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## FAT ASSOCIATED WITH STARCH.

By T. C. TAYLOR AND J. M. NELSON.

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It is well known that the starches as they are obtained ordinarily from the plants have a certain amount of fat associated with them. It has been assumed generally that this fat can be removed by solvents, and that its presence is a contamination of the starch with other constituents which occur intimately associated with the starch in the plant.

In the manufacture of glucose commercially from corn starch an insoluble product known as "refinery mud" occurs in the sugar liquor after the hydrolysis, and is separated by filtration. This refinery mud as recovered contains about 50% of fat, chiefly the higher fatty acids, and after washing and other treatment is sold as soap stock.

Considering the process employed in the commercial hydrolysis of starch it is improbable that the source of this fatty material can be attributed to extraneous matter accompanying the starch from the kernel, since its presence is observed only during or after the destruction of the starch by hydrolysis. The occurrence of free fatty acids during the hydrolysis and disruption of the complex starch molecule leads to the interesting question of whether the fatty material constitutes an inherent part of the starch itself.

The present article is an account of an attempt to answer this question, and the results obtained lead to the following conclusions.

I. The major part of the fatty material present in starch cannot be removed by solvents before hydrolysis.

II. Hydrolysis of corn starch freed of extraneous fat liberates fatty acids.

III. The liberated fat is principally palmitic acid, but an unsaturated substance of unknown structure also occurs with it.

IV. The fat is liberated when hydrolysis has reached the erythrodextrin stage.

V. It is possible to obtain from starch residues containing relatively large amounts of fat combined with carbohydrate.

VI. The palmitic acid apparently is attached indirectly to the carbohydrate, but directly to the unsaturated component.

VII. Starches from other sources than corn also contain combined fat.

### I. Extraneous Fat Accompanying the Starch.

The corn starch used was the best alkali-washed product available in the open market. A 82.26 g. sample of this material was extracted, first with ether, then with petroleum ether and finally with carbon tetra-

chloride. The duration of the extraction in each case was 36 hours. The amount of soluble matter, chiefly fat, obtained by means of each successive solvent was

Solvent.	%.
Ether (dry).....	0.057
Petroleum ether.....	0.012
Carbon tetrachloride.....	0.046
	<hr/>
Total.....	0.115

Further extraction gave no weighable residues upon evaporation of the solvent.

The residues, obtained by evaporation of the solutions from the first extractions and the weights of which are given in the table above, were yellow and gummy. Upon solution in alcohol and titration with alkali, the combined residues gave an acid value<sup>1</sup> of 95.1. A comparison of this acid number with 186.0, that of the fat described under II, indicates the presence of considerable foreign matter, probably nitrogenous in character.

**Purification of the Corn Starch.**—Since starch is separated mechanically from the other substances which occur with it in the plant,<sup>2</sup> the separation is never complete, even after repeated treatments. For this reason, it is necessary to resort to other methods of removing the remaining impurities,<sup>3</sup> chiefly nitrogenous. The presence of the latter is indicated both by the nitrogen content 0.06% (Kjeldahl method), and the presence of dark yellow particles when the starch, stained with iodine, is examined under the microscope.

**Removal of the Nitrogenous Material and Extraneous Fat.**—About 500 g. of dry powdered corn starch was placed in a 3-liter round-bottom flask provided with a reflux condenser, then enough 95% alcohol added to make a thick paste and finally 500 cc. of 90% alcohol containing 0.23 g. of hydrogen chloride per cc. After refluxing on the water bath for  $\frac{3}{4}$  hour with continuous agitation, the contents of the flask was filtered through a Büchner funnel and the residue washed, first with hot 95% alcohol and finally with several portions of ether.

The purified starch obtained in this way was clean and white. When stained with iodine, it showed under the microscope no indications of protein, and the granules appeared to be still intact, although slightly enlarged, with evidence of incipient rupture at the hilum. That no profound change in the starch had occurred was shown also by the fact that when a sample of this starch, treated with alkali to neutralize the hydrochloric acid which still adhered, was gelatinized by stirring into

<sup>1</sup> Mg. of potassium hydroxide neutralized by one g. of the fat.

<sup>2</sup> Commercial method, T. B. Wagner, *J. Soc. Chem. Ind.*, 28, 343 (1909).

<sup>3</sup> Composition of corn kernel, *THIS JOURNAL*, 25, 1166 (1903); and Forst, *Eighth Inter. Cong. Appl. Chem.*, 12, 205.

boiling water it gave a "thick boiling" paste which is characteristic of unchanged or "raw" starch. The amount of hydrochloric acid adhering was determined by drying the purified starch at  $40^{\circ}$  and then suspending in water and titrating with standard alkali, and was found to be 0.52%. By means of Kjeldahl determinations, the nitrogen content was found to have been decreased in the process of purification from 0.06 to 0.02%.

When the purified starch was stained with iodine and examined under the microscope a stratified structure of alternating dark blue and transparent concentric rings, was observed. This is in accord with previous observations of Duclaux<sup>1</sup> and others and indicates a layer structure of the starch granules, which might be of importance in connection with the question of the presence of 2 kinds of starch material in the granules, as held by Baker and Sherman and others.<sup>2</sup>

The starch purified by the latter method contains no extraneous fat. Eighty g. of dried starch was extracted in a Soxhlet for 40 hours with dry ether. Upon evaporation of the ether extract no weighable residue remained, showing the starch to be free from extraneous fat.

In order to make sure that no free fat was adsorbed so firmly to the starch that it could not be removed by the above method of extraction the following procedure was employed.

An ether solution of fat, previously obtained from hydrolyzed starch, was thoroughly mixed with finely powdered purified starch and this mixture, containing the starch in suspension, evaporated, then heated on the water bath with stirring. This fat-impregnated starch was then extracted for 10 hours in a Soxhlet with absolute ether and the amount of fat determined.

SAMPLE, 20.0 G.		
	Fat added. G.	Fat recovered. G.
1.....	0.4566	0.4501
2.....	0.4035	0.4057
3.....	0.1400	0.1465

In Numbers 1 and 2 the fat added was much in excess of total fat usually found, while in 3 the amount was comparable with the usual fat content of the original starch.

On hydrolysis of these starch samples with aqueous hydrochloric acid, after the added fat had been removed by extraction, the usual amount of fatty material was recovered, as in II.

## II. Liberation of Fatty Acids.

When the starch freed from extraneous fat as described in I is hydrolyzed, fat is liberated, exceeding in amount the extraneous fat extracted from the original starch.

<sup>1</sup> Duclaux, *Ann. Inst. Pasteur*, 9, 836 (1895).

<sup>2</sup> *THIS JOURNAL*, 38, 1885 (1916).

**Fat by Hydrolysis.**—One hundred cc. of conc. hydrochloric acid was added to 200 cc. of boiling water, and immediately upon addition of the acid a starch suspension of 25 g. of starch in 100 cc. of water, was poured slowly into the boiling acid solution with continuous stirring and heating. The mixture soon liquefied and the starch was hydrolyzed with the liberation of an insoluble, flocculent material. The heating was discontinued when a drop of the liquid no longer showed color with iodine, and the mixture was allowed to cool. It was then poured on a large filter paper, allowed to drain, and the residue washed with cold water until free from hydrochloric acid. The paper and residue were dried at 50° in an air-bath, placed in a thimble and extracted in a Soxhlet for 3 hours. After removal of the solvent from the extract, by evaporation on the steam-bath, the flask and the remaining residue were dried and weighed.

Both ethyl ether and petroleum ether were used in the above. The former seemed to give slightly higher results and always carried with it more coloring matter, besides a small amount of reducing sugar, while the latter gave a cleaner product but it was found difficult to get rid of the last traces of the solvent.

Four samples of purified starch were subjected to the above treatment and the fat liberated by hydrolysis determined and given below.

SAMPLE, 25 G. OF STARCH.

	Solvent.	Extraction.	Fat by hydrolysis. %	Acid number.
1.....	Petroleum ether	Dry	0.56	181.3
2.....	Petroleum ether	Dry	0.49	186.5
3.....	Petroleum ether	Wet	0.54	...
4.....	Ethyl ether	Dry	0.64	184.7

Wet extraction with ethyl ether of the hydrolyzed starch liquor necessitated drying the ether layer due to the relatively large amount of water taken up by it. As this entails some loss, and further since in the presence of fat, reducing sugar passes into the ether layer, only extraction of dried fat should be attempted with this solvent.

The experiment corresponding to the data marked 3 in the above table was carried out slightly differently, in that the liquor resulting from the hydrolysis was extracted directly with petroleum ether, without first filtering or drying the residue.

The fat obtained by the hydrolysis of the starch will hereafter be designated as "fat by hydrolysis" to distinguish it from the small amount of extraneous fat, extracted directly from the starch.

Gelatinization and destruction of starch granule does not affect combined fat. In order to make sure that none of the fat obtained by the hydrolysis of the starch, the amounts of which are given in the table in

Part II, was held mechanically as extraneous fat between the layers in the starch granule, the latter were destroyed by gelatinization. One hundred parts of dry powdered starch were added to 80 parts of a 50% aqueous solution of ammonium thiocyanate to which had been added previously 40% of its weight of alcohol.<sup>1</sup> The gelatinized paste thus obtained was stirred with an excess of acetone which precipitated a white amorphous starch containing no granules. Practically all of the ammonium thiocyanate was then removed by repeated washing with acetone and alcohol and the dried product finally extracted with ether. A sample of this ether-washed starch contained no directly extractable fat, but did contain 0.41% of "fat by hydrolysis" and the latter had an acid number of 182.7. Since the amount of fat and its acid number agree very well with the values in the above table for starch in the form of granules, it is evident that the "fat by hydrolysis" cannot be attributed to retention of fat between the layers in the granules. Had this been the case then when the granules were destroyed the fat would have been removed by the acetone and alcohol, and the fat obtained upon subsequent hydrolysis of the amorphous material would have been much lower than 0.41%.

### III. Nature of the Fatty Material Liberated by the Hydrolysis of Maize Starch.

The fatty material obtained by the hydrolysis of the starch as described under II, is semi-crystalline, forms soap with alkali, produces grease stains on paper and has a "tallow"-like odor which becomes more pronounced on heating.

In order to obtain more definite data as to the exact nature of the fatty material, a much larger quantity was necessary than could be obtained by the hydrolysis of batches of starch in ordinary chemical laboratory apparatus. Therefore, the "refinery mud" mentioned in Part I was used.<sup>2</sup> This mud corresponds to the flocculent precipitate which remains after acid hydrolysis of the starch in the method for "fat by hydrolysis." The commercial production of glucose from starch is simply this aqueous acid hydrolysis modified to fit large scale production.

Due to the fact that the mud is removed by filtration in iron filter presses, a considerable amount of iron soap is formed from the action of the fatty acids on the apparatus. A proximate analysis of this "refinery mud" shows the following.

<sup>1</sup> *J. Soc. Chem. Ind.*, 28, 213 (1909).

<sup>2</sup> The authors wish to thank Dr. C. E. G. Porst for procuring from the Corn Products Refining Company the starch and "refinery mud" used in this investigation, and for his valuable suggestions concerning the determination of the amount of combined fat.

	Per cent.
Moisture.....	29.5
Protein.....	10.9
Ash.....	3.5
Red. sugar.....	2.4
Fat.....	48.8
Remainder.....	Undetermined

The fat content is high because included in this are other substances, especially iron soaps which dissolve with the fat, but these probably do not amount to more than 2.0% of the total extractable matter.

When the fat is first recovered it is a light yellow mass with an odor suggesting furfural, but on standing exposed to air it soon darkens and becomes gummy. After a few days the odor is typical of oleic acid which has been exposed to the air for a time.

**Recovery of Fat from the Refinery Mud.**—A kilo of this mud was thoroughly mixed with about a liter of low boiling petroleum ether and then poured into a 2-liter round-bottom, short-neck flask connected with a spiral condenser. The flask was heated on a water bath while vacuum was applied to the system, water and petroleum ether distilled over together and separated into 2-layers in the receiver. The ether layer was removed and returned to the flask. On distilling over the ether repeatedly the water was removed from the mud without undue exposure of the fat to the air and in addition the drying was uniform throughout the mass. Finally the last suspension of the starch in the petroleum ether was filtered through a Büchner funnel under suction and the ether distilled off, leaving the crude fat in a dark brown mass whose surface showed, as the fat became cold, a peculiar crystalline structure. This crude fat was dissolved in boiling alcohol and repeatedly treated with bone black and then re-crystallized from alcohol until, on cooling, a perfectly white crystalline fatty acid separated. Several more crystallizations were made until the product showed no iodine number. A weighed sample of this final product was dissolved in neutral alcohol and titrated with standard alkali.

Subs., 0.2788, 0.5000: NaOH required, 0.0437, 0.0739. Found: Mol. wt. (monobasic acid): 254.8, 256.5.

The iodine value for this material was zero.

A silver soap was prepared by slightly over-neutralizing the free fatty acid with ammonia and then adding silver nitrate solution until no further precipitate formed. The precipitate was filtered off, washed free of salts, dried, and a weighed portion ignited in a tared porcelain crucible. A residue of silver resulted which was weighed and from this value the molecular weight of the substance calculated as a monobasic acid.

Subs., 0.212: Ag, 0.0061.

Calc. for palmitic acid: 256.6. Found: 268.3.

The acid, after remaining in an ice box for several hours, melted at

61.6°, with a rate of heating of about 1° per minute. Lewkowitsch gives 62.6° as the melting point for palmitic acid. The anilide of our acid melted at 90.2° which is the melting point given for the anilide of palmitic acid. The substance, upon combustion, agrees in composition with that of palmitic acid.

Calc. for palmitic acid: C, 74.92; H, 12.54. Found: C, 75.00; H, 12.36.

The freshly isolated fat mentioned in II, however, has a melting point between 38° and 43°, an acid number of 186, and an iodine number of about 92, while palmitic acid, as pointed out above, has a melting point of 62°, an acid number of 219, and a practically zero iodine number. There is, therefore, present in the fat, liberated in the hydrolysis of the purified starch, besides the palmitic acid an unsaturated constituent of relatively low acidity.

In order to get some insight into the nature of this other constituent, the lead salts were made according to the usual method and the ether soluble portion (the lead salts of certain unsaturated acids are soluble in ether) decomposed with hydrogen sulfide. The liberated fatty material was liquid at ordinary temperature and had an iodine number of 136.0. On cooling, some solid separated, leaving a light yellow liquid. The solid was removed from the liquid and dried between filter paper and was apparently palmitic acid, since it melted at 62° and had an acid number of 202. The light yellow liquid remaining was highly unsaturated, had an iodine value of 136, and showed practically no acidity. It was insoluble in cold aqueous alkali, coagulating as a gummy mass, showed the presence of small amounts of reducing sugar when treated with Fehling's solution, and gave no test for nitrogen by the sodium fusion method, but did show the presence of small amount of phosphorus, and gave no residue on ignition. This material will be referred to again as X in the latter part of the article.

#### IV. Liberation of the Fatty Acids at the Early Stages.

**Erythrodextrin of the Hydrolysis of the Starch. Hydrolysis by Mineral Acids.**—Fifty g. of the purified starch, suspended in water, was treated with 3 *M* hydrochloric acid, and when the erythrodextrin stage of hydrolysis, indicated by plum color with iodine, was reached, the operation was arrested, and the mixture poured into an excess of 95% alcohol. The alcohol caused a separation of the dextrin, which was removed by filtering and then washed with alcohol and ether. The combined alcohol-ether filtrates and washings were evaporated, the residue remaining extracted with ether and the extract evaporated, weighed, and found to amount to 0.53% of the original starch. The dextrin was hydrolyzed and the "fat by hydrolysis" from this was found to be negligible. When it is recalled that the "fat by hydrolysis" from the original starch is be-

tween 0.5 and 0.6% (see table under II) it becomes evident from these results that practically all the fat is liberated in the early stages of hydrolysis of the starch by aqueous acids. By repeating the above procedure with various concentrations of hydrochloric and nitric acids the same results were obtained except that the lower concentrations of acid seemed to favor the formation of insoluble retrograded starch which resisted further hydrolytic action. It was found, however, that a short treatment with 10% aqueous sodium carbonate solution rendered the retrograded starch soluble so that acid reagents could act in the usual way to give glucose.

**Diastatic Hydrolysis.**—The starch was hydrolyzed by means of diastase also instead of by acid. It was thought that possibly the fat might be liberated at a later stage in the hydrolysis than the erythrodextrin when the enzyme was used. The procedure was practically the same as in the acid hydrolysis except that in order to secure optimum conditions for the diastatic action, the hydrogen ion concentration was adjusted according to the directions of Sherman, Thomas and Baldwin.<sup>1</sup> Two experiments were carried out and in each case free fat was obtained. In one case the hydrolysis was interrupted at the erythrodextrin stage and the alcohol-ether residue similar to that described for acid hydrolysis, amounted to 0.19%. In the other case the hydrolysis was allowed to continue until a test portion gave practically no color with iodine, and then the liberated fat amounted to 0.41% of the original starch. It therefore appears, as far as one can judge by means of the iodine color, that at least some fat is liberated at the erythrodextrin stage when starch is hydrolyzed by the diastase just as was observed when acid was the hydrolytic agent.

## V. Cleavage of Starch into Products of Relatively High Fat Content.

(A). **Hydrolysis or Cleavage of Starch by Low Hydrogen-Ion Concentration in the Presence of *Bacillus Aceto-ethylicum*.**—One hundred g. portions of starch were gelatinized by stirring into 2 liters of boiling water containing 2 g. of ammonium phosphate and 0.5 g. of magnesium sulfate as nutriment for the bacteria.<sup>2</sup> Starch purified as described above formed in this way a very limpid paste.<sup>3</sup> The last traces of foreign material such as cell fragments still present, settled and were removed by the decantation of paste through a layer of cotton. The decanted paste was then placed in a 5-liter flask and the hydrogen ion adjusted to approximately  $10^{-8}$  by adding acid or alkali until a test sample showed no pink color with phenolphthalein and no yellow with phenol red.<sup>4</sup> The

<sup>1</sup> Sherman, Thomas and Baldwin, *THIS JOURNAL*, 41, 231 (1919).

<sup>2</sup> Schardinger, *Centr. Bakt.*, 22, 98 (1909).

<sup>3</sup> Small, *THIS JOURNAL*, 41, 113 (1919).

<sup>4</sup> Lubs and Clark, *J. Bact.*, 4, 107 (1919).



flask was then stoppered with cotton, and the contents sterilized at 15 pounds steam pressure for one hour, cooled to 40° and inoculated with *bacillus aceto-ethylicum*,<sup>1</sup> and allowed to remain in an incubator at 40° until there was no more apparent action. A very limpid liquid, containing a small amount of insoluble material, resulted. This was filtered and the residue obtained was washed with hot water and allowed to drain. The residue then was stirred into an excess of absolute alcohol, the alcohol filtered off and the material dried *in vacuo* at 40°. In this way a gray powder was finally obtained which was digested with boiling ether, filtered through a Büchner funnel and washed with several small portions of ether to remove any fat which might have been freed in the fermentation. The residue thus obtained produced a plum color with iodine and showed no starch granules under the microscope.

Three such residues, ranging in weight from 10 to 15 g., were hydrolyzed by acid as in II and were found to contain still a considerable quantity of fat. The values given below were calculated on the dry weight of the residues.

No.	Fat of hydrolysis. %
1.....	1.6
2.....	3.8
3.....	2.2

(B). Hydrolysis or Cleavage of Starch by Low Hydrogen Ion Concentration without Addition of Aceto-ethylicum.—On allowing solutions, prepared for inoculation as above, to stand, a separation into layers was noticed, an upper clear and a lower turbid one. The latter was similar in appearance to retrograded starch. This observation led to the carrying out of the above partial hydrolysis of the starch without the addition of the salts or *aceto-ethylicum*, the procedure otherwise being identical with that in (A). After standing for about 6 hours a rather complete settling of the insoluble portion had taken place. The residue obtained by this latter method differed from that described in (A) in that it was more voluminous and had the appearance of retrograded starch. It was removed by filtration and treated in a way analogous to the procedure employed for the residue in (A).

Three 100 g. portions of purified starch containing 0.52 g. of "fat by hydrolysis" were analyzed by this method.

Residue No.	Residue from 100 g. of starch G.	"Fat by hydrolysis" in residue. %
88	5.0	5.5
89	8.1	3.6
90	8.0	3.3

<sup>1</sup> The authors wish to thank Dr. J. H. Northrop for the culture of the *Bacillus aceto-ethylicum* and directions for applying it to starch. This is the bacteria used by Northrop, Ashe and Morgan for the production of acetone from starch and glucose See *J. Ind. Eng. Chem.*, 11, 723 (1919).

A sample of residue No. 88 gave on ignition an alkaline ash of 0.28%, which showed the presence of phosphorus and magnesium, while residue itself showed 0.04% of phosphorus.

Since these residues contained as high fat content as those described in (A), the method of preparation in (B) is to be preferred due to its being simpler. The fat content of the residues from both methods, (A) and (B), being about 6 times that of the original starch, this appears to offer an avenue to the isolation of a simpler compound and information concerning the structure of the fat-carbohydrate linking. Work is now in progress on this particular phase of the problem. That the fat in the residue might be occluded in the residue, is very improbable on account of the method employed in precipitation of the residue by alcohol and ether, both of which are good fat solvents. The argument that the residue material was so insoluble as to prevent the solvents from coming into contact with the fat and hence the latter being retained, although free, in the residue, can have very little justification in the light of the following experiment.

Two g. of residue No. 90 was refluxed for one hour with 20 cc. of 0.1 *M* alkali. This treatment gave a slightly opalescent liquid similar in appearance to colloidal starch. On pouring the liquid into an excess of alcohol a precipitate was formed which gave a blue color with acid iodine solution, indicating unchanged starch. The precipitate was filtered, dried and extracted with boiling alcohol and ether, and upon analysis showed 1.9% of "fat by hydrolysis" with an acid number of 175.7.

By this alkali treatment the residue was brought into solution again, and if the fat had been free fatty acid it would have been removed completely, yet the results showed that only 1.4 of the 3.3% fat present in the residue was removed. The reason why the 1.4% was removed can be explained most satisfactorily by assuming that the fat is present in the original residue as ester and that under the conditions of the above experiment the saponification of ester by the alkali was not complete.

**Fat not Accounted for in Residue Described in B.**—It is evident that only about  $\frac{1}{2}$  of the total combined fat in the original starch remains as such in the residue No. 89. The aqueous filtrate, obtained in the preparation of this residue, was extracted with ether to make sure that no free fat was present, and subjected to acid hydrolysis. In this way, the filtrate was found to contain 0.085 g. of fat still combined with the soluble starch contained in the filtrate. Subtracting the sum of the combined fat held in the residue and aqueous filtrate,  $0.292 + 0.085$  or 0.38 g., from 0.56 g., the "fat by hydrolysis" in the original 100 g. of starch, gives 0.18 g. of fat which must have been liberated in the process and lost by being extracted by the alcohol-ether treatment. Qualitative test of the filtrate with Fehling's solution showed reducing sugar, and iodine test indicated erythrodextrin.

### Decomposition of the Starch by Alcohol Solution of Hydrogen Chloride.

—In the preparation of some methyl glucoside in this laboratory by the method described by Fischer,<sup>1</sup> maize starch was heated in an autoclave with an absolute methyl alcohol solution of hydrogen chloride. An insoluble residue, similar in appearance to the sediments described above, together with an oily liquid floating upon the surface of the alcoholic solution, were formed. This residue and the oily liquid were examined in the hope that further light might be cast upon the way the fat is combined within the starch.

Since the reaction of the starch with the alcoholic hydrogen chloride had taken place in a copper autoclave, there was some contamination with copper salts. It was necessary to remove the latter by washing with glacial acetic acid before the residue could be examined. The residue showed microscopically only flocks of amorphous material, which produced a red color with iodine similar to that from erythrodextrin. The "fat by hydrolysis" in this residue was 0.56% which is practically the same as in the original starch (see table under II). It is of interest in this connection to compare these results with those obtained in the aqueous acid hydrolysis. Although the iodine color of the various cleavage products of starch can not be considered absolutely reliable, it serves to show roughly that a change has taken place in the starch molecule. Comparing this color reaction of the material from the aqueous hydrolysis with that obtained from the alcoholic-hydrochloric acid treatment, the following facts can be set down.

In the aqueous hydrolysis (see Part III) as soon as there was any change in the starch complex, noted by the iodine test, the entire amount of bound fat was liberated. In the alcoholic cleavage, although apparently all the starch had undergone a change as shown by the red color with iodine and by the amorphous condition of the residue as revealed by the microscope, the erythrodextrin-like residue retained the same quota of fat as is present in the original starch. It would seem, therefore, from these rather limited data that the cleavage of the starch molecule takes place at different linkings according to the method used, and furthermore it serves as additional evidence that the fat is an inherent part of the starch molecule.

When the above alcoholic filtrate is evaporated and extracted with ether a dark brown oil is obtained. It was non-acidic, decolorized bromine, was insoluble in cold alkali but on warming practically all of it dissolved and gave a soapy solution which reduced Fehling's solution. Apparently this substance is the unsaturated constituent "X," mentioned in Part III.

### Treatment of Residue Containing Relatively a Large Amount of Fat,

<sup>1</sup>Fischer, *Ber.*, 28, 1151 (1895).

with **Absolute Alcohol-hydrogen Chloride**.—Since the residue from (A) and (B) still contained carbohydrate very similar to starch itself, and cleavage with hydrolytic reagents and the bacillus was apparently impossible, it occurred to us that treatment with alcoholic hydrogen chloride might offer a means of breaking down this still complex compound to a simpler one. Accordingly the following experiment was made.

Five g. of residue No. 99 was refluxed for 50 hours with 160 cc. of absolute alcohol containing 1.971 g. of hydrogen chloride. After this treatment there remained 2.24 g. of apparently unattacked starch-like residue which gave a red color when tested with iodine instead of the purple color given by the original sample. Analysis of this sample, after washing with ether, showed 3.2% of "fat by hydrolysis" referred to the weight of the dry product, a fat content essentially the same as that of the original sample No. 89 (see table) and therefore indicating that only about half of the original sample had been affected by the treatment. On partial evaporation of the alcoholic solution containing the glucoside, there appeared on the surface small droplets of dark brown oil. These were removed by solution in ether; and on evaporation of the ether a product remained which was similar in properties to the unsaturated liquid called "X" described above.

#### VI. The Fatty Acid is Combined Indirectly to the Starch.

(A) **Saponification of Fat Liberated by Acid Hydrolysis**.—A sample of 0.3058 g. of fat liberated by aqueous acid hydrolysis was refluxed for one hour with 5 cc. of *M* alcoholic sodium hydroxide, while as a blank analysis 5 cc. of sodium hydroxide was given exactly the same treatment. After refluxing, the flasks were cooled and titrated back with 0.05 *M* sulfuric acid.

##### CC. OF 0.05 *M* SULFURIC ACID REQUIRED.

Blank.....	48.9
Sample.....	38.0
Difference.....	10.9

This was equivalent to a saponification number of 199.5. Another portion of same sample gave an acid number of 182.3, while that of palmitic acid is 219.1.

(B) **Saponification of Fat Liberated by Diastatic Hydrolysis**.—A sample of 0.1290 g. of fat liberated by diastatic hydrolysis was dissolved in petroleum ether and 10 cc. of 0.1 *M* alcoholic sodium hydroxide added. The mixture protected from carbon dioxide by a soda-lime tube was allowed to remain at room temperature for 18 hours. Back titration of the excess alkali required 5.1 cc. of 0.05 *M* sulfuric acid, indicating therefore a saponification number of 212.7. The acid number of the same sample was 147.8.

It is evident that the fat from both methods of hydrolysis of the starch

contains combined fatty acid or an ester. This ester has suffered more hydrolysis when acid instead of diastase was used in decomposing the starch. In the light of these data, very likely the unsaturated constituent present in the fat, labeled "X," is combined with the palmitic acid and accounts for the presence of the ester.

The fact that the palmitic acid, which is a mono-basic acid, is linked as an ester to the unsaturated compound ("X") precludes the possibility of direct carbohydrate fatty acid union, and since it has been shown that the entire fatty material (fatty acid and unsaturated substance "X") is combined with the carbohydrate and only liberated by hydrolysis, the substance "X" must serve as a connecting link between the acid and the carbohydrate.

Starches from other sources than corn also contained combined fat.

Samples of various representative starches were purified as described under Part I and the "fat by hydrolysis" determined. In the following table are enumerated the results of these analyses. Fifty g. samples were used, except in the case of potato starch.

Starch.	"Fat by hydrolysis."	Acid No.	Iodine No.
Corn.....	0.61	182.5	92.5
Rice.....	0.83	283.4	84.7
Sago.....	0.11	151.0	..
Cassava.....	0.12	168.0	..
Potato (200 g. sample).....	0.04	109.4	..
Horse chestnut.....	0.56	21.9	..

From the above results it is evident that corn starch is not unique in having combined fat, but that starches of widely differing genera also contain combined fatty material.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, UPJOHN COMPANY.]

### THE RAGWEED POLLEN PROTEINS.

BY FREDERICK W. HEYL AND HARRIS H. HOPKINS.<sup>1</sup>

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In considering any drug from a pharmaceutical point of view, two distinct schools of thought are always in evidence. First, the progressive school which is largely dominated by chemistry and to whom pharmacy owes much in the matter of the isolation or preparation of pure or crystalline principles. Secondly, there is the conservative group, dominated

<sup>1</sup> Holder of The Upjohn Coöperative Fellowship at Kalamazoo College (1919-1920). This paper is based upon the thesis presented by Mr. Hopkins to the Faculty of Kalamazoo College, in partial fulfillment of the requirements for the degree of Master of Science.