[CONTRIBUTION FROM THE RESEARCH LABORATORY OF CORN PRODUCTS REFINING COMPANY]

Non-carbohydrate Substances in the Cereal Starches

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In a preliminary communication,[1] the author has reported the removal of fatty acids from the cereal starches by extraction with certain hydrophilic fat solvents. Recently, Evans and Briggs[2] have investigated the solvent extraction of corn starch, particularly as regards the identity of the fatty acids so liberated, and their development during ripening of the corn kernel. The present paper offers further data to indicate that the lipids of the common cereal starches are merely adsorbed on the starch. This is in contrast with the earlier views of Taylor and his co-workers,[3] who maintained that the fatty acids of the cereal starches were esterified with the alpha-amylose, contributing to the insolubility of that fraction.

The extent of removal of fatty material from corn starch depends on the nature of the solvent. Hydrocarbons or chlorinated hydrocarbons have little effect, while hydroxylated fat solvents remove much of the lipid material. This is illustrated by a series of Soxhlet extractions of corn starch with various solvents (Table I). While anhydrous dioxane gives inferior extraction, it will be noted that the constant boiling mixture of 80% dioxane with 20% water is among the most effective solvents.

Soxhlet extraction technique is limited to small quantities of material and to the use of pure solvents or constant boiling mixtures. Hence, extractions were likewise run by suspending one part of corn starch in three parts of the specified solvent, refluxing for one hour on the steam-bath with mechanical stirring, then filtering and digesting twice further in similar fashion with fresh solvent (Table II). Both dioxane and methanol were found to function more effectively when diluted with a small amount of water.

TABLE I

SOXHLET EXTRACTION OF CORN STARCH

Solvent	Hours extracted	Total material removed[a]	Fat content of extracted starch[b]
Mixed petroleum and ethyl ethers	18	0.04%	
Carbon tetrachloride	18	.04	
Ethyl acetate	18	.07	0.84%
Dioxane (anhydrous)	18	.15	.78
Isopropyl alcohol	18	.40	.72
Isoamyl alcohol	18	.42	
Methanol	18	.90	.10
Dioxane (80%)	18	.95	.06
Dioxane (80%)	3	.91	.26
Methyl cellosolve	3	.99	.18

[a] Determined by drying the extract to constant weight. Coloring matter and a portion of the protein are extracted from the starch by some of these solvents, and are included with fatty material under this heading. [b] The original raw starch analyzed 0.80% fat. All analyses for fat in starch reported in this paper were run in duplicate by the acid hydrolysis procedure,[4] and are corrected to dry starch basis. The precision of results by this method of analysis is of the order of ±0.02%. However, values tend to run slightly high, due to carry-over of dextrins by the mixed ethers used for extraction.

TABLE II

EXTRACTION OF FATTY MATERIAL FROM CORN STARCH

Solvent	Fat content of extracted starch[a]
Dioxane (anhydrous)	0.70%
Dioxane (80%)	.12
Dioxane (60%)	.34
Methanol (anhydrous)	.30
Methanol (85%)	.18
Methanol (75%)	.38
Methanol (65%)	.38

[a] By acid hydrolysis method. Original raw corn starch analyzed 0.84% fat.

The fat content of corn starch can be reduced by cold percolation with solvents, though this procedure is much less effective than hot extraction methods. Leaching with a large volume of cold 85% methanol decreased the fat content from 0.84 to 0.41%, or to 0.44% using 80% dioxane. Coloring matter is removed readily by this method.

The lipids extracted from corn starch prove to be free fatty acids, various samples ranging in acid number from 134 to 153. Exhaustive extraction with hot 85% methanol decreased the phosphorus content only slightly, from 0.017 to 0.015%. Most of this phosphorus must be esterified with the carbohydrate, since prolonged electrodialysis of an autoclaved paste of defatted corn starch at 1300 volts direct current potential merely reduced

(1) Schoch, THIS JOURNAL, **60**, 2824 (1938).

(2) Evans and Briggs, *Cereal Chemistry*, **18**, 443, 465 (1941); Evans, *ibid.*, **18**, 468 (1941).

(3) Taylor and Nelson, THIS JOURNAL, **42**, 1726 (1920); Taylor and Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926); Taylor and Werntz, THIS JOURNAL, **49**, 1584 (1927); Taylor and Walton, *ibid.*, **51**, 3431 (1929); Taylor and Sherman, *ibid.*, **55**, 258 (1933).

(4) *Assoc. Official Agr. Chem.*, Official and Tentative Methods of Analysis, 4th ed., p. 208, 1935.

the phosphorus content to 0.013%, indicating the absence of any considerable amount of inorganic phosphate.

As estimated by Kjeldahl determinations, only a portion of the total protein is extracted from corn starch by 80% dioxane or by 85% methanol (Table III). It is assumed that the remainder of the protein either is of a different type, or is heat-denatured during extraction.

TABLE III

EXTRACTION OF PROTEIN FROM CORN STARCH

Treatment	% Protein
A, Raw corn starch	0.34
B, After four digestions with 85% methanol	.24
C, Product B, after twenty-four hours of Soxhlet extraction with 80% dioxane	.21
D, Raw corn starch, after forty-eight hours Soxhlet extraction with 80% dioxane.	.26

The fatty material of rice starch can be similarly removed, but as the soap rather than the free fatty acid. It is presumed that the sample here employed was manufactured under alkaline steeping conditions.

Fat content of raw rice starch	0.59%
After seven successive digestions with 85% methanol	.03%
After forty-eight hours of Soxhlet extraction with 80% dioxane	.07%

In the case of wheat starch, the phosphorus appears to be present as a phospholipid, since both phosphorus and fat are removed concurrently (Table IV). After purification by solution in carbon tetrachloride, the extracted lipid analyzes high in phosphorus.

TABLE IV

EXTRACTION OF FATTY MATERIAL AND PHOSPHORUS FROM WHEAT STARCH

Treatment	% Fat[a]	% P[b]
Raw wheat starch, Sample A	0.50	0.054
Sample A, Soxhlet extracted forty-eight hours with 80% dioxane	.08	.008
Sample A, after seven digestions with 85% methanol	.04	...
Raw wheat starch, Sample B	.64	.059
Sample B, Soxhlet extracted forty-eight hours with 80% dioxane	.16	.022
Sample B, after seven digestions with 85% methanol	.03	...
Extracted fatty material from Sample B	...	2.25

[a] By acid hydrolysis. [b] Phosphorus was determined alkalimetrically, on the various starch samples after the dry-ashing procedure of Howk and De Turk,[5] and on the fatty extract by the A. O. A. C. method.[6] All analyses in duplicate.

These results are of particular interest since Stamberg and Bailey[7] have characterized the amylopectin fraction of wheat starch on the basis of its phosphorus content. From the above results, it appears that the phosphorus is present merely as an adsorbed phospholipid, and hence cannot be considered as truly characterizing either fraction. In contrast, prolonged Soxhlet extraction of potato starch with 80% dioxane does not materially reduce its phosphorus content.

Raw potato starch	0.095% P
After forty-eight hours of extraction with 80% dioxane	.087% P
After one hundred and twenty hours extraction with 80% dioxane	.084% P

Just as lipids can be removed by certain hydrophilic solvents, so it is also possible to impregnate starch with fatty acid by use of those same solvents. While this added fat cannot be removed by hydrocarbon solvents, it is readily extracted by hydrophilic solvents. Under similar conditions, carbon tetrachloride is inferior as a medium for impregnating starch with fatty acid:

A, 50 g. raw corn starch was suspended in 100 ml. of methanol containing 25 g. of oleic acid and evaporated to dryness on the steam-bath. The pasty mass was washed with hot xylene, then Soxhlet-extracted for twenty-four hours with carbon tetrachloride, dried and analyzed. Initial fat content of raw corn starch = 0.84%; final fat content = 2.26%.

B, Similar procedure, using defatted corn starch. Initial fat content = 0.17%; final fat content = 1.27%.

C, Similar procedure, but impregnating defatted corn starch with a 25% solution of oleic acid in carbon tetrachloride, instead of methanol. Initial fat content = 0.17%; final fat content = 0.26%.

D, Same procedure as in A, but using potato starch. Initial fat content of potato starch = negligible; final fat content = 0.77%. A portion of this fatted potato starch was Soxhlet-extracted for an additional twenty-four hours with carbon tetrachloride, thereafter analyzing 0.81% fat. A second portion was similarly extracted, but with 80% dioxane, which reduced the fat content to 0.04%.

The concept of a carbohydrate fatty ester is considered untenable. The selective removal of lipids by certain solvents cannot be attributed to ester exchange, since the free fatty acid or the soap is isolated. Also, no such mechanism could be formulated for dioxane. To ascertain whether a simple sugar fatty ester might decompose under the conditions of starch extraction, glucose penta-

(5) Howk and De Turk, *Ind. Eng. Chem., Anal. Ed.*, **4**, 111 (1932).

(6) *Assoc. Official Agr. Chem.*, Official and Tentative Methods of Analysis, 4th ed., p. 455, 1935.

(7) Stamberg and Bailey, *Cereal Chemistry*, **16**, 309, 319 (1939).

palmitate was refluxed for forty-eight hours with methanol and with 80% dioxane. In each case, the sugar ester was recovered quantitatively and unchanged. To clarify further the specific action of hydrophilic solvents in removing fatty material, oven-dried samples of corn starch were confined in closed containers over various solvents until constant weight was attained. The increase in weight of the starch due to solvent–vapor adsorption is directly related to the hydrophilic nature of the solvent, and hence is interpreted as a measure of solvent adsorption into the granule (Table V).

TABLE V

ADSORPTION OF VARIOUS SOLVENT VAPORS BY DRIED CORN STARCH

Solvent	Gain in weight, %
Benzene	0.6
Xylene	0.9
Carbon tetrachloride	1.1
Ethyl acetate	1.6
Dioxane (anhydrous)	3.3
Acetone	6.4
Methanol	19.6
Methyl cellosolve	20.6
Water	25.8

The lipids of the cereal starches appear to be distributed through the granule, loosely bound to the carbohydrate by polar adsorption, as suggested by Lehrman.[8] These lipids can only be displaced by fat solvents which can penetrate into the granule and adsorb preferentially on the starch by virtue of their hydrophilic loading. With dioxane and methanol, the hydrophilic qualities are markedly enhanced by the addition of a small amount of water.

The defatted cereal starches give clearer pastes possessing more pronounced gelling qualities than the original raw starches. The alkali lability is not increased by removal of lipid material. With corn starch, the apparent alkali number[9] drops slightly (*e. g.*, from 12.1 to 10.7), due to removal of fatty acid and protein, which consume a small amount of alkali in this test. Extraction of fatty acid raises the *p*H of corn starch to 6.0, within the range of minimum hydrolysis at elevated temperatures.[9] While raw corn starch pastes undergo slight hydrolysis during autoclaving, due to the acidity imparted by the presence of free fatty acid, no such effect is noted with pastes of defatted corn starch. At concentrations of 1–3%, defatted corn starch pastes may be dispersed by autoclaving for two hours at 19 pounds pressure, without any rise in the alkali number. The presence of lipid material appears to promote retrogradation of the cereal starches, and for this reason may interfere with enzymatic conversions. To avoid these effects, and for purposes of purification, it is the general practice in this Laboratory to defat all cereal starches intended for fundamental investigations. Recommended procedure is as follows: one part of starch is suspended in three parts of 85% (by volume) methanol, refluxed for several hours on the steam-bath with adequate stirring, filtered hot, then resuspended in fresh 85% methanol and extracted four times further in similar fashion. Five such extractions are sufficient to reduce the fat content of corn, wheat and rice starches to the vanishing point.

(8) Lehrman, THIS JOURNAL, **61**, 212 (1939); Lehrman has recently shown [*ibid.*, **64**, 2144 (1942)] that the adsorption of palmitic acid from methanol solution by potato starch or defatted corn starch follows a Freundlich isotherm, and that this adsorbed fatty acid is not removed by extraction with carbon tetrachloride.

(9) Schoch and Jensen, *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).

Summary

The fatty acids of corn starch are removed without hydrolytic degradation by extraction with suitable hydrophilic fat solvents. Similarly, the fatty material can be removed from rice starch as the soap and from wheat starch as a phospholipid, together with substantially all the phosphorus of the original raw wheat starch. Consequently, these lipids cannot characterize any amylose fraction, but must be considered as natural impurities adsorbed on the starch. By the use of such hydrophilic fat solvents, corn starch or potato starch can be impregnated with fatty acid, and this added fat can only be removed by the same type of solvent used to introduce it.

Prior to use for any fundamental studies, it is recommended that the common cereal starches be purified of such lipid material by successive extractions with hot 85% methanol.

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