was 0.89, and after 82 hours, 0.96. Chlorination was stopped at this point.

The unchanged ether was distilled off under reduced pressure and the remaining material fractionated. There was obtained 375 g. of dichloro-ether, boiling from 66 to 69° at 45 mm., representing a yield of 24.2% of the theoretical amount. The material in the distilling flask remained colorless till near the end, when it became black and there was left a small residue of 65 g.

It had previously been determined that chlorination should be stopped at about the point where the specific gravity was 0.96. In one trial during which it was carried on to the specific gravity 1.11 it was impossible to separate a pure fraction of dichloro-ether from the product.

INDIANAPOLIS, INDIANA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, NO. 322.]

ACTION OF ENZYMES UPON STARCHES OF DIFFERENT ORIGIN.

BY H. C. SHERMAN, FLORENCE WALKER AND MARY L. CALDWELL.

Received May 5, 1919.

O'Sullivan,[1] working with malt extract and precipitated malt diastase, reported that potato starch was less readily digested to maltose than were the starches of the cereal grains when all were gelatinized by heating in water at 97° and tested under like conditions.

Stone,[2] also working with malt extract, judging the action by the disappearance of the blue coloration with iodine, found potato starch more digestible than wheat starch, and maize starch less so than either. With saliva, Stone found potato starch more digestible than the cereal starches, and among the latter maize and rice starches appeared more digestible than that of wheat. Stone also reported potato starch more readily digestible than cereal starches by pancreatin and by taka-diastase.

Ford[3] found the purified starches of rice, barley, maize, wheat, potato and arrow root when similarly prepared to be equally digested by malt extracts and attributed the differences found by other observers to the probable impurities in their starches.

In view of the conflicting results thus shown by the earlier work and the fact that previous observers have in general worked with but few forms of amylase and these usually with no attempt at isolation, a more comprehensive study which should include observations upon purified enzyme preparations, as well as secretions and extracts, seemed desirable.

[1] O'Sullivan, *J. Chem. Soc.*, **85**, 616 (1904).

[2] Stone, U. S. Dept. Agr., Office of Expt. Sta., *Bull.* 34.

[3] Ford, *J. Soc. Chem. Ind.*, **23**, 414 (1904).

The present investigation includes experiments upon the hydrolysis of wheat, maize, rice and potato starches by means of saliva, pancreatin, purified pancreatic amylase, malt extract, purified malt amylase, taka-diastase, and purified amylase of *Aspergillus oryzae*.

Earlier investigations in this laboratory have shown that, at least with some amylases, the results of comparisons based upon disappearance of material giving blue coloration with iodine are apt to be misleading.[1] On the other hand, the gravimetric method which we have commonly employed for the determination of the amount of reducing sugar formed from "soluble" starch was not applicable because of the difficulty in filtering the starch dispersions here studied. We therefore adopted the method of allowing the enzyme to act upon a 1% dispersion of boiled starch for 30 minutes at 40°, employing in each series of measurements such an amount of the enzyme solution as would result in the formation of about $^1/_3$ to $^1/_2$ of the theoretically possible amount of maltose, and finally determining the reducing sugar formed by titration against Fehling's solution according to the well known volumetric method.

The general plan of our work has been to obtain or purify each starch by several methods and to compare the digestibility or rate of transformation into maltose of different starches when prepared by the same method and purified in the same manner and under the same conditions.

Starches.—The starches used in these experiments were prepared in the laboratory from wheat flour, corn meal, white rice, and mature potatoes. Each of the 4 starches was prepared in 4 different ways: (1) washing with water only, (2) with very dilute alkali, (3) with ether after washing with water, (4) with ether after washing with alkali.

In the preparation of starch from wheat flour or corn meal, the material was mixed with distilled water to a stiff dough and kneaded in a cheesecloth bag under distilled water. The starch suspension was then filtered through 4 thicknesses of cheesecloth to remove particles of cellulose or flocks of insoluble protein, then the starch allowed to settle and washed 5 times by decantation with distilled water. A portion of each of these starches was then stirred thoroughly with cold 0.3% solution of sodium hydroxide and allowed to stand overnight at ice box temperature after which the alkaline liquid was decanted and the starch washed 4 times by decantation with ordinary distilled water and twice with the triply distilled water especially prepared for our enzyme work. In the case of wheat starch a second portion was washed with a more dilute alkali, 0.15% instead of 0.3% sodium hydroxide solution; and a duplicate portion of the corn starch received two treatments with 0.3% sodium hydroxide solution, the subsequent washings with water being as stated above in all cases.

[1] Sherman and Schlesinger, THIS JOURNAL, 35, 1784 (1913).

Rice starch was obtained by mixing finely ground, white rice with water and filtering through cheesecloth, then washing with water, 0.3% sodium hydroxide solution, water and triply distilled water as just described for the wheat and corn starches.

Potato starch was prepared from pared and grated potatoes by filtering and washing as in the preparation of the cereal starches.

After final washing, the starches were filtered on Büchner funnels by suction, dried first at room temperature and then in dry air at 30 to 40°. Portions of the water-washed and alkali-washed starches were then washed with ether in Soxhlet extractors for 16 hours, then repeatedly with triply-distilled water and filtered and dried as just described.

The actual starch content of each preparation was found by hydrolyzing with hydrochloric acid and determining the amount of glucose formed. The amount of 0.01 *N* acid or alkali required to render each starch preparation exactly neutral to rosolic acid was also determined. For each enzyme experiment so much of any given starch preparation was weighed out as would contain just sufficient actual starch to make the required amount of 1% substrate, and the small amount of 0.01 *N* acid or alkali required for strict neutrality to rosolic acid was added before bringing the starch dispersion to final volume or before mixing it with enzyme solution.

Enzyme Preparations.—The amylase preparations and other enzyme-containing materials used and the amount of each employed per 100 cc. of 1% starch dispersion were as follows: Pancreatic amylase preparation No. 64, 0.1 mg.; pancreatin No. 8, 2.5 mg.; fresh saliva, 0.18 to 0.5 cc.; malt extract, 0.15 cc.; malt amylase preparation No. 155, 0.25 mg.; amylase preparation No. 22 from *Aspergillus oryzae*, 0.4 mg.; taka-diastase, 9.0 mg. Each enzyme was used in the presence of such amounts of chloride and phosphate as were believed to afford the most favorable conditions for its action.[1] Special precautions regarding preparation and use of triply distilled water, selection and care of glassware, avoidance of unnecessary exposure to light, etc., were observed as explained in previous papers from this laboratory dealing with other phases of our study of the amylases.

Method.—Amounts of the starch preparations sufficient in each case to furnish one gram of actual anhydrous starch were weighed out, mixed with a little cold water and gelatinized by pouring into boiling water and boiling for 3 minutes; then transferred to 100 cc. cylinders, neutralized, the necessary "activating" salts added and the mixtures brought to volume, thoroughly mixed by stirring and placed in a 40° thermostat water-

[1] The influence of hydrogen ion concentration and of chlorides and phosphates has been discussed in several previous papers from this laboratory, THIS JOURNAL, 1910 to 1919.

bath. While the starch pastes thus prepared were reaching the temperature of the water bath, flasks containing uniform amounts of the enzyme were prepared. The substrate was then poured upon the enzyme, mixed thoroughly, and maintained for 30 minutes at 40°, both time and temperature being very strictly controlled. Finally the action of the enzyme was stopped by pouring into boiling water and quickly boiling the entire mixture, after which the reducing sugar which had been formed was determined volumetrically as explained above.

Results.—The results of typical experiments comparing different starches, similarly purified, with each other (or in some cases differently purified portions of the same starches) are given in the tables which follow. On account of the nature of the enzyme solutions employed and the character of the manipulations involved in these experiments, only those data belonging to the same set of experiments—in general only the data in the same column of the same table—are directly comparable with each other in a quantitative sense.

TABLE I.

Action of Saliva, Pancreatin, and Purified Pancreatic Amylase upon Water-washed, and upon Purified, Starches.

Description of starch.	% of starch hydrolyzed in 30 min. by		
	Saliva.	Pancreatin.	Pancreatic amylase. Prep. No. 64.
Water-washed:			
Wheat starch	56.8	32.2	42.8
Maize starch	50.6	25.1	31.6
Rice starch	61.5	36.1	45.8
Potato starch	67.7	39.7	47.2
Alkali-washed:			
Wheat starch:			
(0.3% NaOH)	65.5	38.2	46.0
(0.15% NaOH)	..	38.4	..
Maize starch:			
(0.3% NaOH once)	66.3	38.3	46.7
(0.3% NaOH twice)	..	37.9	..
Rice starch	65.7	38.3	46.4
Potato starch	67.0	39.4	34.0

The figures in any one column of Table I show that purification by washing with dil. alkali has a pronounced effect upon the comparison of the different starches with reference to their digestive hydrolysis. Potato starch is practically as pure and as accessible to saliva or pancreatin when merely washed with water as after purification by washing with dil. alkali, while water-washed maize "starch" contains impurities which markedly retard its digestion. To a less extent the same is true of wheat, while rice starch is almost as readily digested in the water-washed as in the alkali-washed condition. It is evident that the increased digestibility of the wheat and maize starches when purified by washing with dil. alkali is due

to the removal of other material such as fatty or waxy matter, and not to predigestion of the starch itself by the alkali, since wheat starches treated with 0.15% and with 0.3% sodium hydroxide solutions are equally digestible and maize starch treated twice with the dil. alkali was not more digestible than that treated once. The low result obtained in the action of purified pancreatic amylase upon purified potato starch suggests the removal during purification of something essential to the optimum activation of the enzyme. This will be discussed in a later paper.

TABLE II.

Action of Pancreatin and Purified Pancreatic Amylase upon Alkali-washed and upon Ether-washed Starches.

Description of starch.	% of starch hydrolyzed in 30 min. by Pancreatin.	Pancreatic amylase. Prep. No. 64
Alkali-washed:		
Wheat starch	41.8	46.0
Maize starch	42.5	46.7
Rice starch	42.5	46.4
Potato starch	43.0	34.0
Ether-washed:		
Wheat starch	36.7	41.9
Maize starch	39.4	43.7
Rice starch	39.1	46.3
Potato starch	43.0	46.3

Examination of Tables II and III shows that the removal of fatty matter by ether tends to render the cereal starches more accessible to the enzymes as does purification with dil. alkali but not always to the same extent. Thus the low results obtained with water-washed maize starch might be attributed chiefly to fat which it contains, but the hydrolysis of rice starch by the purified aspergillus amylase is evidently retarded by material which is not removed by ether but is removed by washing with dil. alkali, as shown by comparison of Tables III and IV.

TABLE III.

Action of Different Enzymes upon Water-washed and upon Ether-washed Starches.

Description of starch.	% of starch hydrolyzed in 30 min. by Pancreatin.	Pancreatic amylase. Prep. No. 64.	Malt amylase. Prep. 155.	Aspergillus amylase. Prep. No. 22.
Water-washed:				
Wheat starch	40.4	39.1	38.3	42.5
Maize starch	29.1	27.3	36.8	31.5
Rice starch	40.5	42.4	39.6	15.2
Potato starch	44.9	43.8	42.2	42.9
Ether-washed:				
Wheat starch	43.5	39.7	38.6	42.6
Maize starch	44.3	41.8	39.0	39.9
Rice starch	44.0	44.0	39.8	21.9
Potato starch	44.6	44.0	42.2	40.1

TABLE IV.

Comparative Digestion of Different Starches when Purified by Washing with Dil. Alkali.

	% of starch hydrolyzed in 30 min. by						
Kind of starch.	Saliva.	Pancreatin.	Pancreatic amylase. Prep. No. 64.	Malt extract.	Malt amylase. Prep. 155.	Taka-diastase.	Aspergillus amylase. Prep. 22.
Wheat.....	40.2	38.2	41.8	54.5	34.5	48.2	37.8
Maize.....	40.6	38.9	42.4	54.8	35.6	44.7	39.7
Rice.....	41.5	38.9	42.1	54.5	35.4	48.3	39.7
Potato.....	43.5	39.4	29.0	56.8	35.2	52.4	38.2

TABLE V.

Comparative Digestion of Different Starches when Purified by Washing with Dil. Alkali and with Ether.

	% of starch hydrolyzed in 30 min. by					
Kind of starch.	Pancreatin.	Pancreatic amylase. Prep. 64.	Malt extract.	Malt amylase. Prep. 155.	Taka-diastase.	Aspergillus amylase. Prep. 22.
Wheat.....	40.7	39.0	53.1	38.7	43.5	39.8
Maize.....	41.0	39.8	52.3	39.1	42.7	39.5
Rice.....	41.5	39.8	53.3	39.4	43.2	39.5
Potato.....	44.4	27.7	55.4	40.7	46.0	42.2

Tables IV and V show that wheat, maize and rice starches, when similarly purified by washing with dil. alkali or alkali and ether, are of equal digestibility as measured by the method here used. This is true for each of the 3 typical amylases employed and regardless of whether the amylase was used in commercial or purified form. Except with purified pancreatic amylase[1] potato starch shows a digestibility or rate of enzymic hydrolysis equal to or slightly greater than that shown by the cereal starches. Thus with the single exception already noted, each column in Table V shows a slightly higher yield of maltose from potato starch than from the cereal starches tested (the variations among the latter being probably no greater than the possible error of experiment). In this connection it may be stated that preliminary results obtained in this laboratory by Dr. K. Hattori, in a study designed to compare the rate of hydrolysis of rice and potato starches by means of saliva throughout the course of digestion, have shown a slightly higher yield of reducing sugar from the potato than from the rice starch, the difference becoming perceptible when about 40% of the starch has been transformed to maltose and increasing gradually as the digestion is carried farther, probably because of a larger yield of relatively resistant dextrin from the rice, than from the potato, starch.

Conclusion.

When similarly purified by washing with very dil. alkali, wheat, maize and rice starches show the same digestibility in the sense that under the

[1] The abnormally low results in this single case will be discussed in a later paper.

action of the same kind and amount of amylase they are all transformed into reducing sugar at essentially the same rate. This is true whether the digestive agent be saliva, pancreatin, purified pancreatic amylase, malt extract, purified malt amylase, taka-diastase, or the purified amylase of *Aspergillus oryzae*.

As obtained by washing with water only, potato starch is almost pure, but the cereal starches appear to contain sufficient amounts of fatty or waxy matter to interfere to an appreciable extent with the action of the enzymes upon the starch even after the latter has been dispersed by boiling in water for 3 minutes. This was true to a greater extent of the maize than of the wheat starch, a finding in accordance with the results of natural digestion experiments[1] which emphasize the importance of such preparation of maize products as shall ensure their very thorough mastication and admixture with saliva.

Potato starch shows in general a rate of enzymic hydrolysis equal to or slightly greater than that of the cereal starches. As here tested, it is as readily acted upon in the water washed as in the more highly purified condition. The only case in which the potato starch has shown a distinctly lower rate of hydrolysis than that of the cereal starches, is one in which both starch and enzyme were employed in a highly purified condition. This tendency to abnormally low results is readily corrected by suitable additions to the digestion mixture as will be shown in a subsequent paper.

We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF AMHERST COLLEGE.]

THE ACTION OF CUPROUS CHLORIDE WITH COMPOUNDS CONTAINING THE TRICHLOROMETHYL GROUP.

BY HOWARD WATERS DOUGHTY.

Received May 7, 1919.

In 1917 the present writer published a preliminary report[2] on the "Hydrolysis of Organic Halides and the Corrosion of Metals," in which it was shown that when compounds which contain the trihalogen-methyl group are brought in contact with copper in ammonia water, the copper goes into solution as ammono-cupric halide.

The facts that no evolution of gas is observed during the reaction, and that copper is oxidized to the cupric condition indicate that the reaction is not a simple hydrolysis, but that there is probably a condensation due to the presence of the ammonia, in which the heat of formation of the

[1] Sherman and Winters, *J. Biol. Chem.*, 35, 301 (1918).

[2] THIS JOURNAL, 39, 2685 (1917).