Starch-Complexed Lipids: Differences in Extraction with Various Solvents[1]

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ABSTRACT

The yield and composition of various starch-complexed lipids depend on the choice of solvent used in the extraction. This was observed when the lipids present in starch were quantitated by two different extraction techniques, in one of which carbon tetrachloride was used and in the other, ether. Fractionation on silicic acid columns by the technique of Hirsch and Ahrens (J. Biol. Chem. 233: 311; 1958) has shown additional differences in the components of the extracted lipids. These may be sufficient to account for the discrepancies that appear when starch-complexed lipids are quantitated. This is substantiated by silicic-acid chromatography of both the phospholipid and the glyceride fractions. Marked differences in nature of the lipid material were observed, indicating possible dependence on the polarity of the extracting solvent.

A previous article from this laboratory described a technique whereby acid-hydrolyzed starch lipids were extracted in an ethyl ether system (1). In the course of other laboratory studies, quantitative differences appeared when other solvents such as carbon tetrachloride were used (2). Indeed, other investigators using silica gel chromatography have noted that various extracted lipids show a dependence on particular, selected solvent-extraction techniques (3). Moreover, some degree of confusion as to the exact nature of these components of the starch granule has been reported (4). These lipids can profoundly affect the further reactivity of the starch (5). In this respect, Medcalf et al. (6), in an excellent series of experiments, have shown that the polarity of starch lipids can alter the pasting properties of starch. It is the purpose of this paper to describe the various lipid components that appear in either a carbon tetrachloride (2) or an ether extraction (1,7) and to show that the presence of these is dependent on the solvent used.

MATERIALS AND METHODS

The technique of Hirsch and Ahrens for separation of lipid complexes with the use of silicic acid column chromatography (8) has been used frequently in the quantitation of lipid mixtures (9,10). We have used this technique to study those lipids present in starch which in themselves have led to some discrepancies in the literature (4,11).

Lipids were extracted from three kinds of commercial starches (corn, wheat, tapioca) by two techniques (1,2). Results are given in Table I. Aliquots of each of the extracts, after being dried under vacuum at 100°C. for 1 hr., cooled to room temperature, and reconstituted in petroleum ether (b.p. 60° to 110°C., Baker), were separated with the use of silicic acid (Mallinckrodt) column chromatography (8).

To study the phospholipid fractions (see Figs. 1,2, and 3), the following two procedures were employed:

1. Lipids were extracted from the acid-hydrolyzed starch with ether and separately with carbon tetrachloride. The lipids from each extract were then fraction-

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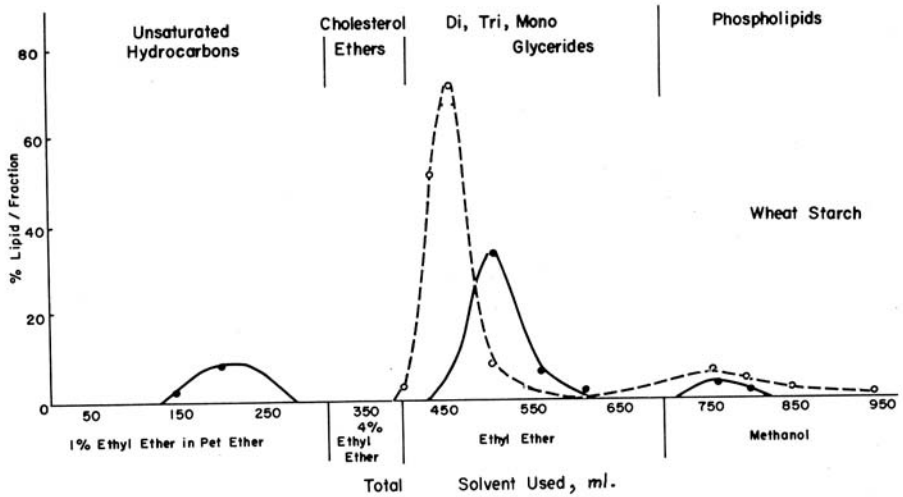

Fig. 1. Separation of extracted lipid constituents from acid-hydrolyzed wheat starch (two separate solvent systems); by elution from a 15-g. column of silicic acid, 1.2 cm. in diameter, 33 cm. long; column temperature maintained below that of room temperature (see ref. 8). Results are from two separate determinations. Solid line, anhydrous ethyl ether; broken line, carbon tetrachloride.

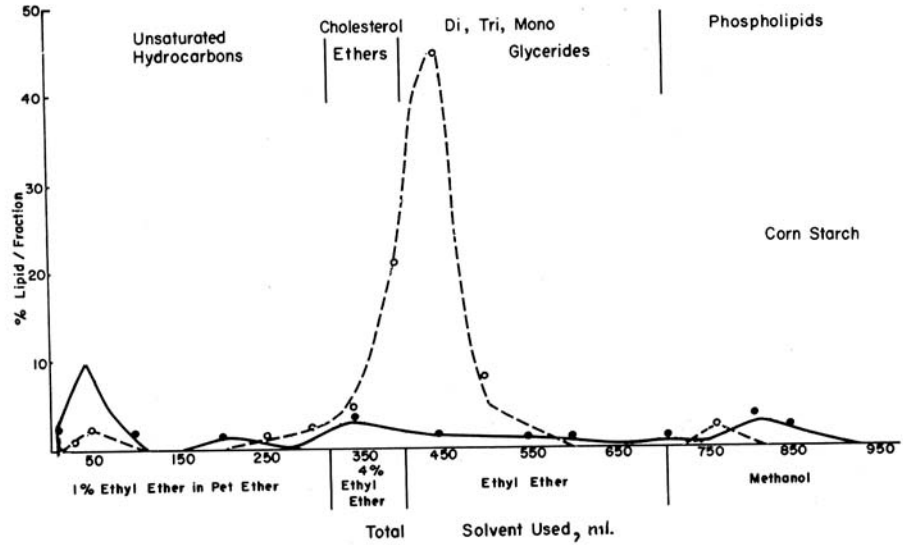

Fig. 2. Separation of two solvent-extracted lipid constituents of acid-hydrolyzed corn starch under the same conditions as those described in Fig. 1.

ated as described (8). The phosphatide fraction from each was then re-chromatogramed on Mallinckrodt silicic acid chromatographic glass fiber sheets ("ChromAR 1000" brand); the solvent used was 20% absolute methanol in chloroform at room temperature for a running time of 15 min. in descending direction.

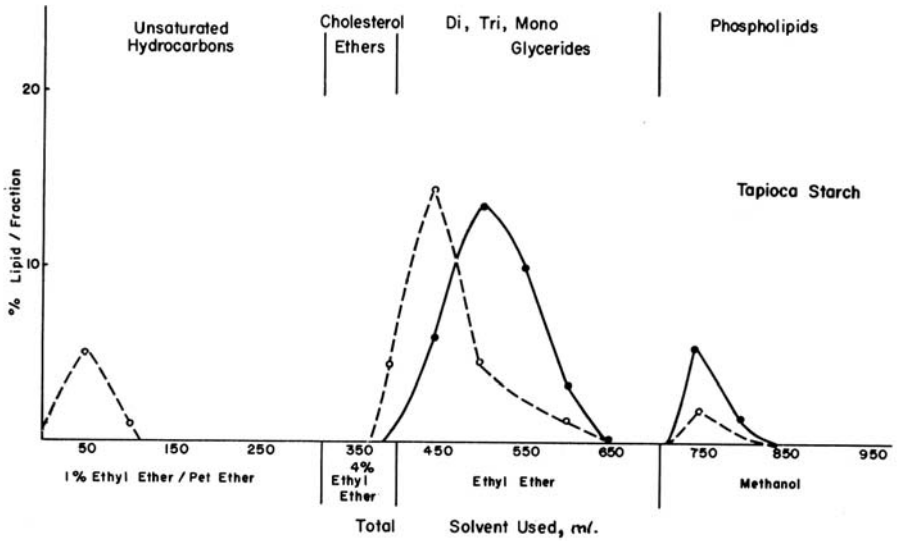

Fig. 3. Separation of two solvent-extracted lipid constituents of acid-hydrolyzed tapioca starch under the same conditions as those described in Fig. 1.

Various detecting reagents were studied. The components of the chromatograms were best visualized with use of Hanes-Isherwood reagent on one side and p-anisidine on the reverse (7).

2. For comparison to naturally occurring phosphatides (see "Discussion"), the starch was extracted three times with frequent agitation in 1-butanol (Baker); each time, three separate portions of 100, 50, and 50 ml. were used for a total of 6 hr. (2 hr. for each extraction), followed by drying at 100°C. for 1 hr. under vacuum. The dried lipids were separated on silicic acid columns and were then chromatogramed as described for the acid-hydrolyzed materials; silicic acid sheets were used.

The glyceride fraction from the carbon tetrachloride and ether extracts of the three starches (as noted in Figs. 1 to 3), was rechromatogramed on the Mallinckrodt silicic acid sheets with a running time of 2 hr., in a solvent consisting of 4% ethyl ether in petroleum ether (b.p. 60° to 100°C.). The components of the chromatogram were detected with the Hanes-Isherwood reagent.

RESULTS

In Table I, yields for the lipids extracted with carbon tetrachloride are higher than those obtained with an ether system. In addition, silica gel chromatography (12) indicated that the lipids from acid hydrolysis of starch showed various patterns, depending on the choice of extracting solvent. Furthermore, we had seen that those fatty materials obtained with an ether system were lower in phosphorus (13)

[2] The nomenclature for the vertical axis in Figs. 1, 2, and 3 is explained by the following equation:

$$\text{Percent lipid/fraction} = \frac{\text{weight of solute (lipid solids) in 50 ml. eluting solvent (e.g., ethyl ether)}}{\text{total weight of solute recovered (load weight of lipid material)}}$$

than those extracted with carbon tetrachloride. These results and observations led to the use of silicic acid separation techniques.

The stepwise elution of three major classes of fatty acid esters in the starch-lipid extracts can be observed in Figs. 1,2, and 3^2. These represent respectively cholesterol esters, triglycerides, and phospholipids. Further examination has revealed that smaller fractions are present, composed of nonesterified fatty acids and partial glycerides. These fractions are being studied further.

With respect to Fig. 1, it appeared that the use of carbon tetrachloride extracted more of the mono, di-, and triglycerides associated with wheat starch lipid material than did the use of ether. However, interestingly enough, lipids extracted by carbon tetrachloride do not account for various unsaturated hydrocarbons known to be present, but the extraction accentuates the presence of phospholipids. Regardless of the solvent used, cholesterol esters appear, thus far, to be absent from the wheat starch lipid extracts.

Corn starch presents a more complex pattern than does wheat. From Fig. 2 it is apparent that carbon tetrachloride extracts predominantly glycerides plus fatty acids but does not extract other classes. Further analysis indicated that cholesterol esters are present in corn starch lipid material to a greater extent than in wheat starch lipid material.

Figure 3 illustrates a typical pattern for a root starch, tapioca. Noteworthy here has been the relative simplicity of the pattern: unsaturated hydrocarbons, glycerides, and phospholipids are obtained when carbon tetrachloride is used; ether systems elute only the latter two groups. Further indentification appears to indicate that cholesterol esters are absent in these extracts.

Figure 4 illustrates the chromatographic results obtained when the phospholipids from two separate solvent extractions of acid-hydrolyzed starch (ether and carbon tetrachloride) are further separated on Mallinckrodt silicic acid sheets. Observe that phospholipids which are natural in origin are absent in the acid-hydrolyzed preparation. However, butanol extracts show the presence of these in the total lipid fraction for corn starch. At this point in our studies, this phospholipid appears to be lysolecithin. In the illustration, this observation has been tentatively applied to wheat and tapioca starches. This is being confirmed in our laboratory, specifically by the use of various detecting reagents.

Figure 5 indicates the pattern that results when the glyceride fraction (see Figs. 1 to 3) is separated further. Interestingly, the fractions from both extracting systems differed in the type of glyceride present. This is a reflection of both the inherent differences in these systems and the polarity of the extracted materials involved. As was observed by chromatograms of the controls, of which three are shown, neither the carbon tetrachloride nor the ether extracts showed any di- or triglycerides, but rather monoglycerides and free fatty acids.

DISCUSSION

For wheat starch, Medcalf et al. have shown that the amount of lipid that can be extracted will vary according to the solvent used (6) and, equally important, that the polarity of these lipids differs (14). Our results are in agreement with these findings and suggest further that the lipid moieties are distributed among certain specific classes of fatty esters. Thus, wheat starch lipids are composed primarily of unsaturated hydrocarbons and glycerides. Qualitatively, it appeared that the more

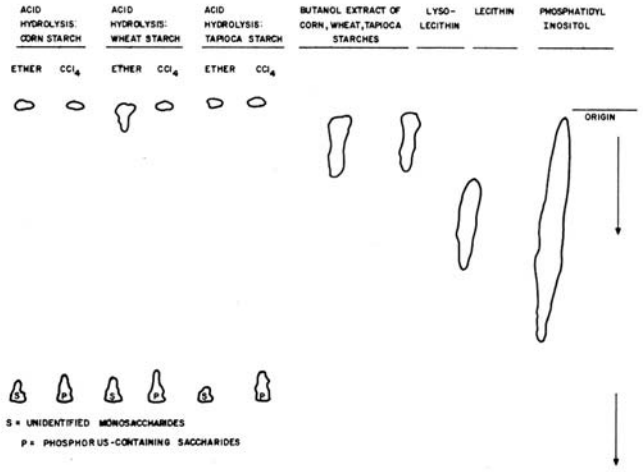

Fig. 4. Silicic-acid thin-layer chromatogram of the phospholipid fraction previously obtained (by the technique in ref. 8) from three starch species. Descending chromatogram on Mallinckrodt silicic acid sheets with 20% absolute methanol in chloroform, running time of 15 min. at room temperature; Hanes-Isherwood reagent was used for visualization.

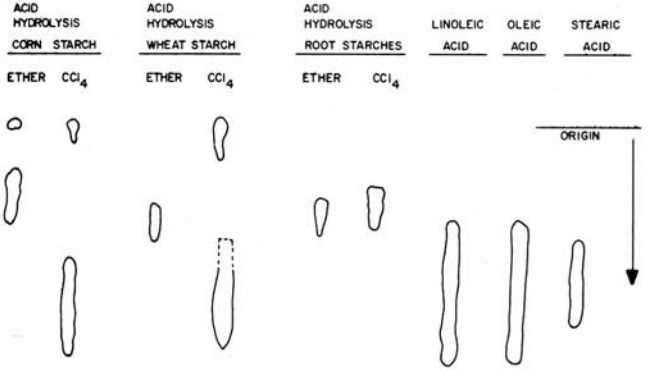

Fig. 5. Silicic-acid thin-layer chromatograms of the glyceride fraction, previously obtained (by the technique in ref. 8) from three starch species; the same media were used as for Fig. 4. Running time of 2 hr. in descending direction; 4% anhydrous ethyl ether in petroleum ether (b.p. 60° – 110° C.) at room temperature; Hanes-Isherwood reagent was used for visualization.

polar the solvent, the more polar are the lipids extracted. Should phospholipids be present, acid hydrolysis would result in extraction of fewer polar compounds. Under the conditions described, however, phospholipids appeared to play a minor quantitative role in the final determination. There was a tendency to produce more polar lipids with use of acid hydrolysis as compared to alcohol extractions. The susceptibility of starch-complexed lipids for particular solvent extraction techniques may explain some of the confusion in the literature (4,11,15). The studies of Medcalf et al. (6) and Youngs et al. (14) concerning the relation between lipid polarity and starch pasting properties become quite consistent, therefore. Specifically, acid hydrolysis would release highly polar groups and would effect hydrogen

bonding. Extraction by solvents with varying polarity (e.g., carbon tetrachloride vs. ether) could influence the actual determination. The findings of Acker and Schmitz (11), which are in agreement with ours regarding the presence of lysolecithin in wheat starch, may be due to the solvent used in the extraction, rather than the specific chromatographic technique employed. Indeed, the presence of polar lipid material, as yet of unknown composition, which appears to be dependent on solvent systems used to extract it, must not be overlooked. Such materials have been described previously for various naturally occurring oils (16).

TABLE I. EFFECT OF SOLVENT (TECHNIQUE) ON ROUTINE LIPID ANALYSES OF STARCHES[a]

Starch	Lipid determined	
	Ethyl ether system (ref. 1) %	Carbon tetrachloride (CIRF std. procedure) %
Wheat	0.480	0.790
Corn	0.350	0.629
Tapioca	0.130	0.250

[a] Results are duplicates for several samples and were collected over a 30-day period.

Corn starch lipids extracted with carbon tetrachloride (Fig. 2) appear to have a predominance of glycerides. Relatively few of these materials (on a percentage basis) appear in an ether extraction. This is reflected in Table I, which shows that on a weight basis, the use of carbon tetrachloride yields a higher final value (0.790 vs. 0.480%) than does the use of ether. This has been confirmed in our laboratory for several species of starch, and the data have been supplemented by silica gel chromatography of the extracted lipid material itself. Initial data showed that carbon tetrachloride (2) is capable of extracting weakly polar or even nonpolar carbohydrate groupings from the starch itself. Although not yet identified, these appear in the final gravimetric determination. In this respect, anhydrous ether systems show the presence of low-molecular-weight carbohydrate groups, probably monosaccharides, in a loose complex with the lipids, which are somewhat polar in nature. Furthermore, ether systems show a decreased tendency to extract very highly polar materials of which phospholipids are a major component.

Tapioca starch (Fig. 3) and root starches in general appear to have three distinct classes of ester groups composing the starch-lipid complex. Esters of the cholesterol type and other quantitatively smaller, minor groups appear to be absent. Both saturated and unsaturated hydrocarbons are accentuated. Their presence may be sufficient to account for the differences that appear in the table, especially when carbon tetrachloride extraction yielded components other than lipids alone. The definite presence of phospholipids for this species of starch correlated with the presence of phosphorus in root starches in general. The positioning of this ion with the amylopectin fraction as described by Holzl et al. (17) remains to be clarified, since specific use of alcohol systems may influence solvent affinity (and therefore extraction) of the phospholipids.

Figure 4 illustrates the chromatographic patterns that resulted when the phos-

pholipid from the acid-hydrolyzed material was studied. When Hanes-Isherwood reagent was used, neither the ether nor the carbon tetrachloride extract yielded structurally natural phospholipids; they did yield phosphorus-containing glycerides. Indeed, the presence of phosphorus was four times as great in carbon tetrachloride extracts as in ether extracts. When unhydrolyzed butanol-extracted starch was subjected to the same separation techniques, naturally occurring phospholipids appeared. Since this technique will not degrade these materials (11), it is assumed that acid hydrolysis did cleave the phospholipids; hence their absence in both carbon tetrachloride and ether extracts. However, these cleaved lipids are not equally susceptible to various solvent extractions, and the amount extracted will vary according to the polarity of the solvent.

Figure 5 points out additional differences which occur in both extraction systems. The qualitative differences which appeared on silicic acid chromatograms of the glyceride moieties would further influence final determined values. This would be expected when the polarity of the extracting solvent is considered.

While the technique was being refined, the reverse side of the chromatograms was examined for sugars with use of p-anisidine. Small quantities of keto sugars which appear to be complexed to lipids were present only in the ether extracts of acid-hydrolyzed starch. Once again, this points out the dependence of starch lipid extractions on the solvent system. It could well be that a low-molecular-weight, atypical carbohydrate fraction which does not interfere with the final determined value exists in close proximal relation to starch-complexed lipids. Its extraction could depend on the polarity of the extracting system. Studies are now in progress to clarify many of these points, especially concerning the true nature of starch lipids.

Acknowledgment

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