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# PROPERTIES OF CORN STARCH. REMOVAL OF COMBINED FATTY ACIDS

BY T. CLINTON TAYLOR AND J. H. WERNTZ

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In previous work in this Laboratory[1] it was shown that corn starch, among other starches, carried high-carbon-content fatty acids combined apparently with some part of the carbohydrate. Later it was found possible, by means of electrophoretic and ultrafiltration[2] methods, to segregate and concentrate the fatty acids[3] in one portion of the starch in the form of an ether-insoluble compound. Throughout the paper this fatty-acid-bearing portion is referred to as $\alpha$-amylose while the fat-free material is $\beta$-amylose.[4]

The facts that the fatty acids are not directly extractable with fat solvents, but may be recovered only after hydrolytic treatment of some kind, and that methyl derivatives of the $\alpha$-amylose can be made which still retain the fatty acids,[5] indicate that this amylose is really a fatty acid derivative of a carbohydrate complex.

In an attempt to develop an analytical procedure which would remove this fatty material from cereal products and starches, Rask and Phelps[6] proposed the use of an alcohol-ammonia-water reagent which does not appreciably gelatinize the starch but extracts some material from it. The extract they designate " lipoid" and report the presence of it to the extent of 0.54% in corn strach. Since this happens to be about the amount of combined fatty acids in pure corn starch,[1] they felt that their treatment had removed all of the combined fatty acids.

The purpose of this paper is to report the experiences in this Laboratory with the Rask and Phelps method on corn starch incidental to the main purpose of the investigation, namely, a study of the properties of corn $\alpha$-amylose. Since this treatment is supposed to remove the fatty acids without affecting the carbohydrate complex, it seemed to offer possibilities of special value for the solution of the problem. As the fatty acids seem, so far, to be the principal distinguishing factor between the alpha and beta corn amyloses, and as the separation from $\beta$-amylose by electrophoresis is apparently dependent on polarity of the fat-bearing $\alpha$-amylose, any method that removes this non-carbohydrate ought to obliterate the differentiating characteristic.

[1] Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920).

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

[3] Taylor and Lehrman, THIS JOURNAL, **48**, 1739 (1926).

[4] Meyer nomenclature, "Untersuchungen über die Starkekörner," Gustav Fischer, Jena, **1895**.

[5] Werntz, *Dissertation*, Columbia, **1926**.

[6] Rask and Phelps, *Ind. Eng. Chem.*, **17**, 187 (1925).

The results of the experiments indicate, first, that the prescribed treatment removes only a small part of the combined fatty acids from raw starch and from corn $\alpha$-amylose; second, that by extension and modification of the treatment, raw starch may be freed of its combined fatty acids, but this is not the case with $\beta$-amylose; third, that in fat-free raw starch it is no longer possible to effect an electrophoretic separation of the amyloses.

This property of the fatty acids in characterizing corn $\alpha$-amylose is shown in another way in some preliminary experiments by Lehrman,[7] who found that the introduction of the palmityl group into the fat-free corn $\beta$-amylose caused the formation of a migratable amylose derivative which acted after the manner of the natural corn $\alpha$-amylose.

## Experimental Part

**Treatment of Corn Starch by Specified Method.**—Five-g., and afterwards 50g. samples of dry, alkali-washed corn starch were treated under the specified conditions[6] with the proportionate amount of reagent[8] and the lipoids determined. The "lipoid-free" starch residue was hydrolyzed with aqueous acid[1] and any material liberated was extracted, weighed and examined In Table I are given the results of the experiments.

TABLE I

EFFECT OF TREATMENT ON RAW STARCH

| Sample, g. | Lipoids, % | Fatty acids by hydrolysis: In lipoid-free residue, % | Fatty acids by hydrolysis: In original starch, % |
|---|---|---|---|
| 5 | 0.46 | .. | 0.6 |
| 5 | .46 | .. | .6 |
| 25 | .53 | 0.26 | .6 |
| 50 | .40 | .31 | .6 |
| 100 | .49 | .23 | .6 |

It is evident that the material designated "lipoid" is a mixture of extraneous material and some of the fatty acids liberated by the mild hydrolytic reagent. An examination of the extracted material showed it to be a gummy mass quite unlike the semi-crystalline fatty acid mixture which is extracted from the hydrolyzed starch pastes.

By using corn starch previously treated with alcoholic hydrogen chloride[1,2] to remove the extraneous nitrogenous and other material, the method gave results which indicated that by extension and modifiation the original object might be accomplished. It will be noticed (Table II) that the sum of the "lipoids" and still combined fatty acids equals almost exactly the total fatty acids liberated on complete hydrolysis of the whole purified starch with aqueous acid.

[7] Lehrman, *Dissertation*, Columbia, **1925.**

[8] For every 5 g. of starch, Rask and Phelps used 10 cc. of 95% alcohol, 2 cc. of concd. ammonium hydroxide and 3 cc. of water.

TABLE II

EFFECT OF TREATMENT ON PURIFIED STARCH

| Sample, g. | Lipoids, % | Fatty acids by hydrolysis: In lipoid-free residue, % | Fatty acids by hydrolysis: In original starch, % |
|---|---|---|---|
| 5 | .. | 0.32 | 0.54 |
| 5 | .. | .33 | .54 |
| 50 | 0.11 | .37[a] | .49 |
| 50 | .12 | .37[a] | .49 |

[a] Proportionately more reagent used, and residue extracted six hours in Soxhlet apparatus before analysis for fatty acids by hydrolysis.

**Treatment with Modified Method of (A) Raw Corn Starch.**—Two hundred g. of dry starch was mixed with 600 cc. of Rask-Phelps reagent and boiled under a reflux with vigorous stirring for 15 minutes. When the starch had settled, the supernatant liquid was decanted and the treatment repeated eight times. The resulting starch residue was thoroughly washed with alcohol and then extracted for eight hours with ether. An analysis for combined fatty acids[1] gave negative results. Apparently the combined fatty acid had been removed without gelatinizing the starch (microscopic examination showed granules unruptured).

When a sample was gelatinized with ammonium thiocyanate[2] and the paste subjected to conditions for electrophoresis in a U-tube,[2] there was no indication of migration of the cloudy material, even after 24 hours.

It should be noted that migration studies can be made only on completely gelatinized pastes to obtain significant results. Partially gelatinized starch will always migrate and it is possible to find the bulk of such a paste at the anode after a relatively brief application of the potential. To effect complete gelatinization is the principal reason for the use of the thiocyanate which is the most efficacious agent[9] for the purpose.

**(B) Purified Starch.**—When corn starch is treated with alcoholic hydrogen chloride[1] practically all of the extraneous nitrogenous and fatty material is removed without affecting the combined fatty acids or gelatinizing the starch. Since treatment of this purified starch by the ammonium hydroxide-alcohol reagent in previous experiments removed some of the combined fatty acids (see Table II), it was thought that with a repeated and prolonged reaction with a large excess of reagent all of the fatty acids might be removed.

Accordingly, 80 g. of dry purified starch containing 0.51% of fatty combined acids (determined by acid hydrolysis) was subjected six times (boiling for six minutes each time) to 160 cc. of the Rask-Phelps reagent.[4] After each six-minute period, the starch was allowed to settle, the supernatant liquid was poured off and a fresh portion of the reagent added. At the end of the treatment, the starch was extracted for eight hours with dry ether and a sample taken for analysis for combined fatty acids. There were no fatty acids remaining and a completely gelatinized sample no longer gave any indication of a migratable portion. Since, however, in the treatment the sample slimed badly due to partial gelatinization of the starch, there remained only 27 g. of dry material on which the above experiments were made. It is conceivable that all of the

[9] Reychler, *Bull. soc. chim. Belg.*, **29**, 118 (1920).

α-amylose was lost in the slimy material decanted and rejected, so the investigation was extended to α-amylose itself.

(C) **Corn α-Amylose.**—Thirty g. of α-amylose containing 0.9% of combined fatty acids was treated eight times, boiling for six minutes each time, with 64 cc. of the Rask-Phelps reagent.[4] The α-amylose was very efficiently suspended by vigorous stirring in the reagent, and because of sliming it was very difficult to decant any supernatant liquid without loss. The residue was thoroughly washed with alcohol, air-dried and extracted for ten hours with ether. This extracted material, after being dried to constant weight, still contained 0.35% of combined fatty acids and retained its property of migration when in the form of a paste in water.

A 15g. sample was then subjected to ten more treatments with 150 cc. of reagent, boiling 15 minutes each time before decanting and adding the new portion of reagent. This was followed with a ten-hour ether extraction, after which the sample was dried to constant weight. Analysis showed 0.35% of combined fatty acids, and the property of migration was still present.

It is apparent that the treatment of corn starch with ammonium hydroxide-alcohol reagent causes the partial hydrolysis of corn α-amylose. The ammonia soaps of the fatty acids resulting are relatively much more soluble in organic solvent than the sodium or potassium soaps.[10] In addition, the low hydroxyl-ion concentration of ammonia minimizes the swelling and disruption of the granule with consequent sliming and possible decomposition of the carbohydrate complex.[11]

Work on the structure of the complex is at present in progress, with a view toward a better definition of corn α-amylose. That fatty acids are part of the amylose and not extraneous and that they play a part in characterizing the corn α-amylose, at least to the extent of making separation from the β-amylose possible,[2] is corroborated by the present findings.

The usefulness of the treatment as an analytical procedure on corn starch for the determination of "lipoid" content may be judged best by the results enumerated.

## Summary[12]

Fatty acids are attached to a complex carbohydrate in corn α-amylose. Their presence characterizes this amylose to the extent at least of giving it polarity. Removal of the fatty acids without affecting the carbohydrate complex apparently removes this polarity. The Rask-Phelps method for determining lipoids, although designed to remove these fatty acids from starch, etc., does so only to a limited extent, and hence is of little

[10] Lewkowitsch, "Chemical Technology and Analysis of Oils, Fats and Waxes," Macmillan Co., London, **1921**, 6th ed., vol. 1, p. 142.

[11] (a) Pfeiffer and Tollens, *Ann.*, **210**, 285 (1881). (b) Demoussy, *Compt. rend.*, **142**, 933 (1906). (c) Schmid and Becker, *Ber.*, **58**, 1966 (1925). (d) Fouard, *Bull. soc. chim.*, **5**, 828 (1909). (e) Ferrand and Block, *Bull. sci. Pharmacol.*, **18**, 207 (1911).

[12] The work embodied in this paper is taken from a part of a thesis presented by J. H. Werntz to the Faculty of Pure Science of Columbia University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

value analytically. More rigorous treatment removes the fatty acids from pretreated starch but not from corn amylose itself.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY OF CORNELL UNIVERSITY]

## THE ABSORPTION SPECTRA OF ORTHO-CRESOLBENZEIN

BY W. R. ORNDORFF AND S. ALICE McNULTY[1]

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In connection with the study being made at Cornell University of the absorption spectra of the phthaleins, the sulfonephthaleins and other triphenylmethane derivatives, it seemed desirable to study the absorption spectra of the benzeins from which the phthaleins and sulfonephthaleins are derived. The absorption of benzaurin (phenolbenzein)[2a,2c] and a study of *o*-cresolbenzein[2b] have already been reported.

A complete description of the apparatus and method employed in studying the visible and ultraviolet absorption of a substance is to be found in the earlier paper on benzaurin.[2a]

When the red-orange crystals of pure *o*-cresolbenzein are dissolved in cold, absolute ethanol, a yellow-orange solution is formed. Several hours are necessary in order to obtain complete solution of the material, and the absorption of this solution is given by Curve *B* in Fig. 1. A week later, and still four months later the solution which had stood in a darkened room gave absorption curves exactly coincident with Curve *B*. Curve *A*, Fig. 1, shows the very similar absorption obtained with the alcoholic solution of benzaurin after the solution had stood ten days and was found to be at equilibrium.

The absorption of fresh alcoholic solutions of fuchsone and aurin[2c] is of an entirely different type from that given by these solutions after they have faded on standing for varying intervals of time. In the fresh solution of these substances absorption is characteristic of the substance in the quinoid state, while the faded solution represents absorption due to the presence of both the quinoid hydrate and carbinol forms of the substance. With benzaurin[2c] the same change in absorption was found, although the fading proceeded with such great rapidity that it was possible to obtain the absorption characteristic of the fresh solution only by making observations as soon as the substance was entirely dissolved. Even then

[1] From a dissertation presented to the Faculty of the Graduate School of Cornell University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy, by S. Alice McNulty, Holder of the Grasselli Fellowship in Chemistry at Cornell University, 1923–1924.

[2] (a) Orndorff, Gibbs and McNulty, THIS JOURNAL, **47**, 2767 (1925). (b) Orndorff and McNulty, *ibid.*, **49**, 992 (1927). (c) Orndorff, Gibbs, McNulty and Shapiro, *ibid.*, **49**, 1545 (1927).