the acid. The product is then dried and sieved. The soluble starch thus obtained is used in measuring the diastatic activity of malt.

With concentrations of less than 2% in water at 100°, this preparation gives a clear, limpid solution which filters readily. Amylodextrin, erythrodextrin and copper-reducing substances are readily identified in such a solution. From this it is apparent that the hydrolysis has, in part, gone beyond the soluble starch stage.

Experimental—Part I.

These experiments were begun with the object of preparing a soluble starch containing a minimum of allied carbohydrates. Raw potato starch prepared by methods described elsewhere[2] was used exclusively. Hydrolysis with aqueous solution of hydrochloric acid under varying conditions of concentration, temperature and period of exposure, was tried throughout a large number of experiments, the results of which indicated that when the amounts of the carbohydrates lower than soluble starch were reduced to a minimum, a considerable portion of the starch was left unchanged. When conditions were so regulated as to effect a more

[1] Lintner, *J. prakt. Chem.*, 34, 378 (1886).

[2] J. C. Small, THIS JOURNAL, 41, 107 (1919).

nearly complete transformation of the starch, the lower carbohydrates again came into evidence. Much of this work was carried out at somewhat elevated temperatures (35 to 65°) and with acid concentrations markedly reduced as compared with the Lintner method (0.2 to 1.6%).

Since these elevated temperatures markedly favor the solution of soluble starch in the medium employed for hydrolysis, relatively large amounts must have gone into solution as soluble starch was formed, especially in the experiments at the higher temperatures. Its solution favors further hydrolysis. Consequently, the hydrolysis of starch in aqueous suspension at elevated temperatures was abandoned and the attempt was made to carry out acid hydrolysis in a medium which would not dissolve the soluble starch at elevated temperatures. Redistilled 95% alcohol was chosen and conc. hydrochloric acid (sp. gr. 1.19) added in varied but small amounts. In the early experiments samples were prepared in bulk. At the end of the period of exposure, the acid alcohol was quickly filtered off and the starch washed with large volumes of distilled water. This procedure was found to entail a slight loss of soluble starch in the wash water. The technique was thereupon modified by the immediate neutralization of the acid with sodium bicarbonate, at the end of the interval of exposure. The neutral alcohol was then filtered off and the starch washed with several additions of fresh alcohol. Fifteen minutes was the longest interval of exposure employed. Table I contains analyses of samples prepared by the former method. The analyses of Table II are those of samples prepared by the latter method.

TABLE I.

Sample No.	Alcohol. Cc.	Acid HCl. Cc.	Starch. G.	Time. Min.	Total starch as dextrose.	Sol. starch as dextrose.	% sol. starch.	Qualitative tests. Unchanged starch.	Erythrodextrin.	Benedict-Fehling reduction.
I-*a*	200	2.0	30	6	2.795	2.436	87.15	+	o	o
I-*b*	200	2.0	30	6	2.779	2.415	86.90	+	o	o
II-*a*	200	2.0	30	10	2.779	2.600	93.56	o	tr.	tr.
II-*b*	200	2.0	30	10	2.795	2.621	93.77	o	tr.	tr.
III-*a*	100	0.75	20	10	2.822	2.837	100.88	o	o	o
III-*b*	100	0.75	20	10	2.841	2.844	100.1	o	o	o
IV-*a*	100	0.5	30	10	2.795	2.569	91.91	+	o	o
IV-*b*	100	0.5	30	10	2.795	2.569	91.91	+	o	o
V-*a*	100	0.5	20	15	2.811	2.744	97.62	?	o	o
V-*b*	100	0.5	20	15	2.821	2.744	97.23	?	o	o
VI-*a*	Lintner's Sol. Starch				2.743	2.620	95.51	o	+	+
VI-*b*	Lintner's Sol. Starch				2.733	2.610	95.49	o	+	+

Detailed Technique of Preparation of Samples in Table I.—The starch was weighed out approximately, transferred to Erlenmeyer flasks and the acid alcohol added for its suspension. The flask was fitted with a reflux condenser, placed in a boiling water bath, and vigorously shaken from

time to time to keep the starch from settling out. At the end of the interval of exposure, the flask was removed and the alcohol filtered off as quickly as possible. The soluble starch was washed with distilled water to free it from the acid. The products thus obtained were dried at room temperature, sieved and preserved as stock samples.

TABLE II.

Sample No.	Alcohol. Cc.	Acid HCl, Cc.	Starch. G.	Time. Min.	Total starch as dextrose.	Sol. starch as dextrose.	% sol. starch.	Qualitative tests. Unchanged starch.	Erythrodextrin.	Benedict-Fehling reduction.
I-*a*	100	0.75	50	10	2.658	1.781	67.0	+	o	o
I-*b*	100	0.75	50	10	2.658	1.781	67.0	+	o	o
II-*a*	100	0.75	50	15	2.672	2.243	83.94	+	o	o
II-*b*	100	0.75	50	15	2.647	2.243	84.73	+	o	o
III-*a*	50	0.5	25	10	2.631	2.569	97.30	⊕	o	o
III-*b*	50	0.5	25	10	2.658	2.569	96.65	⊕	o	o
IV-*a*	50	0.5	25	15	2.631	2.569	97.30	o	⊕	o
IV-*b*	50	0.5	25	15	2.647	2.569	97.05	o	⊕	o
V-*a*	100	1.25	50	10	2.631	1.895	72.02	+	o	o
V-*b*	100	1.25	50	10	2.631	1.912	72.67	+	o	o
VI-*a*	100	1.25	50	15	2.619	2.518	96.14	o	+	⊕
VI-*b*	100	1.25	50	15	2.619	2.535	96.79	o	+	⊕

Detailed Technique of Preparation of Samples in Table II.—The above technique was followed until the end of the period of hydrolysis when, on removal of the flasks, the acid alcohol was immediately neutralized with a normal solution of sodium hydrogen carbonate. The amount required in each case was previously determined by titration, using methyl orange as an indicator. This amount could be added in mass, thus abruptly terminating the hydrolysis interval. As the effervescence of carbon dioxide subsided, the starch rapidly settled out. The bulk of the alcohol was decanted through a filter and several additions of fresh alcohol were used to wash and transfer the starch to the same filter. The samples were dried, sieved and preserved.

Analysis of Samples.

Duplicate 3-g. portions were weighed out, transferred to 350 cc. Erlenmeyer flasks and suspended in about 200 cc. of water. Twenty cc. of hydrochloric acid (sp. gr. 1.125) was added and the flasks, fitted with reflux condensers, were placed in a boiling water bath. At the end of 4.5 hours they were removed, allowed to cool and the volume was made up to 250 cc. The solutions, clarified by filtration, were read on the Fric polariscope using the Ventzke scale. This procedure gave the "total starch as dextrose" values indicated in Tables I and II.

The soluble starch content of similar duplicate 3-g. portions was determined according to the method outlined in a previous paper.[1] The

[1] J. C. Small, THIS JOURNAL, 41, 107 (1918).

readings of the soluble starch fraction after hydrolysis, as above, were taken in the same manner and furnished the "soluble starch as dextrose" values given in Tables I and II.

The results of qualitative tests made on the soluble starch preparations are also tabulated, since they furnish data tending to show whether the percentages of starch unaccounted for in the soluble starch fraction was lost as unchanged starch or as dextrins and lower reducing carbohydrates.

Technique of Qualitative Tests.—Unchanged starch in appreciable amounts is readily recognized by the opacity and viscosity of the solution containing it. For the detection of smaller amounts a few drops of a 4% iodine in potassium iodide solution were added. Where unchanged starch is present, a definite flocculent precipitate of starch-iodine-blue occurs.

Erythrodextrin may be readily recognized by its iodine color test in solutions of the hydrolytic products of starch after the removal of unchanged starch, soluble starch and amylodextrin. This is readily accomplished by the use of a reagent containing 2 g. of iodine and 6 g. of potassium iodide in 1 liter of saturated ammonium sulfate solution. The addition of an equal volume of this reagent to an approximate one % solution of a soluble starch sample, immediately precipitates everything above the erythrodextrin. A clear, red-brown filtrate results. To the filtrate in the test-tube is added, drop by drop, 0.05 *N* sodium thiosulfate solution until the color is just discharged. To this liquid is added a measured amount of an iodine solution sufficient to supply an excess of iodine. An equal volume of water, to which has been added the same measured volume of iodine solution, serves as a control. The dextrin-red stands out on comparison. Very small amounts of erythrodextrin can be recognized in this manner. The reduction tests were made in the usual manner. The boiling over a direct flame was continued for one minute.

Discussion.

In studying these two tables it must be remembered that the percentages of soluble starch recorded represent the soluble starch in a given sample of converted starch and not the amount obtained from a given sample of raw starch. All the samples in Table I (except No. 6, which is introduced for comparison) were prepared at one time and dried under the same conditions. The same is true of those in Table II, which were prepared later. The total starch, while relatively constant in each series, is uniformly lower for the sample in Table II. This is most probably due to less complete desiccation of the latter samples.

Two conditions serve to account for a fall below the maximum percentage of soluble starch in any given instance. They are, first, incomplete conversion of the starch to soluble starch; and second, hydrolysis

of some of the soluble starch. The qualitative tests furnish a guide in recognizing which of these factors is accountable for the diminished yield of soluble starch in any particular instance.

The results given in these two tables represent, more or less, preliminary experiments in the acid alcohol hydrolysis of starch and consequently the amounts of acid, alcohol and starch are not always well chosen. It is difficult to interpret results when all three factors have been varied in the same groups of experiments.

It would appear, however, that for a 20% suspension of starch in alcohol, 0.75 cc. of conc. hydrochloric acid (sp. gr. 1.19) per 100 cc. of alcohol effected complete conversion of the starch in 10 minutes and yielded a product in which no dextrins or reducing carbohydrates could be identified.

It appears further that the heavier suspensions of starch (50%) were not wholly converted in the same interval even when the concentration of the acid was increased to 1.25 cc. per 100 cc. of alcohol. A larger percentage of soluble starch was obtained when the interval of exposure was lengthened in all cases except one, that of Sample I (*a* and *b*) and II (*a* and *b*) of Table II. From a study of the qualitative tests, it would seem that in this instance the loss from incomplete conversion of the starch in Sample I was offset by a loss from hydrolysis of the soluble starch in the case of Sample II.

The only conclusions warranted from the results in Tables I and II are that time of exposure, concentration of acid and density of the starch suspension are three factors influencing the conversion of starch to soluble starch in this process. Closely analogous to the density of the suspension of starch, but differing in some respects, is another factor, that of the total amount of the starch treated. Large amounts of starch are less easily and less uniformly converted than small amounts. This is probably due to the fact that the larger amounts tend to settle out and to form a thick bottom layer, all depths of which are not evenly exposed to the hydrolysis menstruum. Constant agitation tends to obviate this difficulty.

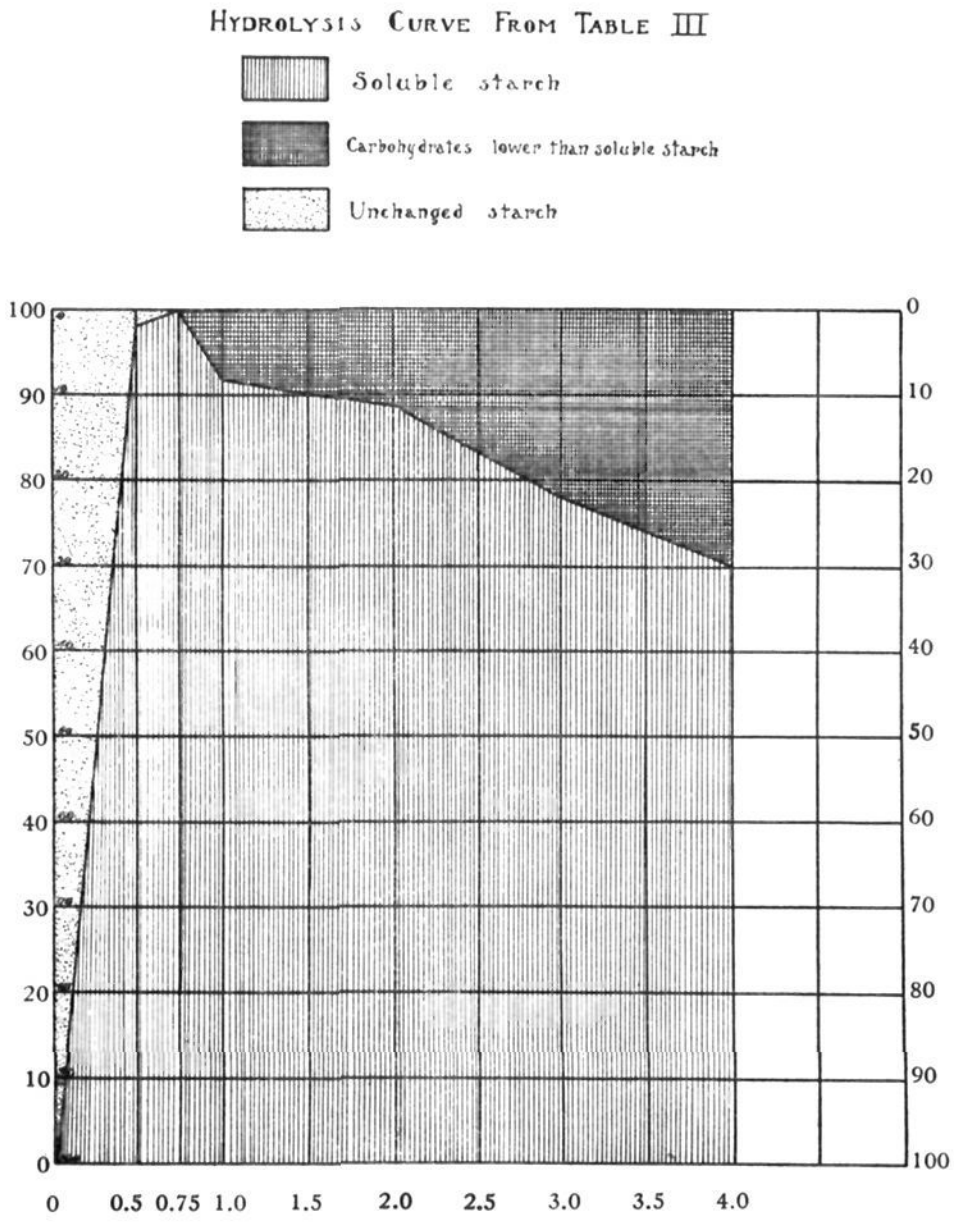
Part II.

The effects of varying one of these factors, namely, the acid concentration, with the others constant, were studied in more detail by beginning with an accurately weighed sample of raw starch and carrying it quantitatively through both the conversion and the analytical procedures.

Details of Method.

Duplicate 3-g. samples of a uniform laboratory preparation of potato starch were weighed out and transferred to 350 cc. Erlenmeyer flasks. These samples were suspended in 100 cc. of redistilled 95% alcohol and the desired amount of hydrochloric acid added. The flasks were then

fitted with reflux condensers and placed in a boiling water bath for 10 minutes. At the end of this interval they were removed and the acid exactly neutralized with a normal solution of sodium hydrogen carbonate, as previously described. The neutral alcohol was decanted through a filter in such a manner as to retain most of the starch within the flask. After draining both flask and filter of the alcohol, the starch caught by the filter and that adhering to the sides and upper part of the flask was washed down into the flask with distilled water. The small amount of alcohol retained in this process did not interfere with the solution of the starch when the volume of water used for suspension and solution was brought up to about 200 cc. and the temperature raised to the boiling point. Care was taken to keep the starch in suspension while raising the temperature of the water to effect solution, so as to avoid any accumulation of starch sediment which tends to adhere to the bottom of the flask



as an opaque gelatinous mass and in this form is difficultly soluble. The solutions of the samples were carried through the various steps of the procedure outlined in a previous paper[1] for separating and determining the soluble starch fractions. Final readings were made with the polariscope as previously outlined.

The total starch in duplicate 3-g. samples of the stock sample of the raw starch was determined by the method previously given in this paper. The results of this study are tabulated in Table III.

TABLE III.

Sample. No.	Alcohol. Cc.	Acid HCl. Cc.	Starch. G.	Time. Min.	Total starch as dextrose.	Sol. starch as dextrose.	% sol. starch.	Qualitative tests. Unchanged starch.	Erythrodextrin	Benedict-Fehling reduction.
I-*a*	100	4.0	3	10	2.713	1.901	70.07	0	+	+
I-*b*	100	4.0	3	10	2.713	1.926	70.99	0	+	+
I-*c*	100	4.0	3	10	2.713	1.901	70.07	0	+	+
II-*a*	100	3.0	3	10	2.713	2.107	77.66	0	+	+
II-*b*	100	3.0	3	10	2.713	2.107	77.66	0	+	+
III-*a*	100	2.0	3	10	2.713	2.415	89.02	0	+	+
III-*b*	100	2.0	3	10	2.713	2.415	89.02	0	+	+
III-*c*	100	2.0	3	10	2.713	2.385	87.91	0	+	+
IV-*a*	100	1.0	3	10	2.713	...	...	0	+	+
IV-*b*	100	1.0	3	10	2.713	2.518	92.81	0	+	+
V-*a*	100	0.75	3	10	2.713	2.723	100.36	0	0	0
V-*b*	100	0.75	3	10	2.713	2.703	99.63	0	0	0
VI-*a*	100	0.5	3	10	2.713	2.672	98.48	?	0	0
VI-*b*	100	0.5	3	10	2.713	2.672	98.48	?	0	0

Discussion of Results.

These results confirm those of Table I in showing that the greatest yield of soluble starch is obtained when 0.75 volume % of strong hydrochloric acid in 95% alcohol is used and the hydrolysis continued at the boiling temperature for 10 minutes.

The results show that the amount of hydrolysis bears a direct ratio to the concentration of the hydrogen ion, confirming the results of Duryea,[2] Noyes and Crawford,[3] de Coninck and Raynaud.[4] The indication that, under the proper conditions, starch seems to be wholly converted to soluble starch before further hydrolysis occurs, is significant. This is out of keeping with the theory that maltose is split from the starch molecule in this change. It supports the idea that soluble starch is a hydrated starch. The soluble starch stage seems to present a momentary barrier

[1] J. C. Small, THIS JOURNAL, 41, 107 (1919).

[2] C. B. Duryea, *J. Soc. Chem. Ind.*, 30, 789 (1911).

[3] Noyes, Crawford, *et al.*, THIS JOURNAL, 26, 266 (1904).

[4] de Coninck and Raynaud, *Bull. acad. roy. méd. belg.*, 1911, p. 213; *Bull. soc. chim.*, 9, 586 (1911).

to further hydrolysis. From soluble starch downward, the hydrolysis again appears to bear a direct ratio to the acid concentration.

It would be interesting to determine the amylodextrin fraction of the over-hydrolyzed samples. With 100% conversion of starch to soluble starch, it would also be highly profitable from a theoretical standpoint to study the hydrolysis of this product in concentrations of acid below 0.75 per 100% alcohol, and to ascertain whether the soluble starch can be quantitatively converted to amylodextrin.

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THE INFLUENCE OF CATALYSTS ON THE CHLORINATION OF HYDROCARBONS.

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The object of this paper is to show the contrasting action of light and of catalysts on the chlorination of hydrocarbons.

It is well known that chlorine acts progressively on aliphatic hydrocarbons in the presence of light, giving products of the nature of CH_3Cl, CH_2Cl_2, $CHCl_3$ and CCl_4 as in the chlorination of methane. On the aromatic hydrocarbons, on the other hand, it acts, under the same conditions, additively in the ring or substitutively in the side chain. Substitution in the ring requires the use of strong halogen carriers.

It is also known that halogen carriers can be employed in the chlorination of aliphatic hydrocarbons, giving the same ultimate products as in the chlorination under the influence of light. Halogen carriers seem to have a selective action in the substitution of aromatic hydrocarbons in preference to aliphatic. Thus in the chlorination of toluene in the presence of catalysts at a low temperature substitution is restricted to the ring without in the least affecting the side chain. At a higher temperature, however, substitution takes place in the side chain in preference to the ring. It is likely that even at a low temperature, when the substitution has gone as far as pentachlorotoluene, the side chain will then be attacked.

Regnault[1] demonstrated that chlorine acts progressively on methane in the presence of light, forming CH_3Cl, CH_2Cl_2, $CHCl_3$ and CCl_4, all of which can be isolated.

Bedford[2] claims to have obtained $CHCl_3$, CH_2Cl_2, CH_3Cl and CCl_4, either singly or collectively, according to the control of conditions. He made methane and chlorine to react in the presence of ice and actinic light. Masland and Sparre[3] claim to obtain mono-, di-, tri- or tetra-

[1] *Ann.*, **33**, 332 (1840).

[2] U. S. P. 1,245,553, Nov. 6, 1917.

[3] U. S. P. 1,148,259, July 27, 1915.