

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

Fractionation of Starch by Selective Precipitation with Butanol

BY THOMAS JOHN SCHOCH

Introduction

The existence of several component amyloses in the common cereal and tuber starches has long been presumed. However, the literature records little agreement on the methods for isolating these components, or on their individual chemical and physical characteristics and relative proportion in the native starch. Most of this confusion can be traced to four principal sources of error: 1, incomplete dispersion of the starch prior to separation studies, thus including swollen and partially fragmented granules in the insoluble fraction; 2, slow separation methods, as by prolonged leaching or electrophoresis, permitting the starch to "retrograde" to less soluble form; 3, hydrolytic changes suffered either preliminary to or during the fractionation, as the use of starch solubilized by ball-milling or by alcoholic acid treatment, or the application of doubtful enzymatic methods to effect a separation; 4, interference by non-carbohydrate impurities, especially by fatty acid in the case of corn starch.

Based on the selective precipitant action of normal butyl alcohol toward starch sols,¹ a new technique of fractionation has been developed which avoids the above difficulties and which yields products² of markedly different physical and chemical characteristics. It has not as yet been established whether this mode of separation isolates two specific amyloses existing in the starch, or whether it fractionates a graded series of starch components. However, it is hoped that the methods here presented will help to clarify the problem of starch structure, by affording two chemically different fractions which may be studied individually and by comparison with one another. The fractionation involves four separate steps: (1) dispersion of the starch without attendant hydrolysis by autoclaving within carefully controlled pH limits, (2) selective precipitation of one fraction under conditions which

prevent retrogradation, presumably through complex formation with the precipitating agent, (3) separation of this product by supercentrifuging, and (4) a system of purification of this fraction by washing or by reprecipitation. The specific application of these methods to corn starch, potato starch and waxy maize will be considered in turn.

Corn Starch

Removal of Fatty Material.—In a previous publication,³ the use of defatted corn starch has been advised for all investigations of a fundamental character, and a method of purification has been outlined, consisting of five successive extractions with hot 85% methanol. This defatted starch is preferred as starting material for fractionation studies, since its pH is stabilized at 5.9–6.0 permitting autoclaving without glucosidic hydrolysis. While raw starch can be used, the lower pH (5.0–5.3) prohibits autoclaving and the presence of fatty acid interferes with the fractionation.

Dispersion of the Starch.—Regardless of whether the several fractions of starch are uniformly distributed throughout the granule, or whether one fraction constitutes an outer envelope, it is imperative to effect optimum dispersion of the granule structure preliminary to any fractionation study. In the opinion of the author, autoclaving affords the nearest approach to ideal dispersion, provided that hydrolytic breakdown is prevented by rigid maintenance of the pH between 5.9–6.3.⁴ If a raw corn starch paste is adjusted within these limits by means of sodium hydroxide, the pH will drift badly during autoclaving. If sufficient buffer salts are added to maintain a stable pH, the presence of this electrolyte interferes with subsequent flocculation of the butanol precipitated fraction. Fortunately, the pH of defatted corn starch stabilizes at 5.9–6.0 during the entire separation. Presence of butanol during the autoclaving aids the breakdown of granule structure, by markedly lowering the gelatinization temperature of the starch, possibly a surface tension effect. A mixture of 14 liters of water and 2 liters of butanol in a 5-gallon Pyrex stock bottle is heated to boiling on the steam-bath, and a suspension of 150–450 g. (preferably 300 g.) of defatted corn starch in 1 l. of water is slowly added with vigorous mechanical stirring (Flow Sheet). This amount of butanol is considerably in excess of the 8% required to saturate the aqueous phase, to provide for loss during autoclaving. Also, it is desirable to have a supernatant layer of excess butanol present during the entire separation, to prevent the formation of insoluble "skins" by surface evaporation. The 1–3% paste is then autoclaved for two to three hours at 18–20 lb. pressure. Tests have shown that the viscosity drops to a minimum (indicative of granule disinte-

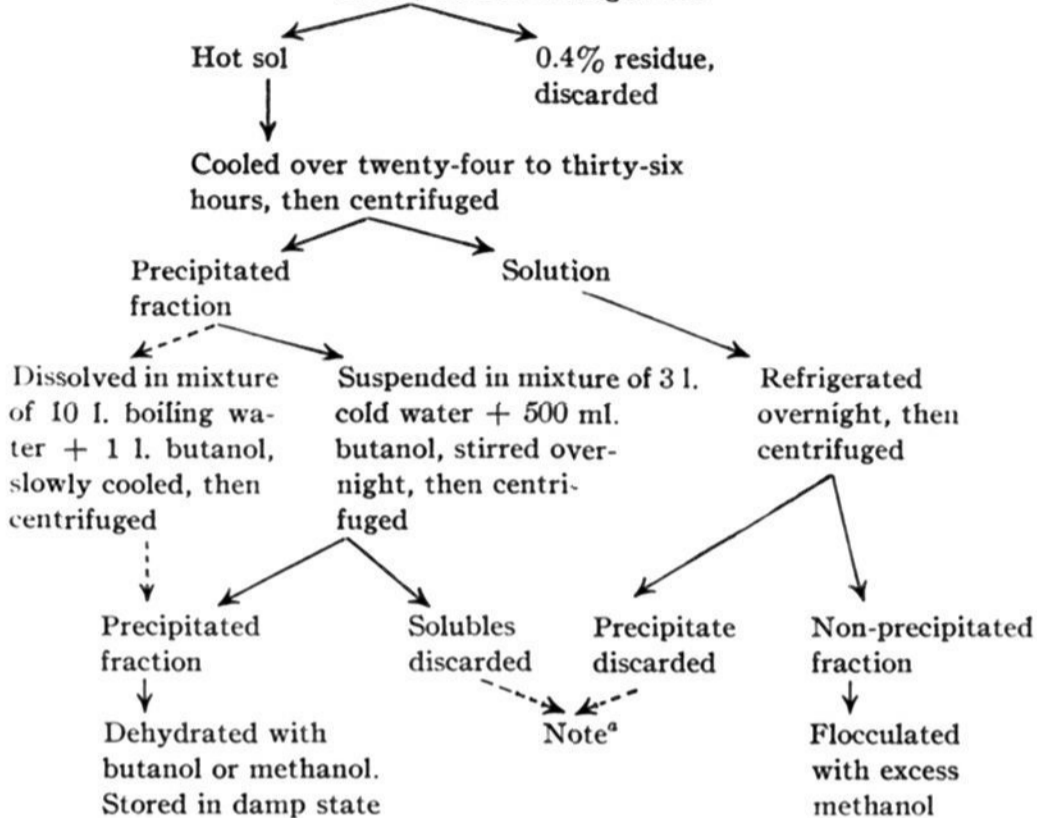
(1) Schoch, *Cereal Chem.*, **18**, 121 (1941).

(2) The author prefers not to identify these fractions as alpha and beta amyloses, nor as amylose and amylopectin, since these terms have been so confused by indiscriminate usage. Until a more exact nomenclature can be devised, based on the identification of specific configurational differences between these fractions, they will be designated merely as the "butanol precipitated fraction" and the "butanol non-precipitated fraction."

(3) Schoch, *THIS JOURNAL*, **64**, 2954 (1942).

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

FLOW SHEET.—FRACTIONATION OF CORN STARCH: 150–450 g. of defatted corn starch pasted in a mixture of 15 l. of water + 2 l. of butanol, autoclaved two hours, then centrifuged hot



Dotted lines indicate alternative procedures.

^a Soluble and precipitated residues are usually slight in amount and may be discarded. Where total recovery is desired, they may be combined at this point, dissolved by autoclaving, then reprecipitated and separated.

gration) in one to one and one-half hours under these conditions. If maximum purity of product is desired, the hot starch sol may be passed through the continuous supercentrifuge at this point, removing impurities and incompletely dispersed starch, totalling approximately 0.4% of the original starch. This insoluble residue is dark in color and may analyze as high as 10% ash. It appears to represent an irreducible minimum of undispersed material.

As an alternative but somewhat less effective method of dispersion, the butanol–water–starch paste may be boiled

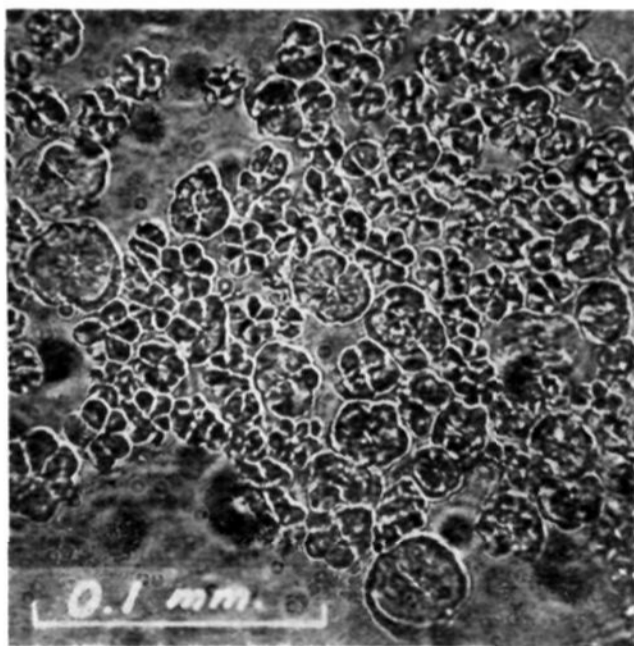


Fig. 1.—Butanol precipitate from corn starch.

under reflux for five to six hours with vigorous mechanical agitation. This digestion time exceeds that required to reduce the viscosity to a minimum. The hot sol is then passed through the supercentrifuge, removing 1–3% of undispersed material. While this method is not recommended, it yields products similar in properties and amount to those obtained from autoclaved pastes, thus justifying use of the more efficient autoclave methods for dispersing the starch.

Flocculation and Separation.—The hot starch sol, by whichever method prepared, is cooled slowly and without agitation to room temperature over a period of twenty-four to thirty-six hours. This can be accomplished by insulating the container with a thick wrapping of cloth. A “crystalline” floc forms in the neighborhood of 50°, usually as more or less perfect six-segmented spherulites, 15–50 microns in diameter (Fig. 1). Separation is preferably effected by means of a Sharples continuous supercentrifuge, fitted with a clarifier bowl, operating at 50,000 r. p. m., and capable of passing 20 l. of liquid per hour. The precipitated fraction is deposited in the rotor

of the centrifuge as a stiff white cream. When operating satisfactorily, the centrifugate should be optically void under the polarizing microscope.

The Precipitated Fraction.—These spherocrystalline formations are birefringent under polarized light, giving an interference pattern somewhat similar to the familiar “Maltese Cross” of native starches. If lightly stained with a solution of iodine in butanol, they become dichroic under crossed nicols. The crystalline form is not disturbed by treatment with cold water saturated with butanol. However, if treated with cold water alone, the spherulites instantly fracture and swell. The product as removed from the bowl of the centrifuge may be dehydrated with methanol or butanol without altering its character, provided that the alcohol is not permitted to dry off. If the butanol precipitated fraction from corn starch is thoroughly dried, the spherulites lose all birefringence, though retaining their outward form. While this product swells in hot or cold water, it does not dissolve. The damp product as removed from the centrifuge, or after dehydration with methanol or butanol, is readily soluble in boiling water, either in the presence or absence of butanol. Relatively clear solutions can be prepared at concentrations as high as 10–15% solids. These are highly unstable, showing exaggerated retrogradation tendencies and forming insoluble skins with great facility. Even at dry solids concentrations as low as 1.5%, solutions of the precipitated fraction will set to hard irreversible gels on cooling to room temperature. At concentrations below 0.5%, solutions are reasonably stable at room temperature, though they eventually retrograde on standing. From its mode of prepara-

TABLE I
BUTANOL FRACTIONATION OF CORN STARCH

Batch	Method	% Yield of precipitated fraction	Alkali no. of precipitated	of fractions Non-precipitated
CA	Single precipitation from autoclaved 1.5% defatted corn starch paste	25		
CB	Similar	29		
CC	Similar	27		
CD	Similar, but at 3% concentration	28		
CE	Precipitated from 1.5% autoclaved defatted starch paste, then dissolved and reprecipitated	21	31.3	8.3
CF	Similar to Batch CE	23	29.5	6.1
CG	Similar to Batch CE, but butanol precipitate was dissolved and re-autoclaved before second precipitation	21		
CJ	Precipitated from 1.5% boiled paste of defatted corn starch, then dissolved and reprecipitated	23	21.9	
CM	Single precipitation from 1.5% boiled paste of defatted corn starch	22.8		
CP	Precipitated from 1.5% boiled paste of raw corn starch, then dissolved and reprecipitated	16	23.6	5.5
CQ	Similar to Batch CP	13	22.7	5.3
CS	Similar to Batch CE	22.4	24.4	4.7
CU	Precipitated from 1.5% boiled paste of defatted corn starch, then washed with butanol-water mixture	20.3	23.2	5.1
CV	Precipitated from 1.5% autoclaved defatted starch paste, then washed with butanol-water mixture	23.6		
CW	Similar to Batch CV, but at 2% concentration	22.2		5.7
CX	Similar to Batch CV, but at 3% concentration	22.8		5.9
CY	Similar to Batch CV, but at 1% concentration	23.3		5.8

tion and solubility behavior, it is suggested that the spherulites may represent a crystalline addition compound between butanol and a specific starch fraction. This product is stable in the presence of excess butanol, but reverts to insoluble form on removal of the latter, whether by treatment with cold water or by drying.

The reason for the selective precipitating action of normal butyl and isoamyl alcohols is obscure, possibly depending on some undefined optimum of molecular volume or "hydrophil balance." No satisfactory separation could be effected with lower alcohols, while octyl alcohol or cyclohexanol precipitated all the starch substance indiscriminately.

After removal of the butanol precipitated fraction, the centrifugate gives no further precipitate, either on long standing, or on refrigeration, or after a second autoclaving. The non-precipitated fraction can be obtained readily by the usual practice of flocculating with excess methanol and triturating with fresh portions of methanol until dehydrated.

Purification.—Either of two methods may be used for purifying the butanol precipitated fraction. The product may be merely suspended in water previously saturated with butanol, thoroughly stirred, then centrifuged. In most cases, this simple washing procedure is adequate. For more thorough purification, the precipitated fraction may be "recrystallized." The moist product as removed from the centrifuge (representing 30–100 g. of dry substance) is slowly added with vigorous agitation to a boiling mixture of 10 l. of water and 1 l. of butanol. On slow cooling, the butanol precipitated fraction flocculates out in minute particles, possibly 1–2 microns in diameter. Efforts to increase the size of these crystals by various cooling procedures have not been successful. However, the

product is readily centrifuged, and recovery is 90–95%. This reprecipitated product is dehydrated by suspending in butanol, then filtered on a Büchner and bottled as a damp product, containing 40–50% dry solids. The yields of butanol precipitated fraction from corn starch have averaged 22% (on dry starch basis), under various conditions of preparation, isolation and purification (Table I).

Properties of the Corn Starch Fractions.—After thorough drying, the non-precipitated fraction from corn starch is soluble in cold water to the extent of 4–5%, and in hot water to 10–12%. When hot 10% solutions are cooled down, they give pasty gels which are readily liquefied by heating. Solutions show no tendency to retrograde on long standing, even at refrigerator temperatures. This behavior suggests that the non-precipitated fraction constitutes the more stable and colloiddally soluble portion of the starch. In contrast, the butanol precipitated fraction might be considered as responsible for the gelation and retrogradation tendencies of corn starch.

An outstanding chemical difference between the butanol separated fractions lies in their respective alkali liabilities. Various samples of the precipitated fraction average an alkali number⁴ of 25, much higher than the original defatted corn starch. The non-precipitated fraction is correspondingly lower, averaging 5.6. Calculating the composite alkali number of the original defatted starch from these average values and from the proportion of each fraction in the starch, an estimated value of 10.0 is obtained, in good agreement with the observed alkali number of 11.0. This is taken as evidence that fractions of high and low alkali liability exist as such in the original starch, and are not produced by some obscure hydrolytic change during the process of fractionation.

It is believed that the alkali number is an index of ter-

minal aldehyde content. However, in view of the possible presence of branched chain configurations in starch, no direct relationship can be deduced between alkali number and molecular weight. In this connection, Bear⁵ has recently reported that the butanol precipitated fraction gives a V-type X-ray pattern, while that of the non-precipitated fraction is relatively diffuse and amorphous. On the basis of the degree of orientation during flow, Rundle and Baldwin⁶ suggest that the precipitated fraction consists of the unbranched component of the starch, while the non-precipitated fraction is composed of branched material.

The Ostwald viscosity of the two fractions can be compared by dissolving the dried products in cold 0.4 *N* sodium hydroxide solution. In this medium, the non-precipitated fraction has been slightly but consistently more viscous than the butanol precipitated material.

Behavior toward butanol must be considered as a specific difference between the fractions. For example, after alkali number determinations on the individual fractions (involving extensive degradation in hot aqueous alkali), if butanol is added to the cooled and neutralized solutions, the butanol precipitated fraction gives an immediate crystalline floc. The non-precipitated fraction remains clear, even on refrigeration.

Pacsu and Mullen⁷ have recently reported an exceedingly interesting method of starch fractionation by selective adsorption on cotton. The butanol precipitated fraction possesses a strong affinity for cellulose, while the non-precipitated fraction is not adsorbed. While not necessarily identical, the two methods of starch separation appear to be very similar, not only in the nature of the fractions produced, but likewise in the adsorption mechanisms involved. A comparative study of the two methods is now in progress.

Periodic acid does not distinguish between the butanol



Fig. 2.—Butanol precipitate from potato starch, stained with iodine.

(5) Bear, *THIS JOURNAL*, **64**, 1388 (1942).

(6) Rundle and Baldwin, *ibid.*, to be published.

(7) Pacsu and Mullen, *ibid.*, **63**, 1168 (1941).

separated fractions, since both oxidize at the same rate and with the same total consumption of oxidizing agent.

Phosphorus does not appear to have any very significant function in characterizing the corn starch fractions. The non-precipitated portion can be separated into sub-fractions of high and low phosphorus content by electromigration of a 1.5% solution at 1300 volts direct current potential. These sub-fractions do not differ materially in solubility behavior or in alkali number (Table II).

TABLE II

DISTRIBUTION OF PHOSPHORUS IN THE CORN STARCH FRACTIONS

Sample	Alkali no.	% Phosphorus ^a
Defatted corn starch	11.0	0.0153
Dried butanol precipitated fraction, extracted with hot water	23.2	.0088
Non-precipitated fraction	5.1	.0107
Migrated sub-fraction (77% recovery from non-precipitated fraction)	5.2	.0116
Non-migrating sub-fraction (20% recovery from non-precipitated fraction)	5.0	.0029

^a Phosphorus determined by the method of Howk and De Turk, *Ind. Eng. Chem., Anal. Ed.*, **4**, 111 (1932).

Potato Starch

Fractionation of potato starch is accomplished in much the same manner as described for corn starch. Obviously, defatting is unnecessary. Samples of potato starch employed in this investigation tested 6.0 pH and no adjustment was required. The concentration of the autoclaved paste should not exceed 2%. Centrifugal clarification of the hot autoclaved sol may be omitted, since undispersed material runs less than 0.1%.

The yields of butanol precipitated fraction from potato starch averaged 22%, identical in amount with that from corn starch. However, several pronounced differences have been noted between the butanol precipitated fractions from corn starch and from potato starch. The latter separates either as large, well-formed, six-petalled rosetts, 50–80 microns in diameter (Fig. 2), or as clumps of hair-like needles. After thorough drying, the precipitated fraction from potato starch remains relatively water-soluble, with little tendency to gel or retrograde. Its alkali number averages 11.

TABLE III

BUTANOL FRACTIONATION OF POTATO STARCH

Batch	Method	% Yield of precipitated fraction	Alkali no. of precipitated	Alkali no. of non-precipitated
PA	Slow precipitation from 1.5% boiled starch paste, washed with butanol-water	21.3	10.6	5.9
PB	Slow precipitation from 3% autoclaved paste, then washed with butanol-water	24.6	10.1	5.0
PC	Slow precipitation from 1.5% autoclaved paste, then dissolved and reprecipitated	21.0	11.5	6.6

The phosphorus content of potato starch is located principally in the non-precipitated fraction. Unlike corn starch, this fraction migrates completely in an electrophoretic cell, retaining substantially all its phosphorus.

TABLE IV

DISTRIBUTION OF PHOSPHORUS IN THE POTATO STARCH FRACTIONS

	Alkali no.	% Phosphorus
Original potato starch	7.5	0.072
Butanol precipitated fraction	11.5	.0103
Non-precipitated fraction	6.6	.079
Non-precipitated fraction, electromi- grated (98.6% recovery)	6.4	.072

These results correct earlier observations by Taylor and Schoch.⁸ In the latter investigation, no fractionation of potato starch could be obtained by electrophoresis, and it was assumed that the phosphorus was randomly distributed, without characterizing any specific fraction. With the more sensitive butanol precipitation technique, the presence of fractions is definitely established, and phosphorus is shown to be preferentially attached to the non-precipitated portion.

The butanol precipitated fraction from potato starch gives a pure blue coloration with iodine, while the non-precipitated portion tends toward the purple.

Waxy Maize Starch

Waxy maize starch has recently occasioned considerable interest, by reason of its red coloration with iodine and its reputed lack of retrogradation tendencies.⁹ Since these qualities are suggestive of the non-precipitated fraction, an investigation of its behavior toward butanol was undertaken.

Waxy maize starch was defatted in the prescribed manner, reducing the total fat content from 0.11 to 0.04%. This defatted starch analyzed an alkali number of 4.1, much lower than any of the common cereal or tuber starches. Butanol treatment of autoclaved pastes gave

no trace of crystalline flocculate. A small amount of slime and impurities (less than 3%) was separated by the supercentrifuge, but most of this material could be dispersed by further autoclaving and did not subsequently precipitate with butanol.

Summary

By means of selective precipitation with butanol, a method of starch fractionation has been developed which avoids retrogradation and hydrolytic degradation. The precipitated fraction (constituting 22% of either corn or potato starch) is isolated in unique spherocrystalline form, probably as an addition compound with the butanol. The butanol precipitated fractions from corn and potato starches are more alkali labile than the respective raw starches, while the non-precipitated fractions are correspondingly more alkali stable, indicative of definite chemical differences.

The butanol precipitated fraction from corn starch tends to revert to insoluble form and appears to be the component of the starch responsible for gelation and retrogradation. The non-precipitated fraction from corn starch constitutes the more soluble and stable component.

With potato starch, the physical differences between the fractions are less pronounced, since the butanol precipitated portion is more soluble and less subject to retrogradation than the corresponding fraction from corn starch. The phosphorus in potato starch is principally associated with the non-precipitated fraction.

Waxy maize starch is peculiar in possessing an unusually low alkali lability and in giving no precipitate with butanol.

(8) Taylor and Schoch, *THIS JOURNAL*, **55**, 4248 (1933).

(9) Hixon and Sprague, *Ind. Eng. Chem.*, **34**, 959 (1942).