1940

Fractionation of starch

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NOTE TO USERS

FRACTIONATION OF STARCH

by

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A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Plant Chemistry

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1940

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INTRODUCTION

From a survey of the literature related either directly or indirectly to the problem of starch heterogeneity, several facts become evident. Many different lines of approach to the problem have been used but none of these taken independently has resulted in a complete picture of starch. Furthermore, sharply contrasting views, based on the same or different techniques, have developed. While some of these are apparently sound, they have yet to be reconciled with one another. One notable weakness of the material has been the failure of many workers to exploit the differences between the various starches.

These considerations seem to warrant the extensive literature summary which follows. This summary consists of these general sections: (1) the evidence for the generally accepted structure of starch, (2) the fractionation of starch into soluble and insoluble portions by physical methods, (3) the distribution of these fractions in the starch granule, (4) the bearing of phosphoric and fatty acid content on the separation of starch into fractions, (5) the methods of determining the molecular weight of starch and its fractions, (6) the theories of starch aggregation, (7) the retrogradation of starch pastes, (8) the fractionation of starch by

enzymes and the significance of their action as to starch structure, and (9) the phenomenon of starch-iodine coloration.

With regard to the present work, the literature on starch chemistry emphasizes the difficulty of selecting a sure means of approach to the question of why starch is heterogeneous. At best the objective can only be the verification of some of the existing data and possibly the addition of some new facts. The results of any one experimental approach cannot be used alone for the elaboration of a theory of starch structure but must be interpreted in the light of the best of the large body of facts already in existence. Thus the knowledge of starch structure grows bit by bit.

REVIEW OF LITERATURE

Chemically starch is best represented as a chain structure of glucopyranose units joined in alpha-1,4 glucosidic linkages. This conception is based on a great deal of evidence of which the most significant points are summarized here.

Glucose is produced from starch in practically quantitative yields by acid hydrolysis and if any other monosaccharide unit exists in starch it must be present in extremely small amounts. That most of the glucoses have free hydroxyls on carbons two, three, and six was convincingly shown by Haworth, Hirst and Webb (1) who obtained an 85 per cent yield of 2,3,6-trimethyl glucose by hydrolysis of a fully methylated starch sample which in turn corresponded to 85 per cent of the original starch. More recently, Jackson and Hudson (2) and Caldwell and Hixon (3) have shown that periodic acid, an oxidizing agent capable of splitting the carbon-carbon bond of glycol groups, is consumed by starch in amounts agreeing with the presumption of adjacent hydroxyls on carbons two and three of the glucose units. Further, Jackson and Hudson (4) have obtained the expected glyoxal and d-erythrose in yields of 25 to 30 per cent and 20 to 25 per cent respectively by hydrolysis of the oxidized starch.

The close relation of starch to maltose is impelling evidence for the pyranose ring structure and the 1,4 glucosidic linkages. Haworth and Percival (5) subjected fully methylated starch in chloroform to degradation by acetyl bromide and from the mixed cleavage products isolated a methylated disaccharide which was definitely identified as maltose and corresponded to 22.4 per cent of the original starch. The presence of beta-maltose in digestion mixtures of starch and the saccharogenic amylases has lead to the suggestions that beta-linkages preexist in the starch, either alternately with the alpha-linkages or in one particular fraction of the starch. That all of the linkages are alpha and not beta is now generally accepted.

This question has received an interesting treatment by Freudenberg and coworkers (6) who formulated a relationship between the optical rotation of a disaccharide and the rotations of higher saccharides containing exactly similar chain units joined together with glucosidic linkages identical with that present in the disaccharide. On the basis of this relationship Freudenberg predicted a linear connection between the optical rotation divided by the number of glucose units (n) and $\frac{n-1}{n}$ for members of the starch series. This was found to hold for members for which n was known by end-group assay and moreover the differences in rotational powers exhibited by the alpha-linked starch series and the beta-

linked cellulose series emphasized the stereoisomeric relationship between these polysaccharides.

Additional evidence for the continuous alpha-glucosidic chain has been given from a study of the kinetics during the hydrolysis of polysaccharides. For a detailed account of this method, see the papers by Meyer, Hopff and Mark (8), Kuhn (9), and Freudenberg, Kuhn, Dürr, Bolz and Steinbrunn (10). A chain of n glucose residues, united by n-1 equal linkings may be hydrolyzed successively by the action of n-1 water molecules. The number of simple glucoses formed increases, while the number of bioses, trioses, etc., passes through a maximum. If it is assumed that a large number of very long chains is present, the yield of the various disintegration products can be calculated.

For example, the highest yield of biose is calculated to be 30 per cent in a homogeneous system, but if each molecule of biose is protected from further hydrolysis, say by insolubility, the highest amount could not exceed 67 per cent. The best yield of biose derivative (octaacetyl cellobiose) obtained by acetolysis of cellulose (degradation by acetyl bromide) is about 40 per cent. A yield of 67 per cent is not attained because the octaacetyl cellobiose is not absolutely insoluble. Estimation of the loss by experiments and addition of this to the yield obtained proved that at least 50 per cent, but in no case more than 67 per cent, of cellobiose is formed during the reaction. Study of the

acetolysis of starch has been more difficult on account of the poor crystallizing properties of the maltose derivatives.

H. Fischer and E. Fischer (11) first noted that acetyl bromide reacted with maltose to give aceto-brom maltose in 80 per cent yield and this in turn on treatment with silver carbonate yielded 30 per cent of the maltose as the hepta-acetate. Karrer et al. (12, 13, 14) extended the reaction to starch and found equivalent amounts of the heptaacetyl maltose formed from both maltose and starch, i.e., 22 to 27 per cent of the theoretical. On the basis of this, these authors erroneously postulated maltose or some maltose anhydride as the building unit of starch. As mentioned before, Haworth and Percival have isolated 22.4 per cent of a maltose derivative from the acetolysis mixture of methylated starch. Freudenberg and Soff (15) obtained 21 per cent of the hepta-acetate from starch triacetate under the best conditions. They reported only 37 per cent yields of the aceto-brom maltose from starch in contrast to the 80 per cent claimed by Karrer.

The kinetics of the degradation of starch measured by the rotation change and by the increase of aldehyde groups is similar to the kinetics of the hydrolysis of cellulose. The hydrolysis curve for cellulose is in best agreement with the set of theoretical calculations of Kuhn which assume one hydrolysis constant for the biose and triose molecules and another for the fragments including tetraose to cellulose.

The hydrolysis constant, K_2, for cellobiose and the initial velocity constant, K_n, for cellulose are strikingly different ($K_2:K_n=3:1$). For starch the proportion is not so favorable ($K_2:K_n=1.4:1$), and while the deviation from the course of a monomolecular reaction (in which $K_2=K_n$) is evident, it is not large enough to warrant the assumption of homogeneous linkages as in the case of cellulose.

However, these workers argue for the exclusion of any other linkage along these lines. If there is in starch in considerable amounts a second linkage other than the maltose union, this linkage must be split more quickly, just as quickly, or more slowly than the maltose union. The first case is excluded because the polysaccharide is hydrolyzed more slowly than maltose; the second and third are excluded because then a second disaccharide must appear along with maltose in tangible amounts and this has never been found. Freudenberg has estimated from the kinetics study that any linkage other than maltose must be present in less than one in every 20 to 25 maltose linkages to escape detection.

Although the evidence is conclusive that starch for the most part is fabricated as a chain structure of glucose units joined by alpha-1,4 glucosidic linkages, it has been recognized from the time of the earliest work that starch is not homogeneous. Fractionation of starch by physical and chemical methods which were assumed to leave the starch chemically intact have led in general to two more or less

distinct fractions. Treatment of starch with certain enzymes has resulted in residues apparently containing structural features which condition their resistance to the further action of the enzymes. These phenomena demonstrate the heterogeneous nature of starch and their eventual clarification will depend on a more thorough understanding of many as yet unsettled points of starch chemistry.

While fractionation of starch by purely physical and chemical means has led in general to so-called soluble and insoluble fractions, there has been little agreement among the various workers as to the quantities of these fractions in starch. The soluble fraction has been termed amylose, beta-amylose, etc., and the insoluble fraction amylopectin, alpha-amylose, amylophosphate, etc. (16).

Mme. Gatin-Gruzewska (17) obtained 55 to 60 per cent of an insoluble and 40 to 45 per cent of a soluble fraction by sedimentation of a potato starch paste treated with dilute sodium carbonate. Samec (18) by a modification of Gatin-Gruzewska's method, found 56 per cent insoluble and 44 per cent soluble. Tanret (19) extracted potato starch grains with hot water to obtain 27 per cent and 70 to 80 per cent of soluble and insoluble fractions, respectively. Sherman and Baker (20) concluded that holding a potato starch paste at 85°C. results first in swelling of the granules and leaching out of the soluble portion and then in gradual dispersion or hydration of the insoluble material. Samec and Mayer (21)

by electrodialysis of a potato starch paste prepared at 120°C. found 17 per cent and 83 per cent respectively of the soluble and insoluble fractions. Taylor and Iddles (22) after pretreating the starch with alcoholic hydrogen chloride, swelled the granules with ammonium thiocyanate and separated the soluble and insoluble fractions from the alcohol washed materials by electrodialysis or ultrafiltration of their aqueous pastes. They obtained 97 per cent of a soluble and three per cent of an insoluble fraction from potato starch and 80 and 15 per cent respectively of soluble and insoluble fractions from cornstarch.

Taylor and Morris (23) found that the insoluble fraction from cornstarch, unlike that from potato, was precipitated from 2.5 per cent alkali on neutralization and concluded that potato starch does not have a true insoluble or amylopectin fraction. Baldwin (24) froze pastes of potato starch, and after melting, obtained 15.5 per cent of a soluble fraction by repeated extraction with water at 60°C. Freudenberg and Rapp (25) by electrodialysis of potato starch swollen in potassium thiocyanate obtained 12 to 15 per cent soluble and 85 per cent insoluble fraction. On retreatment of the insoluble fraction, 25 to 50 per cent separated as a soluble fraction.

Taylor and Beckmann (26) by electrodialysis of a homogenized cornstarch paste got 62.5 per cent of an insoluble fraction. By sedimentation of pastes prepared from corn-

starch, dry-ground in a ball mill, they obtained the results shown in Table I.

TABLE I. Results of Taylor and Beckmann on the fractionation of dry-ground cornstarch.

Hours of Grinding	% Soluble Fraction	Appearance of Starch
130	51.3	Whole granules present
225	63.1	No granules, but birefringent particles
600	84.1	No birefringence

Taylor and Keresztesy (27) in a similar study on cornstarch reported the results shown in Table II.

TABLE II. Results of Taylor and Keresztesy on the fractionation of dry-ground cornstarch.

Hours of Grinding	Insoluble Fraction by Electrodialysis	Insoluble Fraction*	Soluble Fraction by Electrodialysis
168	18.6	15.6	86.0
336	14.5	15.1	87.6
672	8.7	11.1	91.8
1344	6.2	7.1	93.1
1848	--	5.8	--

* Upon neutralization after solution in 2.5 per cent aqueous alkali.

The organization of the starch granule and the distribution of the constituent amyloses are other aspects of the

problem of the heterogeneity of starch which are yet to be agreed upon. Brown and Heron (28) reported that in germinating barley during the late stages of endosperm depletion numerous empty hulls representing former starch granules may be seen. These thin sac-like structures were usually perforated by small holes which appeared to mark points at which the initial corrosion occurred and through which removal of the degraded contents was effected. Maquenne and Roux (29) assumed the outer portion of the granule to represent the insoluble and the inner portion the soluble fraction. Beijerinck (30) observed that if boiled starch granules are treated with strong tannin solution the tannin diffuses into the swollen granules and there forms a precipitate which shows active Brownian movement until the particles become too large.

Alsberg et al. (31, 32) hold the opinion that the starch granule is protected by an impermeable membrane and that when this is injured the contents may be leached out in cold water. Thus with potato and wheat starches, dry-ground about 150 hours, each was soluble in cold water to over 60 per cent. These ground starches do not gelatinize and no paste can be obtained. Granules which were crushed or mechanically injured were observed to swell in cold water at the site of the injury.

Baldwin (24) observed that swollen, unbroken granules when frozen and melted appeared as collapsed sacs. The sacs

were colored violet with iodine and the liquid was colored blue. In warm water the collapsed sacs became swollen a second time and upon crushing, a liquid poured out. The sac was considered to behave as a dialyzing membrane.

Hess and Rabinowitsch (33) could observe Brownian movement inside of swollen granules precipitated with certain concentrations of alcohol and have presented photographs showing the puncture of a granule by a micro-needle and its subsequent collapse with the escape of the inner material.

Hanson and Katz (34) reported that potato and wheat starch granules treated with hydrogen chloride and calcium nitrate were divided radially and tangentially into small blocks of about one micron which were separated by some other substance. The blocks were assumed to be the soluble fraction and the insoluble or amylopectin fraction was interposed between them. Badenhuizen (35, 36) observed the same block-like structure but thought it was characteristic only of gelatinized granules. He was unable to confirm Hess' work and believed that the granule consists of a system of sacs enveloping each other. With dry granules the micro knife slipped off; if they were wetted they became soft and were easily penetrated. Thus the granules are pictured as having a more or less rigid, porous structure which swells in water. Osmotic pressure is not assumed to play a part in the swelling.

In spite of the uncertainty as to the proportions of the soluble and insoluble fractions and their distribution in the starch granule, there is little doubt that two or more different materials are involved. Some of the observations concerning the iodine color and optical rotation of the two fractions are summarized in Table III.

The fat content of cereal starches, particularly of cornstarch has been considered responsible for the separation of these into soluble and insoluble fractions. Taylor and Nelson (38) argued for a chemical combination between the fatty acids and starch on the grounds that the fat is not removed by successive extraction with ether, petroleum ether and carbon tetrachloride, that gelatinization does not affect the combined fat and that it is removed only by hydrolytic agents at the erythro-dextrin stage.

Subsequent papers by Taylor and his students [Taylor and Iddles (22), Taylor and Werntz (40), Taylor and Beckmann (36), Taylor and Sherman (41)] have shown that the acids associated with cornstarch are palmitic, oleic acid and linoleic, and that these differ in their ease of removal from the starch, the unsaturated acids being the easiest to remove. The fatty acids were found associated with the insoluble fraction of cornstarch and were held responsible for its polarity since defatted starches would not undergo electrophoretic separation.

TABLE III. Iodine colors and specific rotations reported in the literature for some of the soluble and insoluble fractions.

Fraction	Iodine Color	Specific Rotation (degree)	Reference
Sol., potato	Blue	+182.4	Gatin-Gruzewska (17)
Sol., potato	Blue	+189	Samec and Mayer (21)
Sol., potato	Blue	+190*	Freudenberg and Rapp (25)
Sol., potato	Blue	+188.8** - +189.6	Baldwin (24)
Sol., potato	Blue	+181.6	Taylor and Iddles (22)
Sol., corn	Blue	+181.6	Taylor and Iddles (22)
Insol., potato	Red-violet	+221	Gatin-Gruzewska (17)
Insol., potato	Red-violet	+195 - +196	Samec and Mayer (21)
Insol., potato	Red-violet	+221	Ling and Nanji (37)
Insol., potato	Red-violet	+195.1 - +195.7	Baldwin (24)

* With the Hg yellow line, the specific rotation of the soluble fraction was $+203^{\circ} \pm 1^{\circ}$ in 51 per cent sulfuric acid, $+164^{\circ} \pm 1^{\circ}$ in normal sodium hydroxide, and $+197^{\circ}$ in water; specific rotation of the insoluble fraction was $+206^{\circ} \pm 1^{\circ}$ in 51 per cent sulfuric acid, and $+169^{\circ} \pm 2^{\circ}$ in normal sodium hydroxide.

** The specific rotation was found to decrease as the temperature increases, the effect for the insoluble fraction being about ten times greater than that for the soluble fraction.

Other workers have argued that the fat is not chemically bound to the starch and is merely extraneously distributed throughout the granule. Rask and Phelps (42) reported that the fat could be removed from cornstarch by extraction with alcoholic ammonia. Schoch (43) has found that the fat is completely removed by Soxhlet extraction with water-miscible fat solvents such as methyl alcohol, the cellosolves and 80 per cent dioxane. The defatted starch retains its characteristic granule structure and optical birefringence. It gelatinizes in hot water to give a paste of normal high viscosity. Treatment with oleic acid in ether re-impregnates the starch with fat and this added fat cannot be extracted with hydrocarbon type solvents.

Lehrman (44) advances the argument that since all samples of a given starch give the same fat analysis and since methyl and acetyl derivatives have been prepared which retain part of the original fat it must be chemically held or may be adsorbed.

The phosphoric acid in starch is now generally believed to occur as an ester with the sixth hydroxyls of certain of the glucopyranose units. Northrup and Nelson (45) by treatment of potato starch with hydrochloric acid mineralized 98 per cent of the phosphorus, but were able to isolate a carbohydrate phosphoric acid complex which contained three to five per cent phosphorus. Posternak (46, 47) isolated carbohydrate phosphoric acid complexes from potato starch-

malt-extract digestion mixtures which contained 1.8 to 2.5 per cent phosphorus. Hydrolysis of these with two per cent sulfuric acid led to the isolation and identification of glucose-6-phosphoric acid.

Samec and his coworkers (48, 49, 18, 50, 51) in a series of papers concluded that the phosphoric acid occurs in the insoluble fraction of starches and is responsible for the insolubility, the high viscosity, the mobility under the influence of the electric current, and the gelling properties of this fraction. The increase in conductance and decrease in viscosity which accompany prolonged boiling of starch pastes is attributed to gradual hydrolysis of the phosphoric acid.

Northrup and Nelson (45) contended that little or no phosphoric acid is liberated under the conditions described by Samec and that all of it is liberated only after several hours of refluxing with ten per cent hydrochloric acid. Samec and Mayer (52) have esterified phosphorus-free starch fractions with phosphoric acid to obtain products with markedly increased viscosity and gelling properties. More recently Samec (53) has modified his views on the basis that unless care is used in pasting or autoclaving of starches for fractionation, phosphorus will occur in both fractions, and hence the phosphoric acid does not necessarily render starch insoluble.

Karrer and Krauss (54) have effected separations on potato and tapioca starches by centrifugation of the boiled pastes, repeating the treatment four to six times on the sediments obtained and found that the phosphorus content of these fractions had no relation to their physical properties. Taylor and Iddles (22) reported that the phosphorus content was approximately the same for both the soluble and insoluble fractions of potato starch. Thus it may be accepted that phosphoric acid does have some relation to the physical properties of starch but it does not necessarily occur only in the insoluble fraction.

In considering the question of the molecular weight of starch which obviously has an important bearing on the problem of its heterogeneity, there have been a variety of methods used.

Cryoscopic measurements have been demonstrated to be unreliable. Bergmann and coworkers (55, 56) carried out such measurements on starch acetates in phenol and acetic acid and found abnormally large freezing point depressions. This many times led to the erroneous conclusion that the starch in these solutions is of low molecular weight. For example the normal molecule of starch was assumed to be a monose or biose anhydride which in colloidal solutions existed as micelles.

Hess and Friese (57) noted that cellulose by mild acetolysis was converted to a product of low reducing power

which appeared to have the molecular weight of an acetylated disaccharide by freezing-point lowering in acetic acid. Subsequent investigators have established that this is really a mixture of acetylated cello-dextrins of relatively long chain lengths (58, 59, 60). Samec, Knop, Lavrenčič and Premrl (61) observed that freezing point depressions gave molecular weights smaller than by other methods probably on account of persistent traces of impurities.

Similarly osmotic pressure measurements, while theoretically sound, suffer from large experimental error when applied to high molecular weight substances. Samec and coworkers in a series of papers (62, 63, 64) have used both osmotic pressure measurements and diffusion measurements in the investigation of the mole sizes of certain starch fractions. Molecular weights calculated from diffusion velocity measurements were generally twice those found by osmotic pressure measurements, a discrepancy which was attributed to the fact that the relation between diffusion velocity and molecular weight holds for only spherical particles. Molecular weights of the order of 10^5 are reported by Samec for starch and some of its fractions.

Viscosity measurements while experimentally precise have an uncertain relationship to molecular weights. Staudinger and Eilers (65) working from the premise that the viscosity of long chain molecules is dependent on chain length and not molecular weight have studied both cellulose

and starch and have reported for a series of degraded starches molecular weights varying from 10,000 to 100,000. Using the relationship $K_m M = N_{sp}/C_{gm}$, where N_{sp} is the specific viscosity, C_{gm} the concentration in basic moles per liter, K_m a constant, and M, the molecular weight, Staudinger has evaluated the constant K_m using values of M determined by osmotic pressure measurements and has shown that the value of this constant remains the same for both high and low molecular weight members of the cellulose series. Thus cellulose and its degradation products are seen to be thread-like molecules. With starch the constant K_m is not the same for both high and low molecular weight members and has values about one-tenth that found for cellulose. Hence Staudinger believes that starch has a relatively high molecular weight with a short chain length. In any event the values given by Staudinger can be only provisional since the K_m constant is based on values of M derived from osmotic pressure measurements.

Higginbotham and Richardson (66) feel that Staudinger's method is unreliable since the viscosity and average chain length curves, while approximately linear, do not coincide due to differences in types of distribution of chain lengths. The longest chain molecules make the chief contribution to viscosity.

The most recent and what may prove to be the most satisfactory of the physical methods for studying materials

of high molecular weight employs the ultracentrifuge. This method gives estimates not only of particle size, but of particle shape and, for mixtures, the distribution of particle sizes. Lamm (67) observed that acid degraded starch was separable into two components with possible molecular weights of from 12,000 to 29,000. Starch degraded by heating with water has a particle weight of 28,000 to 54,000. In cold 40 per cent zinc chloride solution starch showed a particle weight of 940,000. Beckmann and Landis (68) have studied cornstarch dry-ground in a pebble mill and show that it is obviously heterogeneous. The soluble or beta-amylose fraction obtained by electrophoresis was fractionated by methyl alcohol precipitation into fractions with molecular weights ranging from 17,000 to 225,000 with a preponderance of material of molecular weight 31,000 to 60,000. The ratio of the particle size of the insoluble or amylopectin fraction to the soluble fraction was given as roughly 1000:1.

The most widely used chemical method for determining the molecular weight of starch is that of Haworth who estimates chain length on the basis of the amount of 2,3,4,6-tetramethyl glucose obtained from fully methylated starch by hydrolysis. The general procedure employed by Haworth and his coworkers consists of acetylation of the starch with acetic anhydride using either pyridine or sulfur dioxide and chlorine as catalyst, simultaneous deacetylation and methylation of the triacetate by treatment with dimethyl sulfate and sodium

hydroxide in acetone solution, hydrolysis of the trimethyl starch and conversion of the resulting mixture of methylated glucoses into the methyl glucosides, which are then separated by fractional distillation in vacuo.

The percentages of tetramethyl and trimethyl methyl glucosides in the first few fractions are estimated by reference to the refractive index of the fractions. This method has been applied to the soluble and insoluble fractions of potato starch (69), waxy maize starch (70), wheat and horse chestnut starches (71), and rice starch (72), giving in every case values of from 25 to 30 glucose units for the length of the chain molecules.

Freudenberg et al. (73, 25, 74) methylated their samples directly in liquid ammonia using sodium and methyl iodide to get products of 45 per cent methoxyl content. The methylated methyl glucosides were subjected to a preliminary distillation to get a fraction containing all of the tetramethyl and some trimethyl methyl glucoside. This fraction was benzoylated before proceeding to a sharper separation by fractional distillation. These workers report values agreeing with those of Haworth et al. for potato starch and its soluble and insoluble fractions.

Hassid and Dore (75) separated the trimethyl and tetramethyl glucoses gotten from methylated canna starch by repeated extraction of their aqueous solution with chloroform and give a chain length of 27 glucose units for this starch.

Hess and his coworkers (76, 77, 78) have criticized the Haworth technique on the grounds that acetylation, particularly with sulfur dioxide and chlorine, leads to an increase in reducing power and thus degradation of the starch: that refractive index measurements while satisfactory for analyzing the top fraction give erroneous results with the middle fractions since it also contains dimethyl methyl glucoside. These workers employ a separation of the pentaether from the lower methylated products by phosphorylation of the latter and removal as the barium salt, which is insoluble in ether. This procedure has the advantage that a completely methylated material is not necessary; thus the disturbing influences of long methylation can be cut down. Air is excluded during the methylation since oxygen in the presence of alkali causes degradative changes leading to increased amounts of the end group. A value of 52 glucose units was reported by these workers for the chain length of starch.

Averill and Peat (79) and Hirst and Young (80) have replied to Hess' criticism of the Haworth technique and in turn have questioned the Hess procedure. They contend that phosphorylation of the trimethyl methyl glucoside is never complete and that there is considerable loss of tetramethyl methyl glucoside during the evaporation of its solutions in light petroleum ether and during the removal of water in which the tetramethyl methyl glucoside and the barium salt

of the phosphorylated trimethyl methyl glucoside are dissolved at one stage.

Richardson, Higginbotham and Farrow (81) working with the opposite end of the molecule have demonstrated that starch has a slight reducing value. These authors assume that all the chain molecules terminate in reducing groups and that their copper reduction method is an accurate measure of these groups. Thus they are able to calculate the average length of the chains from the ratio between the reducing power of the sample and that of a substance of known chain length, such as maltose. With this method they suggest values of from 460 to 1470 glucose units for unmodified starches.

In support of his assumption that starch consists of chain molecules of various lengths terminated at one end by a free aldehyde group, Farrow has shown that with the acid modification of starches the reducing power is a linear function of time, without an initial lag period, and finally attains a value almost equal to that of glucose. Fractionation of different members of a series of acid modified starches by freezing gave soluble fractions with reducing powers greater than the original starches and insoluble fractions with reducing powers lower than the original starches. The reducing power of both fractions increased as the reducing power of the original starch increased.

The validity of the assumptions of Farrow has been partly confirmed by Caldwell and Hixon (3) who have studied the periodic acid oxidation of fractions obtained from a series of six commercial dextrins. These dextrins were designated by the letters A, B, C, D, E and F, the order of increasing conversion being from A to F. The dextrins were fractionated according to their solubilities in alcohol-water mixtures. The terminal reducing glucoses of these molecules, containing a primary alcohol group adjacent to a secondary alcohol group ($-CHOHCH_2OH$) would be expected to produce formaldehyde on oxidation with periodic acid. The results of Caldwell and Hixon are shown in Table IV.

The agreement between the experimentally determined amounts of formaldehyde produced by periodic acid oxidation and the amounts calculated from the average chain lengths as determined by Farrow's method indicates that Farrow's values are an accurate measure of the reducing groups.

The assumption that each molecule terminates in a reducing glucose residue while uncertain in the case of unmodified starch as is pointed out later, seems unavoidable in the case of these acid modified starches. One hydrolytic scission of a non-reducing starch molecule would lead to one reducing and one non-reducing molecule. One scission of each of these would lead to a total of three reducing molecules and one non-reducing molecule. Thus it becomes clear that an accurate measure of the reducing groups in starches

TABLE IV. Correlation of quantities of formaldehyde produced by periodic acid oxidation of various dextrin fractions with chain lengths as determined by Farrow's copper reduction method.

Dextrin Sample	Reducing Values*	Average Chain Length**	Amounts of Formaldehyde	
			Calculated from Chain Lengths***	Determined Experimentally****
			mg.	mg.
B, fraction insoluble in H_2O	30.65	134.0	0.53	0.47
C, fraction insoluble in H_2O	42.70	96.2	0.74	0.94
D, fraction insoluble in H_2O	71.25	57.75	1.24	1.59
D, fraction soluble in H_2O, insoluble in 52% alcohol	55.70	73.50	0.97	0.79
E, fraction insoluble in H_2O	108.90	37.80	1.89	1.64
E, fraction soluble in H_2O, insoluble in 52% alcohol	75.33	54.50	1.31	1.41
E, fraction soluble in 52% alcohol, insoluble in 75% alcohol	124.63	33.00	2.16	2.10
F, fraction insoluble in H_2O	150.20	27.30	2.64	2.85
F, fraction soluble in 52% alcohol, insoluble in 75% alcohol	160.13	25.60	2.81	2.39

* Expressed as R_{Cu} values. R_{Cu} is defined as the number of mg. of copper reduced by 1 gm. of starch. For modified starches and sugars the unit of weight is taken as the weight equivalent to 1 gm. of starch.

** Calculated from R_{Cu} values. The R_{Cu} of maltose is 2055. Hence to find the average chain length of the molecule of a sample whose R_{Cu} is 100, for example, use the equation $100X = 2 \times 2055$.

*** Theoretical amounts calculated from chain lengths on the basis of a 0.3 gm. sample.

**** Determined on the basis of a 0.3 gm. sample.

modified by hydrolysis must be just as reliable as any other end-group method for determining chain length. If starch possesses a branched or other structure than a straight chain, then end-group measurements can give no indication of mole size. Even in this case, however, hydrolysis should very soon reduce the starch to a state approximating a mixture of thread-like molecules whose average chain length would be given by an estimate of the reducing groups. From the table it is seen that the most highly converted sample has a reducing value corresponding to a chain length of 25 glucoses. If the molecules of these samples have still not attained the state of unbranched chains, then these values for mole size are too low.

The ideas presented above should be kept in mind when the other methods of determining mole size and the theories of aggregation are considered.

Wolfrom et al. (82) got a value of 20 $\pm$ 4 for the chain length of potato starch by mathematical extrapolation to zero time of data obtained by subjection of the polysaccharide to hydrolysis at 0°C. with fuming hydrochloric acid under conditions of continuous mercaptalation and determination of the amount of combined sulfur at stated time intervals.

The discrepancy between the values for the molecular weight of starch as determined by end-group assay and as determined viscosimetrically has been the subject of much

debate. Haworth attributes the lack of agreement to the fact that the end-group method measures the fundamental repeating unit while the various physical methods measure aggregates of these units. The soluble and insoluble fractions, since both have the same end-group values, are explained by differences in degree of aggregation (69). Interesting in this connection are the results of Freudenberg et al. (25, 74) who found that the acetates of the soluble and insoluble fractions differed greatly in their solubility in acetone and chloroform while the methylated materials could not be distinguished from one another. This probably has some relation to the method of methylation. Preparations methylated directly in liquid ammonia as well as those which had been previously methylated with sodium hydroxide and dimethyl sulfate have lower viscosities than those methylated only by the latter method.

An explantion of these facts might be indicated by the work of Reich and Damansky (83). These workers found that acylation of potato starch yielded 82 per cent of a diacetate and 16 per cent of a triacetate which were quantitatively separable by solution of the triacetate in chloroform. Hydrolysis of the diacetate led to "amylogene" with the properties of the natural starch while the triacetate led to "amylon" with properties of a soluble starch. On the other hand acetolysis of the diacetate produced the triacetate or "amylon" acetate. Amylopectin on the above basis con-

sisted mostly of "amylogene" with some "amylon" while amylose was entirely made up of "amylon". The "amylogene" was found to be easily transformed to "amylon", e.g., by treatment with hot water and the change was thought to consist of the liberation of one extra esterifiable hydroxyl group per glucose unit. The authors postulated that in "amylogene" the chain of glucoses is compressed by folding so that one hydroxyl in each pair of units engages with that above and below it. Such a structure rather emphasizes the maltose unit and the splitting off of successive maltose units by enzymes.

Baird, Haworth and Hirst (84) noted that acetylated and methylated derivatives having widely varying viscosities could be prepared and attempted to prepare a disaggregated starch by treatment with 0.5 per cent hydrochloric acid in ethyl alcohol. This starch gave acetylated and methylated derivatives whose molecular weights by viscosity measurements approached the value of 25 glucose units found by the end-group method. Haworth, Hirst and Plant (85) outline properties of a dextrin, prepared by heating starch in glycerol, which showed both by end-group assay and viscosity measurements a chain length of eight glucose units. Hess (78) has gotten values by viscosity measurements 30 to 60 times those found by end-group assay and concluded with Haworth that the viscosity difference represents the difference in degree of aggregation.

Taylor and his coworkers have advanced a theory of aggregation based on studies of the effect of alkali on the iodine consumption of different starch samples. Taylor and Salzmann (86) discovered that when any starch had an appreciable reducing value this was increased many times by treating the starch with aqueous alkali. The amount of increase in the reducing power has been termed the "alkali-labile" value and has been used as an analytical method for characterizing starches (87). Aqueous acid, certain heat treatment, and grinding in the presence of moisture were found capable of producing a high alkali-labile value.

Taylor and Keresztesy (27) have theorized that each hydroxyl oxygen atom is a donor and each hydroxyl hydrogen is an acceptor by which a coordinate link can be formed. By an elaboration of this pattern and a possible dovetail fitting end to end of one bundle with another it might be expected that terminal aldehyde groups, although primarily chemically free, might be occluded or shielded. Thus reducing power as reflected in the alkali labile value could increase after a given treatment from two causes. One would be dissociation of the coordinately linked chains from one another to uncover existing terminal aldehyde groups and the other would be hydrolytic scission of glucosidic linkages giving short chains and consequently new aldehyde groups. Since chain shortening through hydrolysis causes a break in continuity of the skeleton on which the coordinately linked

groups function, it is probable that dissociation takes place more readily with fragments than with the original longer chains.

After dissociation, the slower hydrolytic breakdown of glycosidic linkages becomes the principal producer of smaller molecular weight reducing sugars. Thus the initial sharp increase in alkali-labile value observed at the start of the dry-grinding of starch could be explained as due to the production of easily dissociated fragments while the slow increase in reducing power during the later stages of grinding would be due to hydrolysis of glucosidic linkages. Likewise could be explained the heterogeneity of the soluble or beta-amylose fraction prepared from dry-ground starch.

Staudinger (65) is opposed to all theories of aggregation since he has been able to show by viscosity measurements that starch can be transformed into polymerically analogous derivatives like the triacetate, the trimethyl derivative, and the trinitrate, and furthermore that starch regenerated from the triacetate has the same viscosity as the original starch. The constancy of viscosity at different temperatures and in different solvents as well as the linear relation between viscosity and concentration in relatively dilute solutions are cited by Staudinger as additional evidence that starch has a macromolecular rather than a micellar structure.

Farrow (81) argues that with any end-group method of determining molecular weight it must be assumed that no degradation takes place since a slight increase in reducing value corresponds to a large decrease in average chain length. He has shown that the reducing power of the disaggregated starch of Baird, Haworth and Hirst is 37.4 as compared with 3.1 of the original starch and must indicate considerable degradation.

In his opinion the methylation procedure measures the molecular weight of degraded chains rather than of disaggregated units. With the acid hydrolysis of starch the chains are shortened by random attack so that in modified pastes there is a wide distribution of chain lengths. As hydrolysis proceeds the proportion of short chains increases leading to larger amounts of the soluble fraction obtained by freezing and smaller amounts of the insoluble fraction. The average chain length of both fractions decreases. With all the starches examined except farina, Farrow has found a linear relation between time and reducing value. An initial rapid increase in reducing value would be expected if according to Taylor the relatively easy disaggregation process to expose free aldehyde groups takes place.

Hirst and Young (72) have elaborated on the Haworth theory of aggregation along the following lines. Since starch gives by end-group assay a unit of 24 to 30 glucoses regardless of the method of preparing the methyl derivatives

and irrespective of their molecular weights by viscosity measurements, the proportion of end groups cannot be explained by random hydrolysis of long main chains of similarly united residues. These authors conclude that viscous methyl starches are composed of a large number of repeating units joined together laterally forming side chains.

In support of this view they have disaggregated a viscous methyl starch by controlled hydrolysis into a non-viscous, homogeneous methyl derivative which, on further methylation to etherify the hydroxyl groups liberated during the disaggregation process, gives the fully methylated derivative. Osmotic pressure and ultracentrifuge measurements showed the latter to have a molecular weight corresponding to about three repeating units. On hydrolysis the methylated disaggregated derivative yielded precisely the same amount of tetramethyl glucose as did the viscous derivatives, but the yield of dimethyl glucose was now very small. Taking into account the decrease in yield of dimethyl glucose and in viscosity and the increase in reducing power which accompany the disaggregation process, these authors conclude that the repeating chains are linked to a non-terminal glucose of another chain by primary valencies of the glucosidic type. Hence from highly viscous methyl starches, dimethyl glucose should be isolated in amount equal to that of the tetramethyl glucose.

Freudenberg et al. (73, 25, 74) emphasized the difficulty of estimating the dimethyl glucose but likewise reported equivalent amounts of tetramethyl and dimethyl glucoses. Irvine (88) also has reported equivalent amounts of the two methyl sugars. He found the dimethyl glucose to consist of approximately equal proportions of the 2,3- and 2,6-isomerides when starch was methylated directly, but this ratio was disturbed to give 24 and 76 per cent respectively of the 2,3- and 2,6-dimethyl glucoses as the result of preliminary acetylation. He has suggested that the groups responsible for the formation of tetramethyl glucose form part of a complicated fragment of the starch complex, as otherwise no dimethyl glucose could be formed from a fully methylated derivative.

Hess and Lung (78) attribute the production of dimethyl glucose to incomplete methylation of the starch but do not exclude the possibility that the tetramethyl glucose may come from only one fraction of the starch.

The retrogradation or aging of starch pastes as described by Taylor and Schoch (89) is characteristic of clear dispersions of starch, which on standing rapidly become cloudy and deposit the bulk of the solid material in an insoluble form. The material so deposited cannot be redispersed successfully by boiling. This process was explained at one time by Hirst, Plant and Wilkinson (69) as a gradual aggregation of the soluble amylose fraction to the

insoluble or amylopectin condition. Such a picture seems unlikely in view of the marked dissimilarity between the retrograded soluble material and the insoluble fraction. The retrograded amylose, for example, after solution in 2.5 per cent aqueous alkali, will not precipitate on neutralization as does the amylopectin (23). Sherman and Baker (20) found no evidence of retrogradation in solutions containing the insoluble or amylopectin fraction of potato starch while solutions of the soluble fraction retrograded to a marked degree. This has been the experience of a number of workers (89, 69, 23, 20, 24). Sherman and Baker suggested that the insoluble fraction acts as a protective colloid to the soluble fraction since the latter was found to retrograde more rapidly if it alone were present.

Sallinger (90) noted that the amount of material in a starch paste undigestible by saliva increases by aging at 7°C. and found by plotting the logarithm of the time of aging versus the logarithm of the amount of undigestible residue a straight line relationship. The slope of the line and the point at which it cut the abscissa were taken as constants characteristic of each type of starch and were considered as good parameters for the degree of condensation of the starch. Aging was found to proceed fastest with the most homogeneous samples.

Baldwin (24) has pointed out that when starch pastes are frozen, then melted and extracted with water at 60°C.

the amount of insoluble material varies directly with the length of time taken for freezing. The precipitate formed during the more rapid freezing (10 minutes) consisted of finer particles which passed into solution again as soon as the temperature was raised to 55°C. while in the pastes where freezing required several hours the precipitate was so coarse that several days at 50-60°C. were insufficient for its solution.

Staudinger and Eliers (65) from their experience have concluded that in water only low molecular weight starches (10,000 to 50,000) dissolve easily, while particles of higher molecular weight go only slowly into solution, and still higher molecular weight starches never give clear solutions. The starch in these turbid solutions precipitates out on standing. The explanation given is that by warming, the high molecular weight starch is weakly solvated and brought into solution but on cooling the chain molecules come together and so form larger particles which precipitate. This is essentially the view of Farrow (81) who considers a freshly prepared paste to represent a condition of supersaturation which is gradually relieved on cooling by precipitation of the longer chain molecules.

Samec (91) has observed a decrease in viscosity as well as variability of the same by acids and bases, increase in conductance, decrease in amount of material migrating with the electric current and decrease in the amount of material

precipitated by alcohol, in conjunction with the aging of starch pastes. To explain these phenomena as well as the fact that an aged starch paste cannot be restored to its original condition he attributes the aging to an irreversible change of the amylopectin fraction, i.e., to the cleavage of phosphoric acid.

Katz and his collaborators (92, 93, 94, 95, 96, 97, 98) have developed the use of x-ray spectra as a tool for studying the phenomenon of retrogradation. According to them raw starch, gelatinized starch and retrograded starch have characteristic spectra. The spectra for all kinds of retrograded starch are the same although the speed with which this spectrum appeared varied. Wheat starch pastes were found to retrograde much more slowly than those of potato. Addition of alcohol in low concentration (15 per cent) was found to accelerate retrogradation. The effect may be compared with the fact that starch paste shows a maximum retrogradation at a definite water content; with a small amount of water none at all; and with a large amount of water very slow retrogradation. The change in resistance to digestion by malt was found to bear no simple relation to other changes in the retrograding starch. Aging was found to be checked by aldehydes, large amounts of alcohol and heat, and to be completely stopped above a temperature of 60^{o}C.

Samec, Katz, Čanič and Klemen (99) followed the aging of a number of starch fractions and dextrins by observations of changes in turbidity, viscosity, iodine color, conductance, resistance to grinding and x-ray spectra. Changes in viscosity and turbidity differed according to the colloidal condition and chemical characteristics of the different starch substances. The resistance to grinding increased with age for all the products but the velocity and degree of change varied. The x-ray spectra were found to vary according to the way in which precipitation with alcohol was carried out.

Degradation of starch by biochemical methods, as previously pointed out, has also led to the conclusion that the starch molecule is not a homogeneous structure. The action of alpha-amylase, beta-amylase and of the enzyme produced by Bacillus macerans on starch have been considered significant as regards the question of starch structure.

Digestion of starch by beta-amylase is the best understood of these processes. Maltose constitutes almost the sole product of low molecular weight (100, 101, 104, 105, 106) which is formed and is invariably produced in yields of from 60 to 70 per cent. According to Hanes (107) an enzyme preparation exhibiting a sharp limit at 59 to 60 per cent conversion on a 0.5 to one per cent solution of soluble starch gives higher values, up to 66 per cent when allowed to act on very dilute substrates, e.g., 0.2 per cent.

Blom, Bak and Braae (106) noted that at pH 3.4, the hydrolysis with barley extract ceases sharply at the 53 per cent conversion stage but at pH 4.6 proceeds to the normal limit of 60 to 61 per cent and suggested that in barley extract a factor which normally facilitates the resistant stages of hydrolysis beyond 53 per cent is made inoperative at pH 3.4.

Klinkenberg (108) and Hanes (104) pointed out that the limit of hydrolysis is not appreciably affected by wide variations in enzyme concentration.

Solubilized starches from sources other than potato have all been found to be degraded to about the same extent as potato (109, 110, 104, 112). Beckmann and Landis (112) have reported gelatinized potato starch to yield 66.5 per cent of the theoretical amount of maltose, soluble potato starch (Lintner) to yield 63.1 per cent, and dry-ground potato starch to yield 71.2 per cent with the beta-amylase from soft wheat flour.

Ling and Nanji (109) reported the amylose or soluble starch fraction separated by the freezing out method to be completely transformed into maltose by malt-amylase (alpha and beta) or by barley-amylase (beta) and assumed that amylopectin is not hydrolyzed at all, thus introducing a new definition for the amylopectin or insoluble fraction. Hirst, Plant and Wilkinson (69) found the amylose and amylopectin prepared by the Ling and Nanji method to both be hydrolyzed to the same extent and to be indistinguishable

from starch itself in this respect. The soluble fraction of Samec and Mayer (prepared by electrodialysis) is hydrolyzed much less rapidly than soluble starch but is degraded almost to completion by beta-amylase according to Samec and Waldschmidt-Leitz (113), Freeman and Hopkins (105) and Samec (114). After 60 to 70 per cent has been transformed the residual material shows increasing resistance to hydrolysis but reduction values corresponding to 95 to 98 per cent of the theoretical amount of maltose are finally attained. A blue iodine color was reported to persist up to the stage of 80 to 90 per cent apparent conversion and this then changed with further degradation to violet and pink.

The mode of attack on starch by beta-amylase has been deduced from several pieces of evidence. Since maltose is the sole degradation product and since the residual dextrins markedly resemble the original starch an endwise type of breakdown has been postulated. Ohlsson (115) investigated the osmotic behavior of the products when soluble starch was degraded to the extent of 31 per cent of the theoretical amount of maltose and found that the osmotic pressure of the degraded material was only slightly lower than that of the undegraded. Hence it was concluded that no increase in the number of non-dialyzable particles had accompanied the partial transformation of the substrate into maltose. The non-reducing character of the dextrins suggests that degradation proceeds from the non-reducing ends of the starch

molecule. Brown and Millar (116) studied the enzymic degradation of a dextrinic acid, prepared by oxidation of a so-called maltodextrin with mercuric oxide and barium hydroxide, which contained about six glucose units per molecule. In this way short chain fragments terminated at the aldehydic end by a modified carboxylated unit were prepared. This product was readily degraded by malt-amylase yielding maltose and a dextrinic acid of lower complexity. Since the original material was attacked only slowly by alpha-amylase the degradation was attributed to the beta-amylase.

Throughout the hydrolysis of starch by beta-amylase, residual starch-like dextrins are present which are readily precipitated by 50 to 60 per cent alcohol concentrations. The ultimate member of this series which remains at the cessation of hydrolysis in yields of 20 to 25 per cent is the erythroamylose of Wijsman (1889), or the alpha-amylodextrin of Baker (100). These products exhibit iodine coloration ranging from blue to violet, retain the capacity to form molecular aggregates of high dimensions and are practically non-reducing.

According to Hanes the cessation of the degradation process is due neither to inactivation of the enzyme nor to its inhibition by reaction products as is shown readily by the addition of more starch at this stage. Moreover the isolated limit dextrin exhibits high resistance to the action

of the enzyme. However the limit dextrin after precipitation and resuspension in hot water has never been found to be completely resistant. The extent of this further breakdown depends upon the treatment of the dextrin. Mild autoclaving in neutral solution (30 minutes at 120°C.) results in further degradation by beta-amylase, 16 to 26 per cent of the theoretical amount of maltose being formed.

Hopkins, Cope and Green (103) followed the hydrolysis of soluble starch by the repeated action of enormous concentrations of beta-amylase. The resistant material after each digestion was isolated and in preparation for succeeding treatment with enzyme was redispersed by autoclaving. After six treatments the amount of dextrin corresponded to only three per cent of the original starch. The successive dextrin residues, which became progressively more resistant to degradation, retained the iodine coloring property. This changed finally from violet to pale brown. Hence relatively mild hydrolysis removes impedance to further digestion.

Haworth, Hirst and Waine (117) prepared a limit dextrin from soluble starch using beta-barley-amylase which showed by end-group assay a chain length of 16 to 17 glucose units. The molecular weights of the acetylated and methylated derivatives determined by viscosity measurements were considerably greater than those found by end-group assay. Haworth, Hirst, Kitchen and Peat (118) repeated the above work and reported a value of 12 glucose units for the chain

length. The values found by viscosity measurements on the acetate and methyl derivatives were approximately double this. Beckmann and Landis (112) using beta-amylase from soft wheat flour prepared limit dextrins from gelatinized potato starch, soluble potato starch (Lintner) and from dry-ground potato starch and investigated them by means of the ultracentrifuge. The gelatinized potato starch and the soluble potato starch were found to be quite heterogeneous in nature and with both, limit dextrins were obtained which could be resolved into two distinct components. The dry-ground starch on the other hand was homogeneous and so was its limit dextrin. The molecular weights of the limit dextrin fractions from gelatinized potato starch varied from 28,965 to 11,858 (or 178 glucose units to 73); those of the fractions from soluble potato starch varied from 17,496 to 8,553 (or 108 glucoses to 53). The homogeneous dextrin from dry ground potato starch had a molecular weight of 11,858 (or 73 glucose units).

The action of alpha-malt-amylase on the limit dextrins easily degrades them with the destruction of the iodine coloring property and the liberation of reducing products. Klinkenberg (110) reported that certain of the mixed degradation products could be further degraded then by beta-amylase. Hanes (104) confirmed this and observed it to be prominent almost from the beginning of the digestion by the dextrinogenic amylase.

The general characteristics of the action of alpha-malt-amylase on starch have been considered by Klinkenberg (110), Holmbergh (119), Hanes (104) and Freeman and Hopkins (105). When sufficient enzyme is present, the progress curve exhibits two distinct stages: an initial phase in which the reducing power rises rapidly to a value equivalent to 30 to 40 per cent of the theoretical amount of maltose, followed by a second prolonged stage of extremely slow increase in reducing power. The transition appears as an inflection, varying in sharpness in the different curves. By increasing the enzyme concentration not only is the initial velocity increased but the degree of hydrolysis attained after prolonged action is appreciably raised.

In the opinion of Hanes, the most striking feature of the reaction is the more or less abrupt termination of the initial rapid phase of hydrolysis at a reducing level corresponding to 30 to 40 per cent of the theoretical amount of maltose. In a number of cases slope values measured after the transition represented only about one-twentieth of the slopes measured before this point, although there was considerable variation in the general form of the curves and the position of transition. In general the disappearance of the iodine-coloring capacity occurred at or about the same time as the end of the initial phase of rapid degradation.

Ohlsson (115) found that when the iodine color was violet and the reducing value corresponded to a level of

23 per cent of the theoretical amount of maltose, the osmotic pressure stood at 407 millimeters of water as compared with 58 millimeters for the undegraded substance. During the following five days the pressure fell to 277 millimeters which was interpreted as due to the diffusion of particles considerably more complex than maltose. Hence the action of alpha-amylase is pictured as leading to a great increase in the number of non-dialyzing particles which account for the bulk of the reducing value.

Freeman and Hopkins (105) subjected the products of alpha-amylase-starch digestion mixtures to fractionation on the basis of their solubility in alcohol. In two experiments 80 per cent of the material was found to be insoluble in boiling 95 per cent alcohol and to consist of cold water soluble dextrins which would not retrograde. The dextrins freed from maltose showed considerable reducing value, corresponding to a level of about 20.3 to 23 per cent of the theoretical amount of maltose. From fractionation and characterization studies the authors concluded that they consisted for the most part of dextrins of eight and ten glucose units.

It seems then as if the alpha-amylase induces rupture of the linkage responsible for the highly colloidal nature of starch. Hanes has speculated that with this disaggregation the terminal aldehyde groups gain their full reducing value. The fact that this enzyme also liberates esterified

phosphorus may have something to do with its solubilizing action but it seems unlikely since a starch with 0.13 per cent phosphorus would have only one atom of phosphorus to 450 glucose units. Waldschmidt-Leitz and Mayer (120) have shown that extracts from barley and malt in addition to starch saccharification are capable of splitting off phosphorus. This phosphatase can be separated from the saccharifying enzyme by selective adsorption and produces with starch decrease in viscosity and measurable formation of reducing groups as well as complete liberation of all the phosphoric acid.

Hanes and Cattle (121) have measured the absorption spectra at intervals during the action of both the alpha- and the beta-malt-amylases on solutions containing samples of the reaction mixtures with a constant amount of added iodine. Beta-amylase causes a decrease in absorption over the whole spectral range but throughout the digestion the curves display a general similarity in form to that of undegraded starch, i.e., the wave length of the position of maximum absorption is the same throughout. On the contrary the action of alpha-malt-amylase results exclusively in the initial stages in decreased absorption of the longer wave lengths, the values in the violet and blue remaining at first practically constant. Subsequently the values decrease over the whole spectrum and then decrease more pronouncedly in the lower wave lengths; thus as the curves become lower the

position of maximum absorption shifts progressively toward the blue. These workers give curves showing the relationship of the maximum absorption values and the reducing values at intervals during the action of the different amylases which are considered to be characteristic of the type of breakdown rather than the rate. Again the conclusion seems justified that the alpha- or dextrinogenic amylases must attack linkages not attacked by the beta-amylases.

The low reducing value, the capacity for coloring with iodine and the resistance to further degradation by beta-amylase of the limit dextrins, considered in conjunction with the characteristics of the action of both alpha- and beta-amylase seem to warrant the general view that the terminal reducing glucoses in starch exist in some modified grouping which is responsible for the low reducing value of both starch and the limit dextrins and is related in some way with their iodine coloring ability. The action of the beta-amylase would appear then to concentrate these linkages in the residual dextrins as a result of the progressive cleavage of maltose from the non-reducing ends of the molecules.

The coloration of starch with iodine becomes pertinent to the question of the heterogeneity of starch in view of the fact that this property persists in the residual dextrins left by the action of beta-amylase and is destroyed by the

alpha-amylase which reduces the complexity of the residual dextrins.

The color with iodine of starch materials has been found to be independent within wide limits of the mean molecular size although in general the soluble starches and dextrins absorb less iodine the smaller the molecules (122, 123). The presence of electrolytes, particularly potassium iodide, has been demonstrated to increase greatly the amount of iodine absorbed by starch pastes as well as the intensity of the color and in fact the view is held by some that electrolytes are essential to the formation of color (124, 122, 125, 126). The iodine color is not influenced by coupling either with phosphoric acid, silicic acid or phosphoric acid-containing substances (122, 127). The amount of iodine absorbed by a given starch is independent of the concentration of starch within wide limits but increases regularly with increase of the iodine concentration (124). The time of cooking of a starch paste up to about 30 minutes increases its iodine absorption (128).

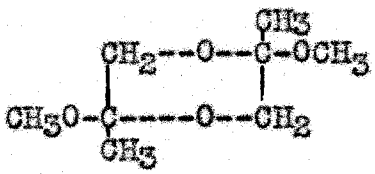
Opinion has been divided as to whether starch-iodine is a true compound or an adsorption complex. Barger and Field (129) argued that the great number of formulae advanced was an argument against the hypothesis of a definite chemical compound. Küster (130) believed that the blue color could satisfactorily be explained by adsorption. This view has been supported by Berczeller (131), Bancroft (132)

and Lottermoser and Ott (133). Euler and Bergman (124) suggested that it is hard to distinguish between adsorption and solution and that these must merge into each other. The presence of adsorption was doubted by them since they could get no optical evidence of surfaces.

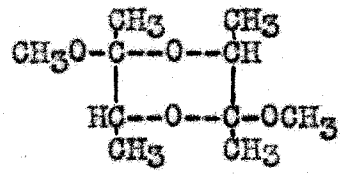
Mylius (134) has studied the absorption of iodine by starch from solutions of iodine in hydriodic acid and has presented apparently sound analytical results to show that iodine and hydrogen iodide are absorbed in the ratio of four moles to one respectively. The iodine content of the isolated complex was estimated to lie between 17 and 19 per cent. Replacement of the hydrogen ion of hydrogen iodide in these complexes with sodium or potassium ions gave soluble starch-iodine products, while replacement with barium or zinc ions gave insoluble products. Bergmann and Ludewig (139) found that acetylated starch in chloroform possesses the typical affinity of starch for iodine and potassium iodide, and takes them up in the ratio of approximately seven moles of iodine to one of potassium iodide. Freudenberg (74) has reported the blue color formed by iodine with methylated starches.

Bergmann (135) discovered that the methyl cycloacetals of acetol and of acetoin existed as dimers which would give colored, unstable, crystalline derivatives with iodine and potassium iodide containing four moles of iodine to one of potassium iodide. These simple acetals have been suggested

by Dirscherl and Braun (136) to possess in the dimer form a dioxane grouping:

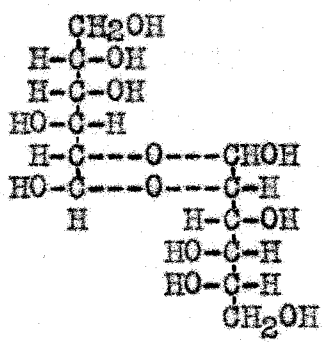


Dimer of Methyl Cyclo-acetal of Acetol



Dimer of Methyl Cyclo-acetal of Acetoin

A similar grouping has been suggested for the unstable dimer of glycerinaldehyde (137) and for the intermediate compound in the transformation of glucose into fructose and mannose by the action of mild alkali (138):



Intermediate Dimer in the Transformation of Glucose to Fructose and Mannose

The above considerations give strong support to the view that the starch-iodine complex represents a definite chemical union, and offer the interesting possibility that the terminal reducing glucoses in starch are involved in some such similar linkage as is found in the simple dimer molecules which is responsible for the iodine coloring ability of starch. This conception likewise might explain the low

reducing value and iodine coloring ability characteristic of the limit dextrins obtained from the beta-amylase digestion of starch. One drawback to such a view is the fact that the various disaggregation processes, e.g., the one of Hirst and Young which leads to decrease in viscosity and increase in reducing value, seem to have little effect on the iodine color. However, the question has not been studied from this viewpoint and it may be significant that the solubilized or "disaggregated" starches as a rule take up less iodine than the original starches.

The action of Bacillus macerans was first observed by Schardinger (140) to effect the solution of various starches. From the resulting products he was able to isolate crystalline compounds which were non-reducing, unfermentable by yeast and possessed the property of forming crystalline iodine derivatives.

The importance of the Schardinger dextrins to the study of the constitution of starch has been recently emphasized by Tilden and Hudson (141) who have shown that the filtrate from a culture of Bacillus macerans contains an enzyme which will produce the dextrins rapidly from starch and in yields of about 40 per cent. This indicates that the dextrins are not synthetic products of the action of living bacteria but are residues from the original starch.

Much study has been devoted to the Schardinger dextrins and in general two distinct constituents have been recognized,

the alpha- and the beta-dextrins. Pringsheim and his coworkers (142, 143, 144, 145, 146, 147, 148) have considered the beta-dextrin to represent a hexasaccharide which could be depolymerized to a non-reducing trisaccharide and the alpha-dextrin to represent a tetrasaccharide which could be depolymerized to a disaccharide. The beta-dextrins were reported to give reddish-brown prisms with iodine and the alpha-dextrins to give fine green needles. Analytical data on the composition of these halogen derivatives showed considerable variation but did bring out the fact that iodides as well as free iodine were involved in the complexes.

The polymerization processes described by Pringsheim have been disputed partly by Karrer (149) and partly by Miekeley (150). Freudenberg and Jacobi (151) appear to have made the most reliable separation of the Schardinger dextrins to get the alpha- and the beta-dextrins and three new dextrins which occurred in slight amounts and were not well characterized. In their opinion the great confusion in the previous work is due to the fact that these dextrins form difficultly separable mixtures, yielding addition products with salt, with water, with alcohol and with other solvents, and like all such substances give with ordinary methods of determining molecular weights false values.

Freudenberg et al. (151, 152, 153) have concluded that the beta-dextrin is a hexasaccharide and the alpha-dextrin is a pentasaccharide and that since the methylated materials

(45.5 per cent methoxyl) give only trimethyl glucose in yields of 96 to 98 per cent on hydrolysis the glucopyranose units must be arranged in large rings of six and five units respectively. This conception was supported by studies on the hydrolysis of the dextrins in 51 per cent sulfuric acid in comparison with similar studies on maltose and starch. With both the dextrins and their methyl derivatives the optical rotations and iodometric titration values passed through a maximum at the beginning of the hydrolysis and then fell to the values for 2,3,6-trimethyl glucose and glucose respectively.

How such units as the Schardinger dextrins can be incorporated into the starch molecule is difficult to see at the present time. Freudenberg, whose work is in agreement with that of the Haworth and Hirst school as to a fundamental unit of 20 to 30 glucoses and as to the production of equivalent amounts of tetra- and dimethyl glucoses from methylated starch (74) has made some suggestions with these ideas in mind (155). If the Schardinger dextrins are produced in a maximum yield of 14 per cent (which may be doubted on the basis of the work by Tilden and Hudson) then such a dextrin must occur in every 35 to 45 glucose units. Inasmuch as two end groups are present in this figure one could assume a five or six membered ring with two side chains, containing a total of 35 to 45 glucose units. No arrangement can be conceived in which more than one Schardinger type exists and thus

starch could possess a degree of polymerization of only 35 to 45 glucose units. He assumes that these forces of association by primary valence are superimposed by a mechanical tying together. This state is unaffected by acetylation, deacetylation, and methylation in accord with Staudinger's polymer analogy. The marked decrease in viscosity when substances of 45 per cent methoxyl content are reached is attributed to the enforced untying of the knots.

Recently Freudenberg et al. (154) have made models of the dextrins with the planes of the glucose units perpendicular to the plane of the ring. No fewer than five glucoses are required to produce such a ring without tension. The ring of the pentosan has the five oxygen atoms of the 1,4-bonds arranged in an equilateral pentagon. Carbon atoms one and four lie in one plane and the other carbon atoms in a second plane. In the beta-dextrin, with six units, the corner oxygen atoms are in two planes. The authors compare these structures with the Hanes screw model of starch which was formulated along the following lines (107).

The production by the action of alpha-amylase of considerable amounts of dextrins containing probably six glucoses (105) has led to the idea that this enzyme needs a large spacing between points of attachment of enzyme and substrate and that such a spacing is provided by a spiral arrangement having six glucoses for each complete turn of the spiral. Thus linkages separated by six glucose units are

brought into lateral proximity and provide a ready basis for interpreting the cleavage of the structure into fragments containing six glucose units.

Assuming the starch molecule to contain 30 glucoses, Hanes pictures the structure as a spiral of five closely packed coils, of which three are readily degraded into maltose by beta-amylase (corresponding to 60 per cent conversion) and the remaining two are resistant due to participation in some obscure type of molecular association.

Further, Hanes suggests that the iodine coloring property must depend on the presence of more than one complete coil of the spiral since degradation by the alpha-amylase destroys this property.

STATEMENT OF PROBLEM

This thesis reports a study of three methods of fractionating starch: electrodialysis, freezing and enzymatic digestion. It was necessary to study the retrogradation or aging of starch pastes to determine the effect, if any, of this phenomenon on the method of electrodialytic separation. A comparative study of the residual dextrins, prepared by beta-amylase digestion of ordinary cornstarch and of waxy cornstarch, was made to throw some light on the question of the heterogeneous structure of starch.

This study has been concerned primarily with cornstarch although, in some instances, other starches have been used for purposes of comparison. Waxy cornstarch, in view of its great dissimilarity to ordinary cornstarch, has been used for comparison throughout.

EXPERIMENTAL

Fractionation of Starch Pastes by Electrodialysis

The electrodialyzer described by Hixon and Dawson (156) was used since it was designed to meet the requirements for quantitative work on starch. This apparatus has the advantages of a large volume (about 1500 cc.), a large electrode area, minimum distance between the electrodes, small electrode compartments and ease of manipulation. It was operated from a source of direct current electricity supplied by a vacuum tube rectifier equipped with resistances in order to vary the voltage. Variation in the current source from zero to 500 volts with a capacity of 100 milliamperes was necessary. The progress of the electrodialysis was followed by noting the voltage and current going through the cell and also by titrating the liquid from the electrode chambers with .1 N acid or base.

The data presented in Table V, taken from the paper of Hixon and Dawson, are characteristic of the operation of the electrodialyzer on a one per cent cornstarch paste. The applied voltage was increased from 200 at the beginning of dialysis to 500 volts in the course of four or five hours, taking care that the current was never more than about 100 milliamperes.

TABLE V. Rate of Dialysis of a One Per Cent Cornstarch Paste

Time Hours	Amperage Milliamps*	Rate of Electrolyte Removal 0.1 N Acid or Base cc/hour	
		Anode	Cathode
0	150		
0.5	105	5.2	9.4
1.0	85	4.2	1.5
2.0	65	3.2	0.9
3.0	55	2.3	0.7
4.0	40	1.9	0.6
5.0	30	1.6	0.6
6.0	28	1.3	0.5
7.0	28**	1.1	0.4
11.0	28***	0.6	0.3
15.0	28****	0.3	0.1

* To get comparative readings, current source was adjusted to 200 volts before each reading.

** Free electrolyte practically all removed.

*** Distinct separation of gel phase is apparent.

**** Separation of gel phase is complete.

Method

The method finally adopted consisted of one electrodialytic separation. The amount of soluble fraction was calculated from the analyses of two 50 cubic centimeter aliquots of the supernatant liquid and the total volume of paste. The material in the aliquot samples was found after the samples had been evaporated to dryness and brought to constant weight in an oven at 100°C. The insoluble fraction was derived by difference.

This procedure was checked by an alternate method which consisted of successive resuspension of the insoluble

fraction by boiling for five minutes in a fresh volume of water followed each time by electrodialysis until the amount of soluble fraction in the supernatant liquid became negligible. The soluble fraction was found from the volumes of supernatant liquid in each separation and the analyses of 50 cubic centimeter aliquots of each. When it was desired to isolate the soluble fraction, the supernatant liquors were concentrated in vacuo to a few hundred cubic centimeters and treated with three or four volumes of alcohol. The precipitated material was allowed to settle and after drawing off of the alcohol-water solution, was dehydrated in absolute alcohol. Finally it was filtered by suction and dried in the vacuum oven at 70°C.

The slimy, gelatinous residue from the final dialysis was, after siphoning off of the supernatant liquid, treated with three to four times its volume of absolute alcohol. After settling and decanting, the insoluble fraction was completely dehydrated in absolute alcohol. It was then filtered by suction, dried in vacuo at 70°C. and weighed.

Results

Comparison of two methods for determining the degree of fractionation by electrodialysis. The following tabulation (Tables VI and VII) shows the agreement of the two procedures for cornstarch paste prepared by heating at 100°C. for thirty minutes. These pastes were passed through a small hand

emulsifier three times which was found by microscopic examination to completely rupture the swollen granules. Pastes of not more than two per cent were used. Electrodialysis of an emulsified four per cent cornstarch paste gave a separation after about 24 hours but the sediment or insoluble material occupied practically the total volume of the cell.

In general about 20 hours were allowed for these separations. Although the amperage fell to a constant minimum value after about ten hours and a separation was observable soon after (about two hours), the dialyses were continued for an additional nine to ten hours to insure complete separation. Considerably less time was needed for the electrodialysis of the resuspended residues.

TABLE VI. Degree of fractionation of emulsified cornstarch paste as determined by analysis of supernatant liquid following first dialysis.

Trial	Concentration of Paste (per cent)	Soluble Fraction (per cent)	Insoluble Fraction by Difference (per cent)
1	1.25	43.5	56.5
2	1.25	40.0	60.0
3	1.25	42.0	58.0

TABLE VII. Degree of fractionation of emulsified cornstarch paste as determined by repeated dialysis and isolation of the insoluble fraction.

Trial	Concentration of Paste (per cent)	Soluble Fraction Removed in Four Successive Dialyses (per cent)	Total Amount of Soluble Fraction (per cent)	Insoluble Fraction by Isolation (per cent)
1	1.33	24.9, 7.8, 3.3, 2.1	38.0	60.0
2	1.33	25.8, 6.4, 4.2, 2.0	38.3	60.0
3	1.25	32.9, 4.8, 2.7, 2.5	42.9	45.6*

* This value was low for some cause.

These results show that the two methods give comparable values for the relative amounts of soluble and insoluble fractions for any given starch paste. In subsequent experiments the more rapid method, consisting of a single electrodialytic separation, was used.

Effect of temperature of preparing paste on the degree of fractionation of cornstarch. Cornstarch pastes of 1.25 per cent concentration, prepared by heating at 80°, 100°, 120° and 129°C. for a period of thirty minutes, were cooled to room temperature and then electrodialyzed. The method of preparing these and other pastes was to suspend the calculated weight of starch, corrected for moisture content, in a small volume of water, then to pour this suspension into a given volume of water already at the desired temperature. The pastes were stirred throughout the period of heating and during the time of cooling to room temperature. In order to

avoid the necessity for washing off the paste adhering to the walls of the vessel a slightly larger quantity of paste than was necessary was prepared and a given volume of this was introduced into the dialyzer. The pastes heated at 120° and 129°C. were made up in boiling water and then placed in the autoclave at the desired temperature.

The results of these experiments are shown in Table VIII.

TABLE VIII. Effect of temperature of preparing paste on degree of fractionation of cornstarch by electrodialysis.

Temperature of Preparing Paste (degrees centigrade)	Soluble Fraction (per cent)	Insoluble Fraction by Difference (per cent)
80	14.0	86.0
100	22.0	78.0
120	25.2	74.8
129	31.1	68.9

With these dialyses it was observed that in from two to three hours the amperage had dropped to a constant minimum value and separation had begun. Twelve to fifteen hours were allowed for complete separation. The insoluble residue was found to collect mostly in the bottom of the cell rather than on the electrode membrane as was observed with the homogenized pastes prepared at 100°C.

Effect of the rupture of the granules on the degree of fractionation of cornstarch. In a preceding section (page 63) it has been shown that mechanical rupture of the granules of

cornstarch paste prepared at 100°C. leads to an increase in the amount of the soluble fraction by electrodialysis. The results shown in Table VI are, for the sake of comparison, included in Table IX with others on pastes which contained no unruptured granules.

With the electrodialysis of the pastes prepared at 120° and 129°C., the initial amperage was very high and considerable time, from ten to twelve hours, elapsed before a constant minimum value was reached. These pastes were dialyzed for about 24 hours and at the end it was noted that much of the insoluble material had collected in the bottom of the cell in contrast to the behavior of the homogenized pastes prepared at 100°C. The paste made from the dry-ground starch was fractionated in about 15 hours to give a quite cloudy supernatant liquor.

Electrodialysis of waxy cornstarch paste. A 1.16 per cent paste of waxy cornstarch prepared by boiling for three minutes was used. Microscopic examination revealed the presence of swollen unruptured granules, a few unswollen granules which still showed the characteristic polarization crosses and numerous small birefringent particles. These were later found to be calcium carbonate. The paste was passed through the small hand emulsifier three times which served to rupture the swollen granules. Electrodialysis of this paste for five days did not effect a separation.

TABLE IX. Effect of rupture of granules on the degree of separation of cornstarch pastes by electrodialysis.

Temperature of Preparing Paste (degrees centigrade)	Concentration of Paste (per cent)	Treatment of Paste	Soluble Fraction (per cent)	Insoluble Fraction by Difference (per cent)
100	1.25	Homogenized after heating	43.5	56.5
100	1.25	Homogenized after heating	40.0	60.0
100	1.25	Homogenized after heating	42.0	58.0
120	1.25	Homogenized after heating	34.1	65.9
129	1.25	Boiled 15', homogenized, then heated at 129°C. for 30'	30.2	69.8
129	1.25	Same as above	29.9	70.1
100	4.00	Starch was dry-ground 600 hrs. before pasting	80.0*	22.5*

* These figures based on the weights of the isolated materials.

Properties of the soluble and insoluble fractions obtained by electrodialysis of cornstarch pastes.

Solubility

Neither one of the fractions is soluble in the real sense of the word. In every case the supernatant liquors containing the so-called soluble fraction were cloudy. The insoluble fraction or amylopectin as it has been called was found to be easily redispersed in boiling water and is obviously responsible for the pasting and gelling properties of starch. The soluble fraction which is known as beta-amylose or amylose was very difficult to redisperse. Even prolonged boiling was not sufficient to effect solution. Both fractions dissolved readily in 2.5 per cent aqueous alkali to give transparent solutions. The solution of amylopectin becomes cloudy white on neutralization while the solution of amylose does not.

Iodine Color

The amylopectin and the amylose obtained by electrodialysis of homogenized cornstarch pastes gave identical blue colors with iodine-potassium iodide solutions when compared in equal concentrations. The amylopectin prepared from the 600-hour ground starch by electrodialysis gave a slightly purple color while the amylose gave the characteristic blue color.

Optical Rotation

Due to the inability to obtain clear solutions of these samples in water the rotations were measured in 2.5 per cent aqueous sodium hydroxide. One-tenth gram samples in 25 cubic centimeters of solution were used. The readings were taken in two decimeter tubes with sodium light. See Table X for the values of optical rotation.

TABLE X. Optical rotations of the soluble and insoluble fractions obtained by electrodialysis of cornstarch pastes.

Sample	$[\alpha]_D^{25°}$
Insoluble fraction, by electrodialysis of homogenized cornstarch paste prepared at 100°C.	+205.0°
Soluble fraction, by electrodialysis of homogenized cornstarch paste prepared at 100°C.	+141.2°

Reducing Value

The reducing values of these dextrins and others reported in this thesis were determined by the method of Richardson, Higginbotham and Farrow (81) which was briefly as follows:

Suitable quantities of the material (.2 to .4 gm.) made into a paste or dissolved in water were weighed into 50 cubic centimeter centrifuge tubes and the weight made up to 15 grams with water. Five cubic centimeters of a .04 per cent

glucose solution were added. Twenty cubic centimeters of boiling Braidy solution, prepared by mixing 3.6 grams of solid mixed carbonates*, 18 cubic centimeters of mixed carbonate solution** and two cubic centimeters of ten per cent copper sulfate solution, were then poured into the tubes which were covered with watch glasses and heated in a boiling water bath for three hours. The tubes were then removed and centrifuged until the cuprous oxide along with any undissolved starch formed a definite layer on the bottom. The supernatant liquid was siphoned off and the precipitate washed by stirring up with water and recentrifuging until the washings were colorless. The precipitated cuprous oxide was dissolved in warm, acidified ten per cent iron alum solution and titrated with .25 N ceric sulfate solution*** with one drop of diluted o-phenanthroline solution**** as internal indicator.

A blank experiment with five cubic centimeters of .04 per cent glucose solution alone was carried out at the same time and the titration figure obtained was subtracted from that of the glucose-starch mixture. The titration difference, expressed as milligrams of copper by multiplying by the

---

* Anhydrous Na_2CO_3 130 parts; $NaHCO_3$ 50 parts.

** Anhydrous Na_2CO_3 130 gms., $NaHCO_3$ 50 gms., water to one liter.

*** In 1.0 N sulfuric acid.

**** 0.695 gms. ferrous sulfate and 1.485 gms. o-phenanthroline in 100 cc. water. Dilute four fold.

appropriate factor was divided by the weight of the starch used to give the reducing power. This is expressed as R_{cu}.

The results of the determination of reducing power are shown in Table XI. The values for chain length were calculated according to Farrow from the ratio of the reducing power of the samples to that of maltose which is reported by Farrow to be 8,055. The acceptance of these values for chain length must be provisional as was brought out in the discussion of the literature on this question.

TABLE XI. Reducing values of the soluble and insoluble fractions obtained from cornstarch by electrodialysis.

Sample	R_{cu}	Chain Length
Cornstarch	3.5	1200
Cornstarch, pasted and precipitated with alcohol	3.4	1200
Soluble fraction, by electrodialysis of a homogenized paste prepared at 100°C.	4.45	970
Insoluble fraction, by electrodialysis of a homogenized paste prepared at 100°C.	1.26	3400
Cornstarch, ground 600 hours in a ball mill	14.00	300
Soluble fraction, by electrodialysis of a paste prepared from the 600-hour ground starch	13.82	310
Insoluble fraction, by electrodialysis of a paste prepared from the 600-hour ground starch	6.85	630

An attempt to determine the reducing value of waxy cornstarch was unsuccessful. Centrifugation of the samples resulted in a voluminous gel in the bottom of the tubes. The titration values for these samples were less than the blank.

Discussion

The results of all the electrodialytic separations are shown graphically in Figure 1. Certain of these are in agreement with those of other workers who used similarly treated pastes of cornstarch. Taylor and Beckmann (26) obtained 62.5 per cent of an insoluble fraction by electrodialysis of a homogenized paste prepared at 100°C. and 84.1 per cent of a soluble fraction by sedimentation of a paste prepared from 600-hour dry-ground cornstarch. Taylor and Keresztesy (27) obtained 91.8 per cent of a soluble fraction from a paste of 672-hour dry-ground cornstarch by electrodialysis.

From these experiments the general conclusion can be drawn that the degree of separation of a cornstarch paste by electrodialysis will depend on the temperature at which the paste is prepared as well as the presence or absence of intact granules. The time of heating naturally would have an effect and has been made the same for all of these samples. Thirty minutes was used as it is generally accepted that little further change in the degree of dispersity of starch takes place after this time of heating at a given temperature.

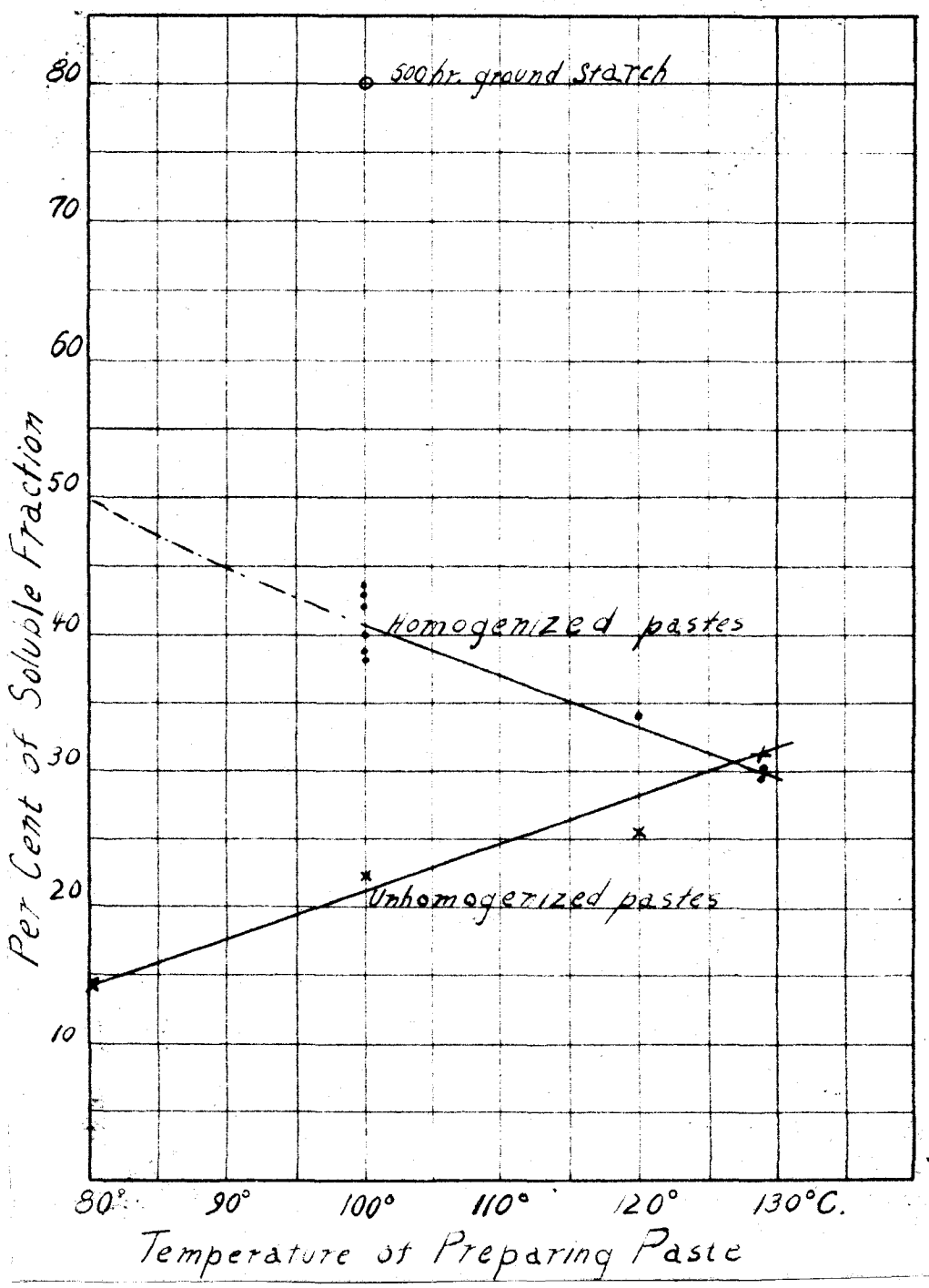


FIGURE 1. Relation of the rupture of the granules and temperature of preparing paste to the degree of fractionation by electrodialysis.

With the unhomogenized pastes the per cent of soluble fraction is shown to increase with the temperature of preparing the pastes. However, this tendency to increase of the amount of soluble fraction with increased temperature of preparing the paste seems to be counteracted by some phenomenon as is shown by the fact that homogenization or rupture of the granules of the 100°C. paste increased the soluble fraction from 22 to 40 per cent while the same treatment of the 120°C. paste increases the soluble fraction from 25.2 to only 34 per cent. Furthermore a paste prepared at 100°C., homogenized and then held at 129°C. for 30 minutes gives only 30 per cent of the soluble fraction. This last checks with the 31.1 per cent of soluble fraction obtained with the unhomogenized 129°C. paste and thus indicates that the degree of disorganization of the granules in the 129°C. paste is not further augmented by homogenization. From the appearance of the curves it would seem probable that rupture of the granules of an 80°C. paste would lead to a larger amount of the soluble fraction than was obtained by homogenization of the pastes prepared at the higher temperatures. This could not be checked as the granules at 80°C. were not swollen enough to undergo rupture in the small hand emulsifier.

The increased temperature of preparing the pastes appears to lead to increased amounts of the soluble fraction only in so far as it brings about greater disorganization of the

granules; the real effect of increased temperature is apparently to decrease the quantity of the soluble fraction obtained by electrodialysis.

This phenomenon cannot be explained by a more rapid reprecipitation or retrogradation of the pastes prepared at higher temperatures. In a later section it is shown that under the conditions of the electrodialytic separations little or no retrogradation takes place and furthermore that retrogradation is slower with the pastes prepared at higher temperatures.

Increased temperature must bring about a change in the colloidal condition of the starch. Significant in this respect are the observations that with the homogenized pastes prepared at 120° and 129°C. a little longer time was necessary for the amperage to reach a minimum value and for separation to begin. In addition, most of the insoluble material settled in the bottom of the cell and did not collect on the electrode membrane as was observed with the homogenized pastes prepared at 100°C. The implication is that increased temperatures serve to liberate more free electrolytes from the starch in accordance with the views of Samec. The starch freed of electrolyte does not migrate to the electrode membrane with the current, nor can it remain suspended. With the unhomogenized pastes the amperage fell rapidly to a minimum value and separation began, indicating relatively small amounts of free electrolytes were present.

It is evident that the fractionation of starch by electrodialysis is partially determined by the distribution and attachment of electrolytes, i.e., phosphoric and fatty acid polar groups. When this relationship is disturbed either by heat or by mechanical rupture of the granules, the degree of separation is affected.

The increase of the soluble fraction to 80 per cent in the case of the 600-hour dry-ground starch must be attributed in part to hydrolytic cleavage into smaller molecules as indicated by the increase of reducing power from 3.5 for the original starch to 14 for the ground starch. If the reducing values of the soluble and insoluble fractions may be taken as an indication of mole size, then it is seen that the insoluble fractions in each case consist of relatively larger mole sizes than do the corresponding soluble fractions even though the degree of fractionation may be shifted in one direction or the other depending on the treatment of the paste before electrodialysis. That the particle size of the insoluble material is greater than that of the soluble material is generally accepted.

Finally, it should be pointed out that the so-called soluble fraction is not actually soluble. Its solutions when taken from the dialyzer are cloudy and, after isolation by precipitation with alcohol, it cannot be redissolved in water even with boiling. It would be better to define it as

the fraction remaining suspended in the mother liquor after electrodialytic equilibrium has been established.

Fractionation of Starch Pastes by Freezing

Method

Five hundred cubic centimeters of 2.5 per cent pastes were frozen in the electric icebox. The time required for freezing was about three hours. The frozen paste was then melted at room temperature and centrifuged. The sediment was dehydrated in absolute alcohol, dried in vacuo at 70°C. and weighed. The supernatant liquor was concentrated in vacuo at 45°C. almost to dryness and was then treated with three or four volumes of alcohol. The precipitated material, after decantation of the alcohol-water mixture, was dehydrated in absolute alcohol. Finally it was filtered by suction, dried in vacuo at 70°C. and weighed.

Results

The data for a number of different samples are shown in Table XII.

Cornstarch pastes either homogenized or unhomogenized show ribbon-like films in the melted pastes. These are stained intense blue with iodine which shows them up more clearly. The material is centrifuged out to give a tightly packed, spongy sediment leaving a water-clear supernatant

TABLE XII. Degree of fractionation of starch pastes as determined by freezing and centrifugation of the melted pastes.

Starch	Treatment	Insoluble Fraction (per cent)	Soluble Fraction (per cent)
Corn	Pasted at 100°C., homogenized	93.2	5.2
Corn	Pasted at 100°C., homogenized	96.8	2.15
Corn	Pasted at 100°C., homogenized	90.0*	9.3*
Corn	Dry-ground 600 hours, pasted at 100°C.	60.6	36.1
Waxy corn	Pasted at 100°C.	0	100.0
Potato	Pasted at 100°C.	79.5	20.4
Tapioca	Pasted at 100°C.	A very slight demarcation was observed but was not sharp enough to allow separation.	
Beta-amylose	Used supernatant liquor from electrodialysis of homogenized cornstarch paste prepared at 100°C.	96.7	3.0

* Melted paste was heated to boiling and cooled before centrifugation, which left a cloudy supernatant liquor.

liquid. The melted paste of dry-ground cornstarch does not show the long ribbon-like forms but consists of granular amorphous particles. These centrifuge out easily to leave a water-clear supernatant liquid.

A melted cornstarch paste brought to boiling before centrifugation sediments only slowly, forming a loosely packed deposit and leaving a slightly cloudy supernatant liquid.

Melted potato starch paste did not sediment to give the fibrous, spongy mass as did the cornstarch pastes. Flake-like forms were observable, however, in the paste before centrifugation.

Waxy cornstarch paste had the same appearance after melting as before freezing and showed no sign of a separation on centrifugation.

Tapioca starch similarly had the same appearance after melting as before freezing. No concrete forms were visible under the microscope. However, a slight line of demarcation was observed on centrifugation but was not sharp enough to warrant separation.

The melted solution of the beta-amylose from cornstarch contained the filmy, ribbon-like forms observed in the melted cornstarch pastes but these were much smaller. Centrifugation gave the same fibrous, spongy deposit leaving a clear supernatant liquid.

Determination of the reducing power of the soluble fraction from the homogenized cornstarch paste and of the soluble

fraction from the dry-ground starch gave values of 12.80 and 19.96 respectively. These correspond to values of 340 and 210 for chain length.

Discussion

The above experiments represent only a cursory study of the fractionation of starch by freezing but the results do show that the treatment of the starch as well as the kind of starch determine the degree of fractionation.

The freezing out process is not satisfactory from the standpoint of obtaining the same fractions as are obtained by electrodialysis. The soluble fraction obtained by electrodialysis, at least with cornstarch, is by freezing transformed into a material which could never be recovered by extraction with water even at 60°C. as is suggested by some workers. Prolonged boiling of frozen cornstarch pastes (2½ hours) is not sufficient to return them to their original degree of dispersity and what is more the mucilaginous amylopectin is more easily redispersed by heating.

Separation of the two fractions by solution of the frozen cornstarch in two per cent alkali and neutralization so that the amylopectin reprecipitates was not satisfactory. The solutions did become cloudy white on neutralization but centrifugation did not effect a separation.

According to Farrow's conception, based on a study of a series of acid modified starches, the freezing of a starch

paste serves to separate all molecules above a certain critical length into one fraction and all those below this size into another fraction. Naturally this critical length would be expected to be subject to variation in the concentration of each mole size as determined by the concentration of the paste and the distribution of the various mole sizes. Also must be taken into consideration the possibility that molecules ordinarily soluble may under the conditions of freezing associate to produce larger insoluble particles.

In any case, it is evident that the freezing process is more truly a separation of soluble and insoluble fractions than is the electrodialytic process. This is indicated by the fact that the soluble fractions obtained by freezing are recovered from water-clear solutions. Taking the reducing values as an indication of chain length, it is seen that cornstarch by freezing gives from two to five per cent soluble fraction of 340 average chain length as compared with a value of 1200 for the original starch. Dry-ground cornstarch gives 36 per cent soluble fraction with an average chain length of 210 as compared with 300 for the original dry-ground starch. Thus dry-grinding serves to reduce the average particle size of the starch to the extent that 36 per cent becomes soluble.

If separation by freezing is an indication of average mole size then the starches examined can be arranged in the order of the increasing proportions of soluble fraction as

follows: cornstarch, potato starch, dry-ground cornstarch, tapioca starch and waxy cornstarch.

With cornstarch it is seen that at least three distinct fractions have been obtained. By electrodialysis a separation based roughly on differences in mole size is obtained. The degree of separation is affected by the attachment of the electrolytes associated with the starch. A third fraction, which obviously constitutes part of the amylose fraction by electrodialysis, is obtained by freezing of cornstarch pastes. This fraction may be defined as the fraction which is completely soluble in water and which does not associate under the conditions of freezing to become insoluble.

Retrogradation or Aging of Starch Pastes

Methods of study

The procedure described by Sallinger (90) slightly modified was used. Pastes of from .5 to two per cent concentration were aged in the refrigerator at 7°C. At intervals, two 50 cubic centimeter portions were pipetted out and to each was added .5 cubic centimeters of filtered saliva. After digestion for 24 hours at room temperature the undigested residues were filtered carefully by suction (under a pressure of not more than 20 centimeters of mercury) through tared Gooch crucibles containing first a layer of fine asbestos and then a layer of sea sand to prevent clogging

and speed up filtration. The residues were washed in the crucibles five times with about five cubic centimeter portions of water. Finally the crucibles were dried at 100°C. to constant weight (about 1½ hours). The samples for zero time of aging were taken immediately after the rapid cooling of the freshly prepared pastes to 7°C. in an ice-salt bath.

The concentration of the original paste was known from the dry weight of the starch used and the volume of paste. In some cases 50 cubic centimeter aliquots were analyzed by evaporation and drying to constant weight in a 100°C. oven.

Results

Aging of soluble potato starch paste. A 1.87 per cent paste, prepared by heating for five minutes at 100°C. was used. The data are shown graphically in Figure 2, by plotting the per cent of undigestible residue against the time of aging. The per cents of residue as determined from both weighings of each analysis are shown to indicate the degree of precision. All subsequent results are plotted on the same scale to facilitate comparison.

Aging of cornstarch paste. A 2.17 per cent paste, prepared by boiling for five minutes was used. The results are shown in Figure 3. Similar results of a second experiment with a 1.59 per cent paste are shown in Figure 4.

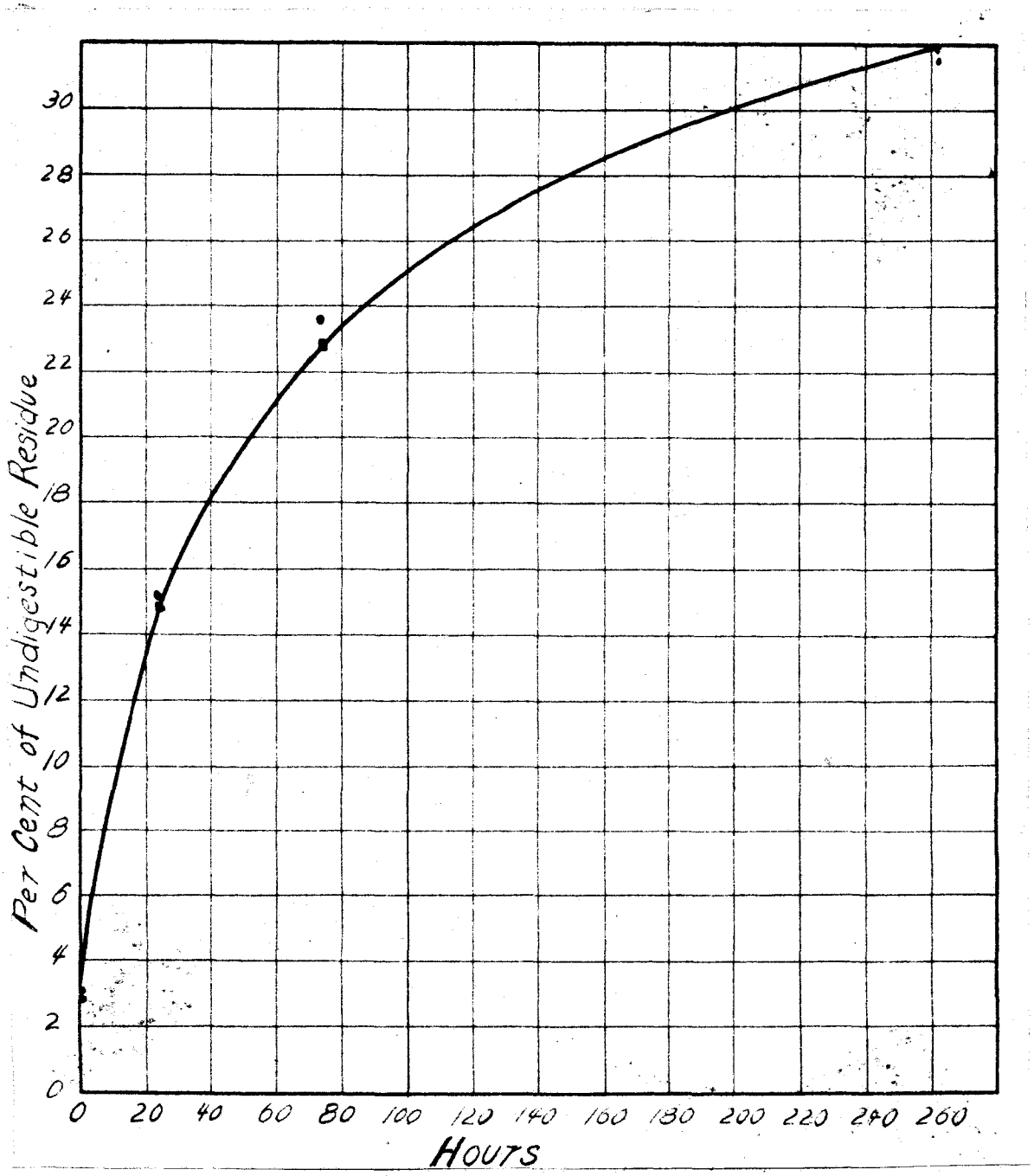


FIGURE 2. Aging of soluble potato starch paste (Lintner).

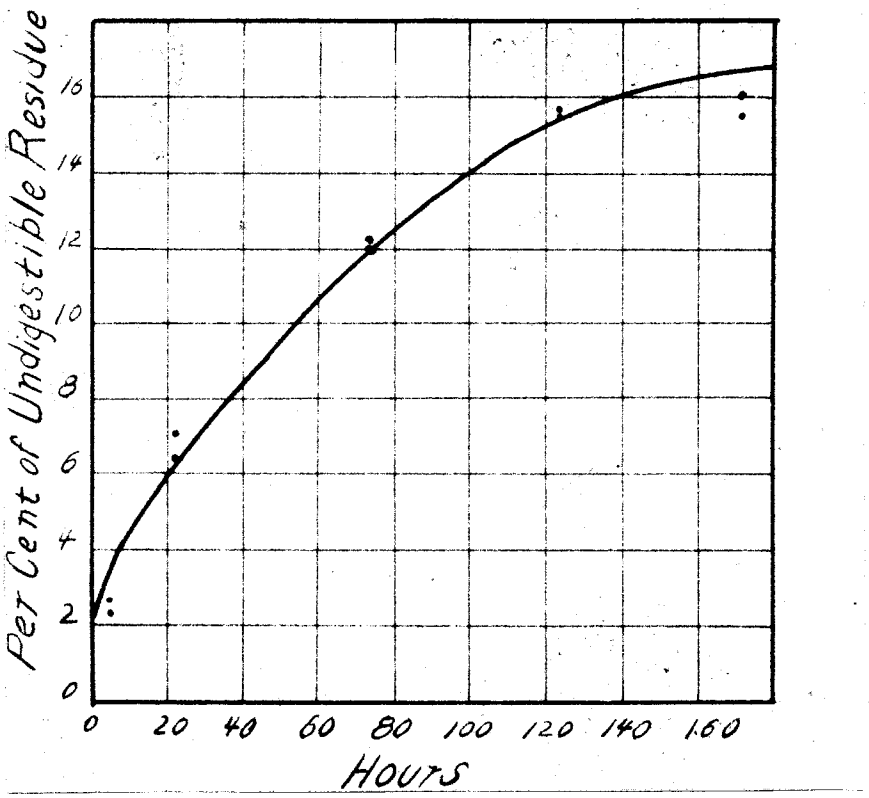


FIGURE 3. Aging of a 2.17 per cent cornstarch paste.

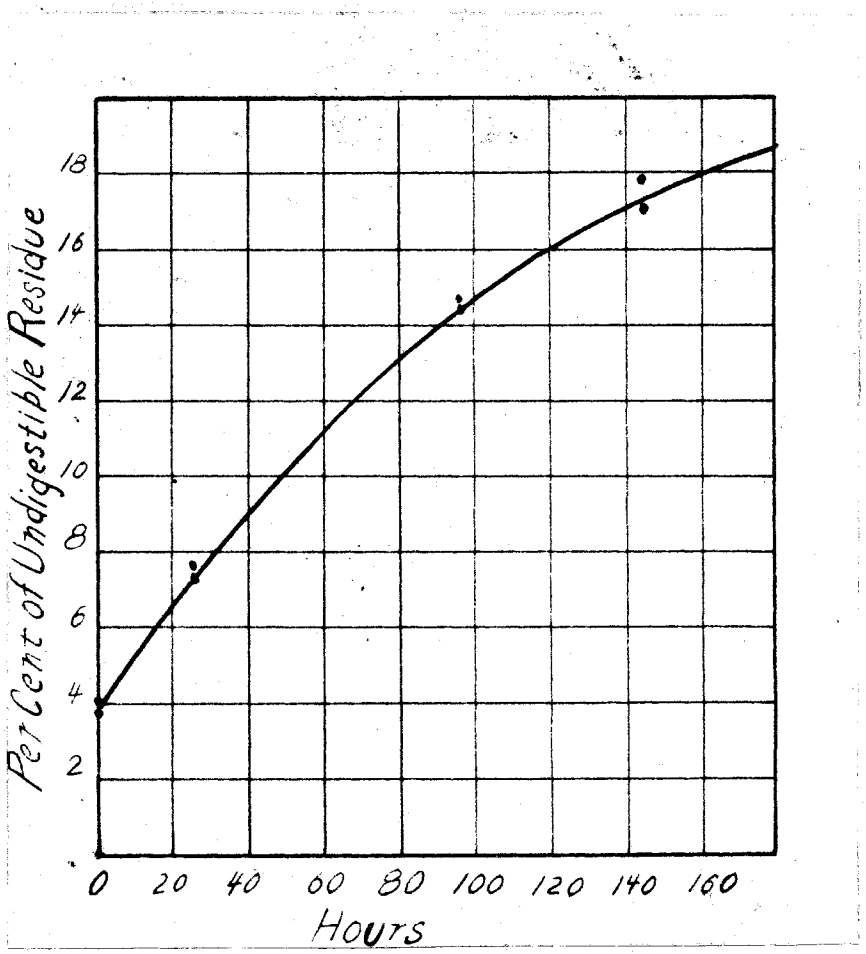


FIGURE 4. Aging of a 1.39 per cent cornstarch paste.

Microscopic examination of the aged paste showed it to be apparently the same as before aging, though the granules may possibly have shrunk somewhat. After digestion the granules still appared as distinct entities but had ragged indistinct boundaries and were only weakly stained with iodine. Boiling of the solutions as well as shaking with glass beads was found to increase their coloration with iodine.

Effect of temperature of preparing paste on the aging of cornstarch pastes. Cornstarch pastes of 1.35 per cent concentration were prepared at 80°, 90°, 100° and 120°C. by heating for a period of 30 minutes. The data are shown in Figure 5.

The paste prepared at 80°C. starts to retrograde almost at once, though much more slowly than the pastes prepared at 100°C. for five minutes. The 90°C. paste apparently starts to retrograde at 180 hours. The 100° and 120°C. pastes retrograde little, if at all, up to 336 hours.

Effect of rupture of the granules on the aging of cornstarch pastes. A 1.39 per cent paste prepared by heating at 100°C. for five minutes was, after cooling, passed through the small hand homogenizer three times which by microscopic examination was shown to have ruptured all the granules. The results of aging (Figure 6) show that this paste retrogrades more slowly than an unhomogenized paste prepared by heating

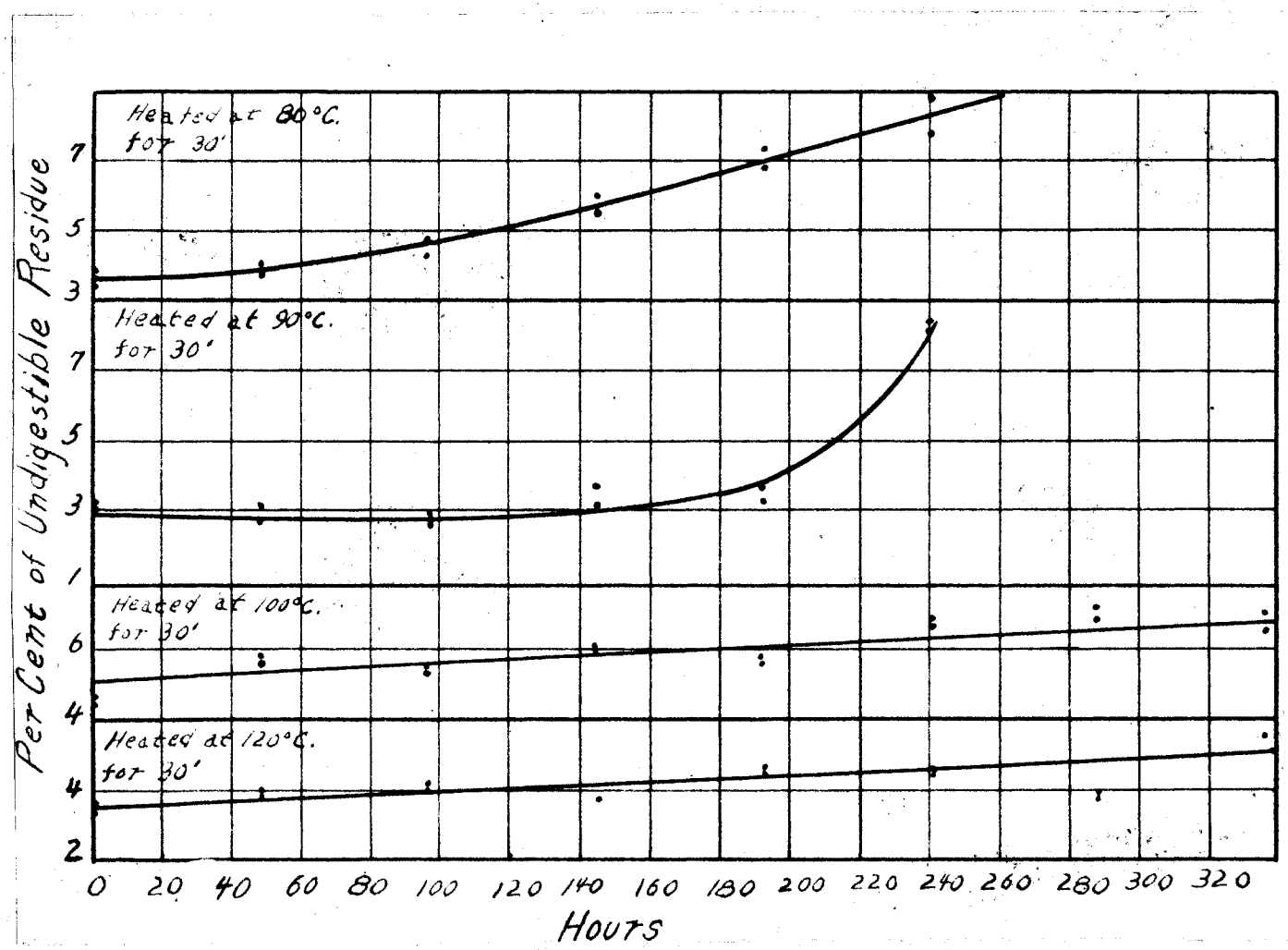


FIGURE 5. Influence of temperature of preparing paste on the aging of cornstarch pastes.

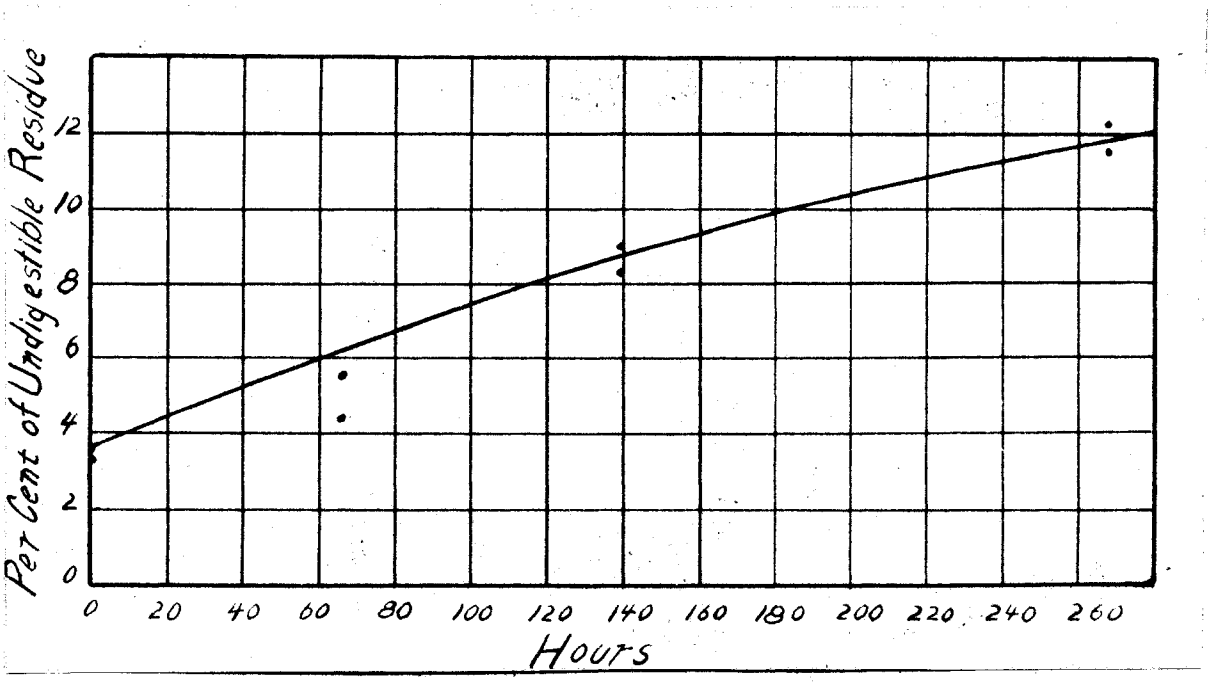


FIGURE 6. Aging of a homogenized cornstarch paste.

for the same length of time (Figure 4) but more rapidly than the paste heated 30 minutes.

Aging of the soluble and insoluble fractions of cornstarch. The insoluble or amylopectin fraction was obtained by electrodialysis of a homogenized cornstarch paste prepared by heating at 100°C. It was suspended in boiling water before aging. The concentration was 1.2 per cent.

Since the soluble fraction or amylose is so difficult to redisperse after isolation, the supernatant liquid obtained by electrodialysis of a homogenized paste was used directly. The concentration was .56 per cent. The data on both these materials are shown in Figure 7.

At zero time the soluble or amylose fraction gives only .65 per cent of undigestible residue in comparison with the 3.25 per cent found with the amylopectin and the 2.5 to 4.0 per cent found with the other samples of cornstarch. The amylose was slower to start retrograding than the amylopectin, but at the end of 300 hours both had retrograded to about the same extent. The curve for the amylopectin is almost identical with the one for homogenized cornstarch paste (Figure 6).

Aging of dry-ground cornstarch and of waxy cornstarch. The cornstarch, dry-ground 600 hours in the ball mill, was pasted by boiling for five minutes. With a 1.39 per cent paste no evidence of retrogradation was seen up to 214 hours.

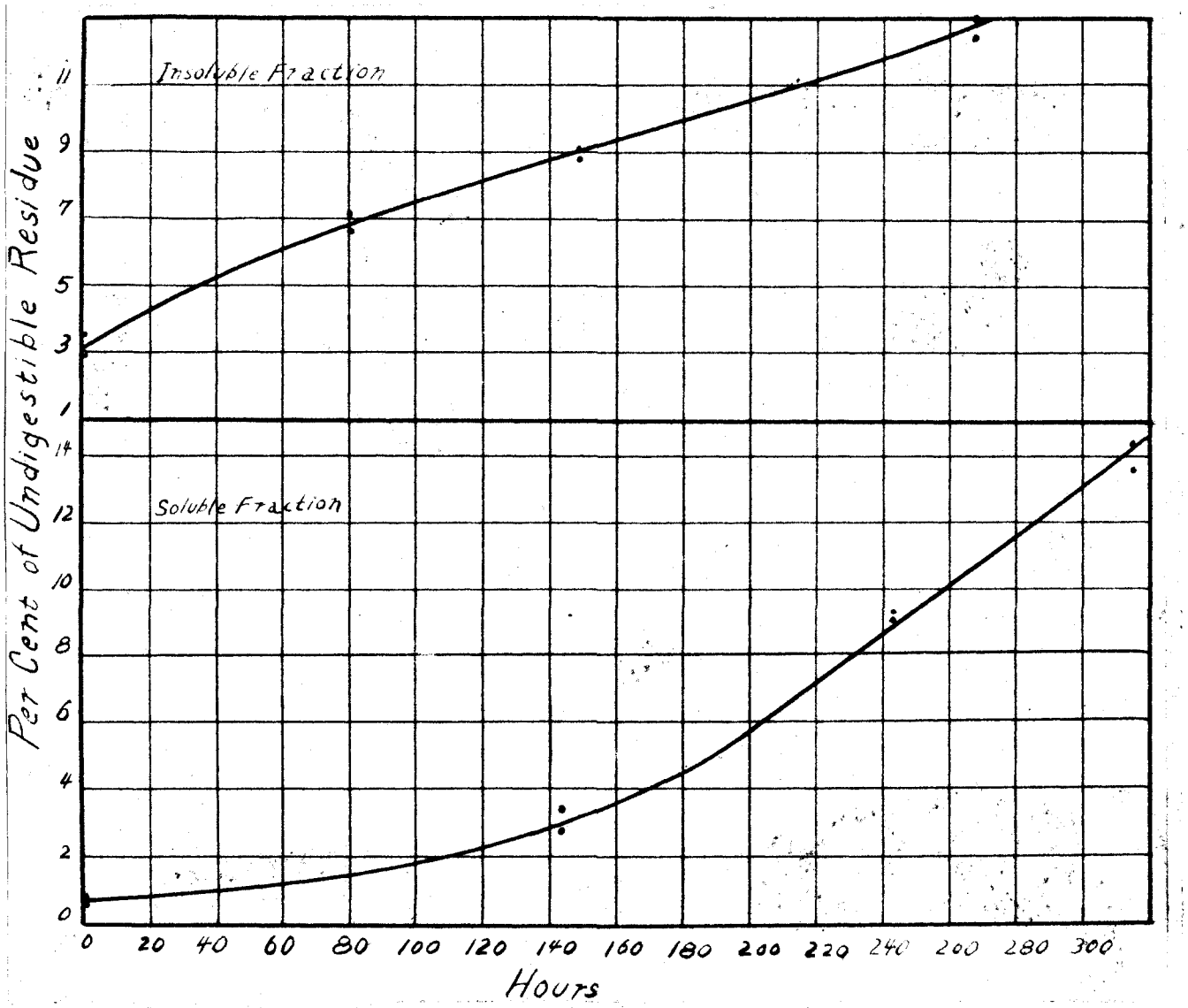


FIGURE 7. Aging of the soluble and insoluble fractions of cornstarch.

A 1.35 per cent paste of waxy cornstarch prepared by heating at a 100°C. for five minutes did not retrograde up to 450 hours.

Effect of heat and mechanical treatment on retrograded cornstarch pastes. A 1.39 per cent paste prepared by boiling for five minutes was kept in the refrigerator 168 hours during which time the per cent of undigestible residue increased from 2.15 to 16.9. A portion of this paste was boiled for 30 minutes with addition of water to maintain constant volume. As a result of this treatment the per cent of undigestible residue fell to 7.9 per cent. The remainder of the paste which now had been in the refrigerator 215 hours and showed 18 per cent of undigestible residue was passed through the homogenizer ten times. No granules could now be detected in the paste and yet the per cent of insoluble residue was found to be 18.3 per cent, i.e., unchanged. The homogenized paste was still cloudy white as is characteristic of the aged pastes. Freshly prepared pastes are more transparent.

Discussion

The effect of retrogradation on the electrodialytic separation of starch would seem to be negligible. A paste prepared at 100°C. for 30 minutes shows no appreciable retrogradation up to 336 hours. A paste heated only five minutes

at 100°C. and then homogenized retrogrades at the most only about two per cent in 20 hours at 7°C. Thus in 20 hours, the upper limit of time necessary for electrodialysis, the amount of retrogradation would be negligible regardless of how the paste is prepared. It must be kept in mind, however, that retrogradation is indicated by the increased resistance to enzyme digestion. This may not necessarily be correlated with the change in physical properties of the starch. In other words, the starch may retrograde to the point of precipitation and still be solvated enough for digestion by saliva.

According to Sallinger (90) logarithms of the value of time and per cent of undigestible residue when plotted against each other give a straight line. The general equation for the line is:

$$\log G = a \log t + \log g$$

G is the per cent of undigested residue, t is time and a and g are constants considered to be characteristic of the degree of condensation and the type of starch respectively. Log g is evaluated by setting t equal to zero in which case log G equals log g. The constant a is found from the slope of the line. Application of this treatment to the aging of the soluble potato starch (Figure 2) is shown in Figure 8. An approximately straight line results with g equal to 3.8 and a equal to .420. These values compare favorably with those reported by Sallinger for soluble potato starch:

$$g = 4.06,\ 3.00$$

$$a = .445,\ .455$$

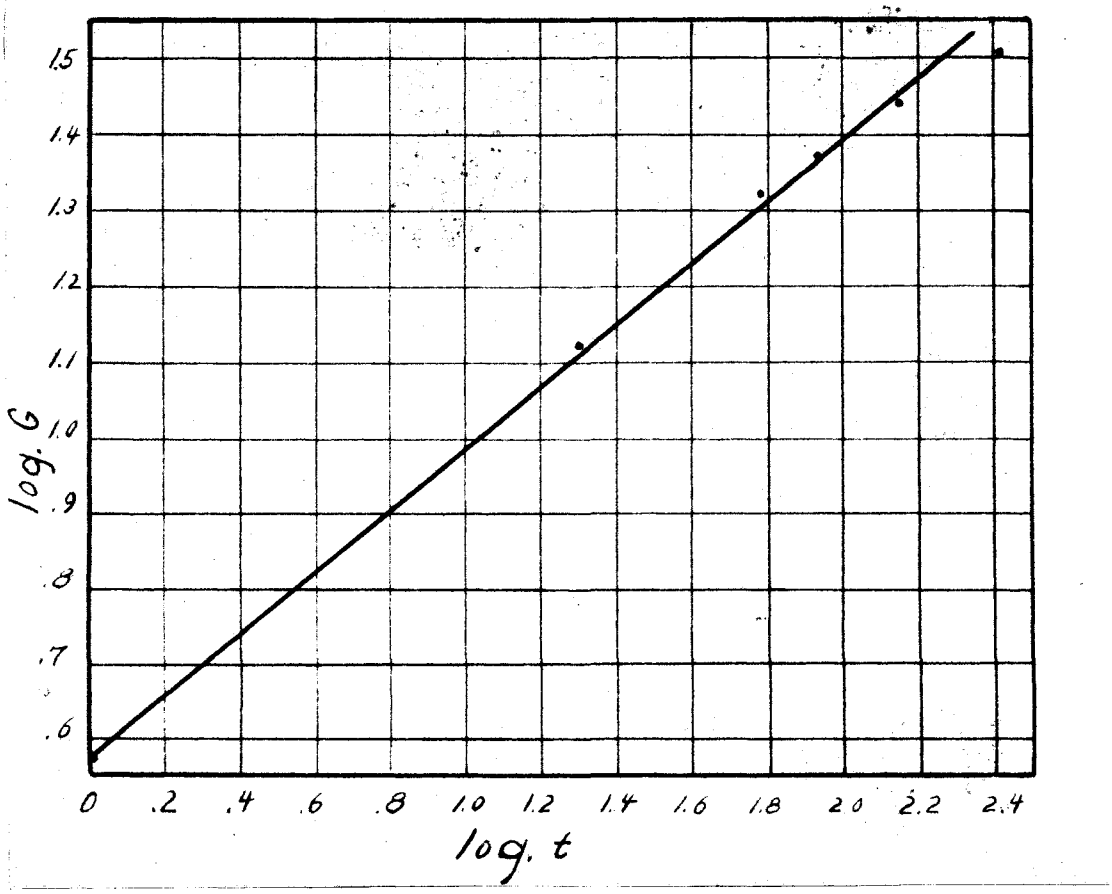


FIGURE 8. Relation of the logarithm of time of aging (t) to the logarithm of the per cent of undigestible residue (G) for soluble potato starch.

With none of the cornstarch pastes studied was a linear relation between log t and log G observed. A typical curve is shown in Figure 9 based on the data for the aging of a cornstarch paste prepared by boiling for five minutes (Figure 3). Sallinger used soluble starches in every case to obtain linear relations between log G and log t and considered them to give more homogeneous pastes than the natural starches. This might explain the failure of a linear relation between log G and log t for the cornstarch pastes.

Retrogradation, as has been pointed out, might be interpreted in one of the following ways:

1. As a change in molecular structure, as is suggested by X-ray studies on frozen starch pastes.
2. As the association of small particles to form entities too large to remain in solution or even to remain suspended.
3. As the gradual precipitation of molecules soluble only in hot water.
4. As the gradual desolvation and consequent precipitation of molecules solvated only at the temperatures of preparing the pastes.
5. As a combination of some of the above.

The results of the aging experiments will be considered in the light of these ideas.

Increasing the temperature of preparing the paste (Figure 5) seems to retard the appearance of retrogradation.

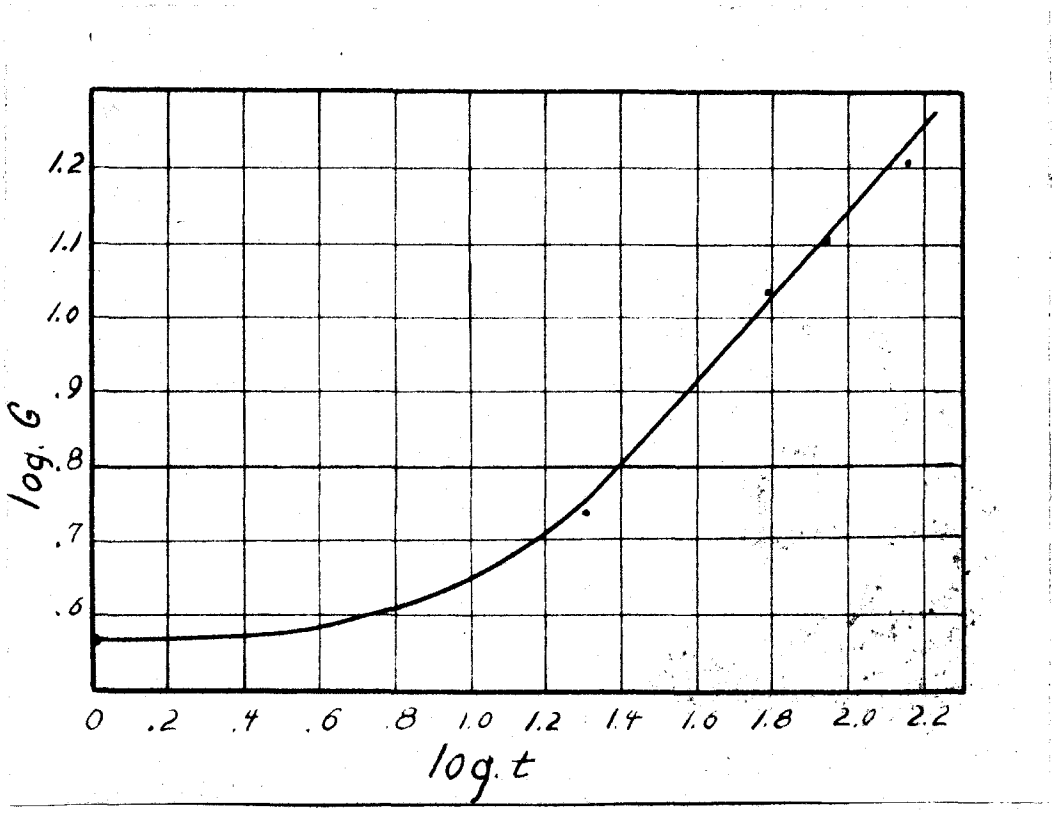


FIGURE 9. Relation of the logarithm of time of aging (t) to the logarithm of the per cent of undigestible residue (G) for cornstarch.

The same effect is seen on comparing the 100°C. paste heated for 30 minutes (Figure 5) with the 100°C. pastes heated for five minutes (Figures 3 and 4). This effect would seem to be best explained by assuming a greater solvation of the starch at the higher temperatures and consequently a longer time for the mechanism of desolvation to result in the precipitation of the starch. This would indicate that with all the pastes examined retrogradation or desolvation sets in at once but is detected by the method used only when actual precipitation begins. If retrogradation consists of a change in structure it is logical to expect that such a change would take place at a given rate regardless of the initial treatment of the paste. The association of small particles to form larger insoluble ones seems unlikely when it is considered that with the degrees of heating used very little, if any, reduction in particle or mole size would be expected. The association of small molecules already in existence in the starch into larger ones does not seem plausible since it appears that the fractions in starch of larger mole size retrograde more quickly. If retrogradation is considered as a precipitation from supersaturated solutions of starch, then increased temperatures would be expected to lead to greater supersaturation and consequently more rapid precipitation on cooling.

The aging of a homogenized paste, which contained only ruptured granules (Figure 6), was found to proceed about half

as rapidly as with an unhomogenized paste. A paste prepared from dry-ground cornstarch did not retrograde at all. The absence of retrogradation with the latter starch may be attributed to the extensive reduction in particle size which accompanies dry-grinding. If it is true that retrogradation consists of desolvation of the difficultly soluble molecules so that they precipitate, then it is seen that in dry-ground starch these have become more soluble, possibly to the extent that they would never undergo desolvation and precipitation. Homogenization of cornstarch pastes ruptures the swollen granules but cannot conceivably lead to any reduction of the particle size of the starch. Thus the more rapid retrogradation of the unhomogenized pastes is accounted for by the fact that as the intact granule retrogrades a portion of the inner material ordinarily digestible by saliva is made inacessible to its action. Since rupture of the granules of an aged cornstarch paste does not lead to a decrease in the amount of undigestible residue, this inner portion must also retrograde eventually. That this is the case may be inferred from the behavior of the amylose and amylopectin (Figure 7) on aging of their pastes. The amylopectin begins to retrograde at once, though rather slowly, while the amylose does not show appreciable retrogradation until about 120 hours of aging.

A reversal of the aging process is brought about by heating the aged starch pastes as is shown by the fact that the amount of undigestible residue decreases and the intensity

of coloration with iodine increases. Indications are, however, that this process is slower and more difficult than was the original pasting process, suggesting that retrograded starch is quite different from the original starch. This has been suggested from X-ray studies and should not be surprising in view of the difference in conditions under which the two are formed.

The failure of waxy cornstarch to retrograde may possibly be taken as an indication that its average mole size is much less than that of the ordinary corn or potato starches.

The influence of the non-carbohydrate constituents of starch should not be overlooked. These certainly have a considerable effect on the solvation of starches and probably play a part in the phenomenon of retrogradation.

Fractionation of Starch by Digestion with Beta-Amylase

The soybean enzyme was selected since it has been confirmed by Newton and Naylor (157) that this enzyme is almost exclusively beta-amylase, and since its concentrates have a higher saccharogenic power than those from other sources. In addition, the action of this enzyme on various starches (corn, rice, wheat, potato and tapioca) has been investigated by Martin, Naylor and Hixon (158) and shown to result characteristically in the production of from 58.5 to 77 per cent of the theoretical amount of maltose and from 30 to 40 per cent of low reducing materials resistant to further

digestion by the enzyme. The low reducing material was obtained in two fractions, one which flocked out directly from the digestion mixtures and one which resulted by precipitation in 60 per cent alcohol. The first fraction amounted to from one to two per cent with the cereal starches and to less than one per cent with the root starches. The fraction obtained by alcohol precipitation is analogous to the "residual" or "alpha-amylodextrins" described by other workers. When prepared from the cereal starches, it was curdy and settled out nicely, but when prepared from the root starches, it formed as a transparent sticky mass. Martin, Naylor and Hixon characterized the precipitates as to further hydrolysis by fresh beta-amylase, by phosphorus and fatty acid content, by reducing power and by iodine precipitation.

Preparation of the beta-amylase concentrates

The concentrates were prepared according to the procedure of Newton and Naylor (157) by extraction of the ground, fat-free soybeans with cold 50 per cent alcohol, followed by precipitation of the active material in cold 70 per cent alcohol. Yields of from .5 to one per cent of enzyme concentrate were obtained.

The saccharogenic power of these concentrates was estimated following the method of the above workers. The amount of maltose produced in 30 minutes at 40°C. by a given weight of the enzyme in the presence of an excess of starch was

determined. The maltose was found by a modified Hagedorn and Jensen method as reported by Gore and Steele (162). The saccharogenic power (milligrams maltose produced per milligram concentrate) varied from 200 to 63 for these concentrates.

With the following preparations of limit dextrins the digestions were allowed to run for 24 hours and consequently a given weight of concentrate may be safely assumed to digest at least twice as much starch as is equivalent to its saccharogenic power determined with digestion periods of 30 minutes. The margin of safety is even greater when it is considered that not more than 60 per cent of the starch is converted to maltose.

Digestion of cornstarch pastes

Experiment I. A temperature of 80°C. was used to paste the starch in accord with the observation of Martin and Newton (163) that this afforded optimum digestion. Eight liters of distilled water in a 16-liter balloon flask were brought to 80°C. by heating with steam in a galvanized bucket. Six hundred grams of cornstarch suspended in two liters of water were introduced into the hot water with vigorous stirring. The temperature of the paste was maintained at 80°C. for 30 minutes. The balloon flask was then removed from the steam bath and its contents allowed to cool to 45°C. when three grams of soybean amylase (saccharogenic power = 63) suspended in a 100 cubic centimeters of water

were added with stirring. After addition of a little toluene the digestion mixture was kept at 40°C. for 24 hours, during which time the paste was markedly thinned. Determination of the reducing sugars in one cubic centimeter samples indicated that 51 per cent of the theoretical amount of maltose was formed.

Centrifugation of the digestion mixture in the Sharple's supercentrifuge (400 R. P. S.) separated 7.7 grams or 1.4 per cent of residue which was designated as undigested starch.

The cloudy centrifugate was found by microscopic examination to contain what appeared to be the transparent hulls of starch granules. For the purpose of removing these, enough 95 per cent alcohol was added to bring the concentration to 14 per cent by volume. The precipitate which formed was recovered by centrifuging in the supercentrifuge, followed by dehydration with several volumes of absolute alcohol, washing with ether and drying. It amounted to 98.6 grams or 18.2 per cent of the original starch. The centrifugate was treated with additional 95 per cent alcohol to bring the concentration to 55 per cent by volume. The white flocculent precipitate was allowed to settle out. The supernatent liquid was siphoned off and replaced by a volume of 60 per cent alcohol equal to twice that of the sediment. After siphoning off the wash alcohol the sediment was dehydrated with absolute alcohol, washed with ether and dried to yield

120.3 grams or 22.3 per cent of material. The percentage recovery of the total starch on the dry basis was as follows:

Calculated as maltose equivalent	51.0 per cent
Undigested residue	1.4 per cent
14 per cent alcohol precipitate	18.2 per cent
55 per cent alcohol precipitate	22.3 per cent
Total	92.9 per cent

The 14 and 55 per cent alcohol precipitates were tested for further digestion by beta-amylase. Samples of 1.3 grams were dissolved in 50 cubic centimeters of boiling water. After cooling to 40°C. the volume was made to 100 cubic centimeters with water at 40°C. and .01 grams of enzyme concentrate (saccharogenic power = 200) suspended in five cubic centimeters of water were added. Immediately after shaking, two duplicate five cubic centimeter samples were removed for a blank determination of reducing sugars by the modified Hagedorn and Jensen method. The samples were digested for 24 hours at 40°C. when two more five cubic centimeter portions were removed for sugar analysis. The 14 per cent alcohol precipitate formed 19 per cent of the theoretical amount of maltose; the 55 per cent alcohol precipitate formed 12.3 per cent of the theoretical amount of maltose.

Redigestion of the 14 and 55 per cent alcohol precipitates

One hundred and fourteen grams of the 55 per cent alcohol precipitate were made up to a 6.5 per cent solution in boiling water; the solution was cooled to 40°C. and one gram of

amylase concentrate (saccharogenic power = 200) suspended in 50 cubic centimeters of water was added. Toluene was added to prevent bacterial decay and the digestion was allowed to run 24 hours at 40°C. Determination of reducing sugars at the beginning and end of the digestion indicated that 8.1 per cent of the theoretical amount of maltose was formed. The solution was made to an alcohol concentration of 60 per cent and the white flocculent precipitate was recovered as previously described. The yield was 80 grams. The percentage recovery of the original material was:

Calculated as maltose equivalent	8.2 per cent
60 per cent alcohol precipitate	70.0 per cent
Total	78.2 per cent

This sample tested for further digestion by beta-amylase yielded only 6.6 per cent of the theoretical amount of maltose.

Eighty-nine grams of the 14 per cent alcohol precipitate were made up to a 4.8 per cent paste in boiling water. Boiling was prolonged for 30 minutes to secure complete dispersion of the material. After cooling to 40°C. the paste was digested as before with beta-amylase for 24 hours. Fifteen per cent of the theoretical amount of maltose was formed. Addition of alcohol to 15 per cent by volume resulted in a flocculent white precipitate which amounted to 50.6 grams upon recovery. This sample tested for further digestion gave 11.4 per cent of the theoretical amount of maltose. The

alcohol concentration of the mother liquor was increased to 60 per cent by volume and resulted in a cloudy white precipitate which settled only slowly (3 days) to give a syrupy residue. This on recovery amounted to 26 grams. The percentage of the total material recovered was:

Calculated as maltose equivalent	15.0 per cent
15 per cent alcohol precipitate	56.0 per cent
60 per cent alcohol precipitate	29.0 per cent
Total	100.0 per cent

The percentage yield of the original starch as 14 per cent alcohol precipitate plus 55 per cent alcohol precipitate was 40.5 per cent. The yield of the redigested materials amounted to 31.1 per cent of the original starch.

Experiment II. The paste was prepared at 100°C. rather than at 80°C. Five hundred grams of starch suspended in a liter of water were poured with vigorous stirring into six liters of boiling water contained in a galvanized pail. Boiling was continued for 15 minutes with constant stirring. Then the paste was poured into a 12-liter balloon flask, with some loss due to the paste adhering to the pail. When the suspension had cooled to 45°C., three grams of the enzyme concentrate (saccharogenic power = 63) suspended in 100 cubic centimeters of water were added with stirring. After adding toluene, the paste was allowed to digest for 24 hours at 40°C. Reducing sugar analyses on one cubic centi-

meter samples indicated that 51.5 per cent of the theoretical amount of maltose was formed. Centrifugation of the digested mixture resulted in ten grams of undigested residue. Microscopic examination of the centrifugate revealed the absence of the granules which were observed with the paste prepared at 80°C.

The addition of 95 per cent alcohol to the centrifugate to bring the concentration to 60 per cent by volume resulted in a rather gummy precipitate. This was allowed to settle out overnight. The supernatant liquid was siphoned off and replaced with absolute alcohol equal to twice the volume of sediment. Finally the material was filtered by suction and dried in the vacuum oven at 50°C. The yield was 142.4 grams or 31.6 per cent. The percentage recovery of the total starch on the dry basis was:

Calculated as maltose equivalent	51.5 per cent
Undigested residue	1.3 per cent
60 per cent alcohol precipitate	31.6 per cent
Total	84.4 per cent

Redigestion of the 60 per cent alcohol precipitate

The 142 grams of dextrin were suspended in two liters of boiling water and boiling was continued for about 30 minutes till all the lumps dispersed. After cooling to 45°C., a solution of one gram of enzyme concentrate (saccharogenic power = 63) in 50 cubic centimeters of water was added with stirring and the digestion mixture was preserved at 40°C.

for 24 hours. Reducing sugar analyses on one cubic centimeter samples at the beginning and end of the digestion indicated that 11.9 per cent of the theoretical amount of maltose was formed. Precipitation with 60 per cent alcohol gave a gummy precipitate which settled out and left a cloudy supernatent liquid. A small amount of additional material was obtained by use of the supercentrifuge. After dehydration in absolute alcohol, the material was filtered by suction and dried in the vacuum oven at 50°C. The yield was 116.4 grams. The percentage recovery of the original material was:

Calculated as maltose equivalent	11.9 per cent
60 per cent alcohol precipitate	81.7 per cent
Total	93.6 per cent

The redigested limit dextrin amounted to 25.9 per cent of the original starch.

Digestion of waxy cornstarch pastes

Experiment I. One hundred grams of the starch were used to make a four per cent paste by heating at 80°C. for 30 minutes as was described in Experiment I with cornstarch. The extremely viscous paste became watery thin after digestion and was converted to 42 per cent of the theoretical amount of maltose. Centrifugation of the digestion mixture resulted in approximately two grams of undigested residue. The 60 per cent alcohol precipitate was white and sticky but readily settled out. On recovery, it amounted to

50.5 grams. The total recovery of the original starch on the dry basis was:

Calculated as maltose equivalent	42.0 per cent
Undigested residue	2.2 per cent
60 per cent alcohol precipitate	55.5 per cent
Total	99.7 per cent

The 60 per cent alcohol precipitate tested for further digestion by beta-amylase yielded 18.25 per cent of the theoretical amount of maltose.

Redigestion of the 60 per cent alcohol precipitate

The above dextrin on redigestion with beta-amylase gave 19.9 per cent of the theoretical amount of maltose. The 60 per cent alcohol precipitate was quite syrupy in nature and was allowed to settle for several days. On recovery it amounted to 34.5 grams. The total recovery of the original material was:

Calculated as maltose equivalent	19.9 per cent
60 per cent alcohol precipitate	68.3 per cent
Total	88.2 per cent

The redigested dextrin amounted to 38.4 per cent of the original starch. Tested for further digestion by beta-amylase it yielded 5.4 per cent of the theoretical amount of maltose.

Experiment II. One hundred grams of the starch were made to a 2.8 per cent paste by boiling as in Experiment II with ordinary cornstarch. With digestion, 42.1 per cent of

the theoretical amount of maltose was formed. Centrifugation of the mixture resulted in approximately two grams of undigested residue. The 60 per cent alcohol precipitate partially settled out as a sticky residue. About ten grams more of syrupy material was recovered by centrifugation in the supercentrifuge. The total yield after recovery was 40 grams. The percentage recovery of the original starch on the dry basis was:

Calculated as maltose equivalent	42.1 per cent
Undigested residue	2.2 per cent
60 per cent alcohol precipitate	44.5 per cent
Total	88.8 per cent

Redigestion of the 60 per cent alcohol precipitate

The 40 grams of the above material were made up to a 6.3 per cent paste in hot water. Digestion with beta-amylase resulted in 10.5 per cent of the theoretical amount of maltose. The 60 per cent alcohol precipitate was recovered in the supercentrifuge as a syrupy sediment. The yield was 35.5 grams. The percentage recovery of the original material was:

Calculated as maltose equivalent	10.5 per cent
60 per cent alcohol precipitate	88.7 per cent
Total	99.2 per cent

The redigested dextrin amounted to 39.5 per cent of the original starch on the dry basis.

Properties of the limit dextrins

A comparison of the yields, solubilities, iodine colors, optical rotations and reducing powers of the various limit dextrins are shown in Table XIII.

The iodine colors were observed in 100 cubic centimeters of .03 per cent solutions of the dextrins. These were prepared by boiling where necessary. The red-coloring samples required five times as much .1 N iodine solution (about one cubic centimeter) to give a plainly visible color. They also lost iodine more easily than the blue-coloring samples, becoming colorless overnight.

The reducing values were determined by Farrow's copper reduction method. The values shown correspond to chain lengths of from 83 to 230.

The redigested 60 per cent alcohol precipitate from the cornstarch (Experiment II) was shown to be separable into fractions by freezing and by electrodialysis.

Freezing, followed by centrifugation of the melted paste, resulted in 78.5 per cent of a soluble, red-iodine-coloring fraction and 21.4 per cent of an insoluble, blue-iodine-coloring fraction. The latter gave a reddish-purple color on increasing the iodine concentration. The soluble fraction showed an R_{Cu} of 33.85 while that of the insoluble fraction was 15.42. The melted paste showed the presence of the characteristic thread-like particles. These were much

TABLE XIII. Properties of the limit dextrins.

Dextrin	Yield (per cent)	Solubility in Water	Iodine Color	$[\alpha]_D^{25°}$ In 2.5% NaOH (degree)	$[\alpha]_D^{25°}$ In HOH (degree)	R_{cu}
Ordinary Corn-Expt. I						
14% alc. ppt.-redigested	10.0	Insoluble with prolonged boiling	Blue	+126.8	---	49.6
55% alc. ppt.-redigested	15.9	Soluble with boiling to give a cloudy solution	Blue*	+154.6	---	30.9
60% alc. ppt. fr. redigestion of 1st 14% alc. ppt.	5.2	Soluble in cold	Red	+144.0	---	26.2
	31.1					
Ordinary Corn-Expt. II						
60% ppt.-redigested	25.9	Soluble with boiling to give a cloudy solution	Blue*	+155.6	---	17.84
Waxy Corn-Expt. I						
60% alc. ppt.-redigested	38.4	Soluble in cold	Red	+156.2	+172.0	31.1
Waxy Corn-Expt. II						
60% alc. ppt.-redigested	39.5	Soluble in cold	Red	+150.0	+178.8	25.5

* These showed a reddish-purple color on increasing the iodine concentration five fold.

smaller than the filmy particles found with the original cornstarch pastes.

Electrodialysis of an .8 per cent paste and analysis of the supernatent liquid showed 92 per cent of the soluble fraction which was blue, then red to iodine. The sediment amounted to 3.6 per cent of the original material and gave a blue color with iodine. The soluble fraction had an R_{Cu} of 9.27 while the insoluble fraction had a value of 23.3.

Discussion

The data on the percentage recovery of the starch from the beta-amylase digestions can only be taken as approximate in view of the large volumes of paste used and the possibility of mechanical losses, etc. The yields of the limit dextrins are in general agreement with those obtained by other workers. For example, Martin, Naylor and Hixon (158) report the following yields of 60 per cent alcohol precipitates from various starches:

Corn	32.3 per cent
Rice	34.4 per cent
Wheat	38.8 per cent
Potato	30.0 per cent
Tapioca	35.5 per cent

It is quite obvious that the limit dextrin obtained from cornstarch is heterogeneous. This is shown by the separation of the digestion mixture of the 80°C. paste into

a 14 per cent alcohol precipitate and a 55 per cent alcohol precipitate and by the fractionation of the 60 per cent alcohol precipitate from the digestion of the 100°C. paste with electrodialysis and freezing. The cornstarch dextrin appears to consist of two fractions, a more insoluble fraction which gives a blue color with iodine and a soluble fraction which gives a red color with iodine. The latter requires much higher concentrations of iodine to show a visible color. Pasting the starch at 100°C. rather than at 80°C. seems to result in a more soluble dextrin. However, that the blue-iodine-coloring precipitate is a limit dextrin is shown by its low reducing value and resistance to further digestion by beta-amylase. The dextrin from waxy cornstarch consists entirely of the soluble, red-iodine-coloring fraction and rather surprisingly is left in greater yields than the dextrin from ordinary cornstarch. These observations support the conception that the resistance to further digestion by beta-amylase and the iodine-coloring ability are due to some structural feature of the limit dextrins.

These ideas are compatible with the results of Beckmann and Landis (112) who obtained similar yields (20, 25, and 23 per cent respectively) of the limit dextrins from gelatinized potato starch, from soluble potato starch (Lintner) and from dry-ground potato starch. The dextrins from the first two of these were found to be resolvable into two mole sizes

by ultracentrifugation, while the dextrin from the dry-ground starch was homogeneous.

The limit dextrins in view of their resistance to further digestion by beta-amylase, their low reducing value and their retention of the ability to color with iodine seem admirably suited for a study of the problem of the heterogeneity of starch. The action of beta-amylase appears to result in a concentration of the structures in starch associated with the above unexplained phenomena. This has been the guiding thought in the work reported in the following section.

Characterization of the Limit Dextrins Left by Beta-Amylase Digestion of Starch

The 60 per cent alcohol precipitate from cornstarch (Experiment I) and the 60 per cent alcohol precipitate from waxy cornstarch (Experiment I) were acetylated and compared as regards the molecular weight of the acetates by the Rast camphor method and by Staudinger's viscosity method.

The acetate of the dextrin from ordinary cornstarch was methylated and compared to the methyl derivative of the original starch with respect to the proportion of di-, tri- and tetramethyl glucoses resulting by hydrolysis.

Oxidations of the dextrins from cornstarch and from waxy cornstarch by periodic acid were carried out. Periodic

acid cleaves carbon chains between adjacent hydroxyl groups and results in the production of formaldehyde from primary alcohol groups adjacent to secondary alcohol groups. The consumption of periodic acid, the production of formaldehyde and the isolation of the oxidized dextrins were the objects of study.

Acetylation of the limit dextrins

Cornstarch limit dextrin acetate. Twenty-five grams of the cornstarch dextrin were stirred at 40°C. for 12 hours in 100 grams of pyridine, then an additional 12 hours at room temperature. One hundred grams of pyridine and 150 grams of acetic anhydride were added and stirring was continued for three hours at room temperature. The mixture was filtered through glass wool and the acetate was precipitated by pouring the filtrate into ten volumes of cold water. The flake-like precipitate was filtered by suction, washed with water until free of acid and dried overnight in the vacuum oven at 60°C. The yield was 35 grams or 79 per cent of theoretical.

The acetate was not completely soluble in acetone or chloroform. Enough fine material remained insoluble in chloroform to make the rotation measurements difficult.

$[\alpha]_D^{25^\circ}$ +157.7° (.2 gm. in 25 cc. $CHCl_3$)

The acetyl content was determined by the micro-method of Friedrich and Rapoport (161). The results were none too satisfactory and the procedure was modified to the extent

that the distillation of the acetic acid from the p-toluene sulfonic acid solution was carried just to dryness each time and heating was not prolonged for the prescribed five minutes.

CH_3CO: Found, 39.6, 39.3; Calculated for $C_6O_5H_7(CH_3CO)_3$, 44.75

Because of the low value for the acetyl content, the above acetate was redissolved in pyridine and treated again with acetic anhydride. The acetate was isolated as before and was completely soluble in acetone and in chloroform.

$[\alpha]_D^{25°}$ +159.0° (.1 gm. in 25 cc. $CHCl_3$)

CH_3CO: Found, 42.7, 42.7; Calculated for $[C_6O_5H_7(CH_3CO)_3]$, 44.75

The acetate was deacetylated as follows: Four grams were shaken for three hours* with 100 cubic centimeters of .5 N alcoholic sodium hydroxide. The alkali was neutralized with N acetic acid and the solid was filtered by suction. It was then ground in alcohol containing a little acetic acid. After filtering, this was repeated. Finally, the dextrin was dissolved in a little warm water, neutralized with acetic acid and precipitated by pouring into alcohol. Filtration, followed by washing with alcohol and drying, yielded 1.7 grams of the dextrin or 73.2 per cent of the amount expected

$[\alpha]_D^{25°}$ +166° (.1 gm. in 2.5% aqueous NaOH)

R_{Cu} = 14.48

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* Titration of the excess alkali with .5 N hydrochloric acid showed that this time was sufficient to remove all of the acetyl groups.

For the original dextrin:

$[\alpha]_D^{25°}$ +154.6° (.1 gm. in 2.5% aqueous NaOH)

R_{Cu} = 30.9

Deacetylation of cornstarch triacetate gives a product which has the same R_{Cu} (3.5) as the original starch. It seems logical to conclude that the change observed in rotation and reducing power of the above dextrin is due to fractionation rather than hydrolytic change.

Waxy cornstarch limit dextrin acetate. This dextrin was acetylated in the same manner as the cornstarch dextrin. The yield was 33.5 grams or 75 per cent of the theoretical. The product was completely soluble in acetone and chloroform

$[\alpha]_D^{25°}$ +157.2° (.5 gm. in 25 cc. $CHCl_3$)

CH_3CO: Found, 43.8, 44.2; Calculated for $[C_6O_5H_7(CH_3CO)_3]$, 44.75

Deacetylation of this acetate resulted in 91.6 per cent of the expected amount of dextrin.

$[\alpha]_D^{25°}$ +162.5°(.1 gm. in 25 cc. HOH); +151.2°(.2 gm. in 25 cc. 2.5% aqueous NaOH)

R_{Cu} = 12.55

Molecular weights of the dextrin acetates by the Rast camphor method

The reliability of this method for polysaccharides is open to question. Freudenberg, Friedrich and Bumann (6) determined the molecular weights of hendekamethyl maltotriose,

tetradekamethyl maltotetraose, dekamethyl b-methyl cellotrioside and tridekamethyl b-methyl cellotetraoside with apparent success as checked by determinations of the total and glucosidic methoxyl content. Ullmann and Hess (162) found the correct molecular weight for cellobiose octacetate but only the value of a monose for maltose octacetate. Freudenberg and Jacobi (151) reported the following values for the Schardinger alpha- and beta-dextrins:

alpha-dextrin in water	4.5 glucose units
alpha-dextrin acetate in camphor	4.95 glucose units
beta-dextrin in water	5.5 glucose units
beta-dextrin acetate in camphor	5.95 glucose units

The methyl derivatives of these dextrins have also been reported to give values of approximately five and six glucose units for the alpha- and beta-dextrins by lowering of the melting point of camphor.

The procedure consisted of dissolving .2000 grams of the dextrin acetates in .5000 grams of camphor (M.P. 176°C.) and of measuring the melting point depression (ΔT). Three readings of the melting point of the acetate-camphor mixture in each of three capillary tubes were taken to obtain an average value. The molecular weight was caluclated from the following formula:

$$M = \frac{(\text{gms. dextrin acetate})\ (1000)\ (40)}{(\text{gms. camphor})\ (\Delta T)}$$

A sample of the Shardinger beta-dextrin acetate was used to check the procedure. It showed a molecular weight of 1,629 or 5.5 glucose units.

The cornstarch limit dextrin acetate showed a molecular weight of 5,170, corresponding to 14.9 glucose units. The limit dextrin from waxy cornstarch had a molecular weight of 3,880 or 13.5 glucose units. It must be remembered that a value for the molecular weight of these dextrins indicates the average mole size.

Molecular weights of the dextrin acetates by Staudinger's viscosity method

From .15 to .3 grams of the dextrin acetates were dissolved in m-cresol at 30°C. and made to 25 cubic centimeters in a volumetric flask. The density of the solutions at 30°C., and the time necessary for the solvent to flow through the Ostwald-type viscosmiter* were determined. Finally the time necessary for the solution of the acetate to flow through the viscosimeter at 30°C. was determined. The times measured occurred in the range of from 100 to 250 seconds.

The molecular weights were calculated from the formula:

$$\frac{N_{sp}}{c} = K \times M$$

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* With capillary of approximately .15 cm. diameter.

The symbol, N_{sp}, represents the specific viscosity and is related to the relative viscosity (N_r) as follows:

$$N_{sp} = N_r - 1$$

$$\frac{N_r}{1} = \frac{(\text{Time for solution})(\text{Density of solution})}{(\text{Time for solvent})(\text{Density of solvent})}$$

The symbol, c, represents the concentration of the solution in basic moles per liter, i.e., the concentration of acetylated glucose units in moles per liter.

A provisional value for K of 1×10^{-4} has been suggested by Staudinger and Eilers (65) for starch and its derivatives and was used for the following calculations.

The cornstarch limit dextrin acetate gave values indicating a molecular weight of 75,745 corresponding to 263 glucose units. The limit dextrin acetate from waxy cornstarch gave values corresponding to a molecular weight of 100,980 or 350 glucose units.

Using the value for K of 1×10^{-3}, used by Haworth, Hirst, Kitchen and Peat (118) the mole sizes would correspond to chain lengths of 26.3 and 35.0 glucose units, respectively, for the cornstarch dextrin and the waxy cornstarch dextrin.

Methylation of the cornstarch limit dextrin acetate

The methylation was carried out according to the general Haworth procedure except for the last treatment which consisted of methylation by Freudenberg's technique using methyl iodide and sodium in liquid ammonia.

Twenty-five grams of the dextrin acetate were dissolved in 350 cubic centimeters of acetone, and then 150 cubic centimeters of dimethyl sulfate and 400 cubic centimeters of 30 per cent sodium hydroxide were added with stirring over a period of five hours. The excess sodium hydroxide was almost neutralized with sulfuric acid, the acetone was driven off by heating on the steam bath and, finally, the solution was brought to boiling. The methyl dextrin collected on the surface of the boiling water in a gummy ball.

An attempt to filter this material through a hot water funnel proved unsatisfactory due to the methyl dextrin rapidly becoming syrupy with only slight cooling. As a result, part of the material was lost and a yield of only 18 grams of the methyl dextrin was obtained from the first methylation.

The methyl dextrin was found to be best recovered by fishing it from the surface of the boiling water with a spoon. It was immediately dropped into a second volume of boiling water for the purpose of washing out the sodium sulfate. If the methyl dextrin was allowed to become syrupy by cooling, part of it was lost through solution in the second volume of boiling water. The product was recovered from the wash water as before, and was dried in the vacuum oven at 60°C.

The subsequent nine methylations of this material were carried out in the same way using appropriate quantities of the reagents.

For the last methylation nine grams of the methyl dextrin were suspended in 200 cubic centimeters of liquid ammonia, contained in the apparatus described by Freudenberg and Boppel (74), which was cooled by immersion in an acetone-carbon dioxide bath. The mixture, under continuous stirring, was treated twice with these quantities of sodium and methyl iodide:

I.	1 gm. Na	2.7 cc. CH_3I
II.	1 gm. Na	3.7 cc. CH_3I

The sodium was added in small pieces and was allowed to react three hours before the methyl iodide was added through a dropping funnel. The side arm through which the sodium and methyl iodide were added was closed with a calcium chloride tube when not in use.

At the conclusion of the methylation the product was allowed to settle (four or five hours) and the solution of ammonia and sodium iodide was siphoned off by inserting a siphon in place of the stirrer, closing the side arm and allowing the reaction vessel to warm up by removing it from the cooling bath. The sediment was washed with one 150 cubic centimeter portion of liquid ammonia. After siphoning off the wash ammonia, the residual ammonia was driven off by warming on a water bath. The dried mixture of methyl dextrin and sodium iodide was washed by suspending in boiling water as before described.

The results of the methylation are shown in Table XIV.

TABLE XIV. Results of the methylation of the cornstarch limit dextrin acetate.

Number of Methylation	Yield of Product (grams)	$[\alpha]_D^{25°}$ (in $CHCl_3$)	CH_3O* (per cent)
2	12.5	+185.2°	38.8
4	10.7	+193.8°	41.7
6	9.8	+198.9°	42.4
9.	9.3	+198.7°	42.7
10	9.0	+201.7°	43.2
11 (with Na and CH_3I)	8.2	+203.8°	44.9

* The methoxyl contents were determined by the micro-method described by Pregl using the volumetric determination of alkyl halides suggested by Vieböck and Brecher (Ber. 63, 3207 (1930). The theoretical methoxyl content calculated from the formula $C_6O_2H_7(CH_3O)_3$ is 45.4 per cent.

Methylation of cornstarch

In view of the difficulty of complete methylation with the Haworth procedure, the starch was methylated directly with sodium and methyl iodide in liquid ammonia using the above cited technique of Freudenberg and Boppel.

The starch was prepared for methylation by pasting in water, homogenization to rupture the granules and precipitation with alcohol. After dehydration in methyl alcohol, it was dried in the vacuum oven at 60°C.

Five grams of the starch were taken for methylation. After soaking in 200 cubic centimeters of liquid ammonia, sodium and methyl iodide were added in four separate treatments:

I.	2.5 gms. Na	6.8 cc. CH_3I
II.	1.5 gms. Na	4.25 cc. CH_3I
III.	1.25 gms. Na	3.4 cc. CH_3I
IV.	1.25 gms. Na	4.25 cc. CH_3I

The sodium was allowed to react with the starch three hours before addition of the methyl iodide.

After the methyl starch had settled, the solution of sodium iodide and ammonia was siphoned off and it was washed with 150 cubic centimeters of liquid ammonia. A fresh 200 cubic centimeter portion of ammonia was added and two more treatments with sodium and methyl iodide were effected:

I.	1 gm. Na	2.7 cc. CH_3I
II.	1 gm. Na	3.7 cc. CH_3I

The methyl starch, after freeing of ammonia, was washed free of sodium iodide by suspension in boiling water. This product in contrast to the methylated limit dextrin was hard and crumbly in hot water and could be easily filtered on a hot suction filter.

Yields of from 90 to a 100 per cent of the theoretical amount of methyl starch can be obtained.

CH_3O: Found, 45.0; Calculated for $[C_6O_2H_7(CH_3O)_3]$, 45.5

$[\alpha]_D^{25^\circ}$ +225.5° (in $CHCl_3$)

Hydrolysis of the methyl cornstarch limit dextrin

Hydrolysis of the methylated dextrin and separation of the cleavage products according to the directions of Hassid

and Dore (75) was attempted, but with little success. Hence, the fractions obtained were converted to the glucosides and an attempt was made to estimate the tetramethyl methyl glucoside by fractional distillation.

A sample of 8.264 grams of the methyl dextrin in 40 cubic centimeters of glacial acetic acid was placed under a vacuum for an hour and then 75 cubic centimeters of five per cent hydrochloric acid were added. The solution was kept on the water bath for two days when all but a small amount of dark flaky material was dissolved. An amount of barium carbonate, ten per cent in excess of the hydrochloric acid used, was added and the solution was evaported to dryness under vacuum at 45°C. with the addition of water from time to time to insure the removal of the acetic acid. The residue was dried by a mixture of alcohol and benzene and was then extracted with dry benzene in Sohxlet for 72 hours to obtain the tri- and tetramethyl glucoses. After evaporation of the benzene and drying in vacuo at 45°C., 8.6438 grams of extract were obtained. This was separated just as described by Hassid and Dore by repeated chloroform-water extraction to give a chloroform soluble fraction of 1.2321 grams and a water soluble fraction of 6.8532 grams.

The chloroform extract which should be tetramethyl glucose was analyzed for methoxyl content:

CH_3O: Found, 44.3, 44.5; Calculated for $C_6O_2H_8(CH_3O)_4$, 52.5

The water extract which should be trimethyl glucose showed approximately the correct methoxyl content:

CH_3O: Found, 41.2, 41.6; Calculated for $C_6O_3H_9(CH_3O)_3$, 41.9.

The residue, containing the barium salts after extraction with benzene, was extracted with ethyl acetate for 72 hours to obtain the dimethyl glucose. After evaporation of the ethyl acetate, a partially crystalline syrup was obtained which amounted to .0611 grams.

CH_3O: Found, 17.6, 17.7; Calculated for $C_6O_4H_{10}(CH_3O)_2$, 29.8.

The total yield of material by hydrolysis of the methyl dextrin was 8.2414 grams or 90 per cent of the expected amount of methyl glucoses.

From these results, it was concluded that the amount of dimethyl glucose was at least less than .0611 grams or .67 per cent of the amount of methyl glucoses expected from 8.264 grams of methyl dextrin.

Since little information was gained with respect to the proportion of end groups, all of the fractions obtained were combined and refluxed for seven hours in hydrogen chloride. The hydrogen chloride was removed by shaking with silver carbonate for several hours. The precipitated silver chloride was filtered off and washed with methyl alcohol. The alcohol was driven off by heating on the water bath to leave 8.4371 grams of a brown-colored syrup which corresponded to 96 per cent of the expected weight of glucosides.

The syrup was transferred to a small Widmer distilling flask of about seven cubic centimeters capacity and with a fractionating column of 4.5 centimeters in length. Transferring was done with a medicine dropper of several cubic centimeters capacity. The residual syrup was washed into the flask with benzene which was removed by heating the flask on the water bath while passing a stream of air through it.

The distillation was carried out at pressures of from .05 to .3 millimeter of mercury. Distillate came over at temperatures of from 56° to 87°C. The trimethyl methyl glucoside distilled in a range from 81° to 83°C. Fractions of the distillate were obtained by changing the weighed receivers during the course of the distillation. The results are shown in Table XV.

The residue remaining in the flask after fraction four was collected was removed and retreated with methyl alcoholic hydrogen chloride. The recovered glucoside was introduced into a smaller distilling vessel (about two cc. in volume) and the fractions five, six and seven were obtained.

The final residue was a dark viscous syrup. It was not practical to reduce this to less than two grams because when the volume of residue was about one-fourth that of the flask bumping occurred and the residue was charged into the receiver.

The value for the amount of tetramethyl methyl glucoside corresponds to an estimated chain length of about 9.5

glucose units for the cornstarch limit dextrin. This estimate takes into account the losses on hydrolysis and glucosidation.

Table XV. Fractionation of the methyl glucosides obtained from the methylated limit dextrin of cornstarch.

Fraction	Wt. (gms.)	$N^{20°}$*	Wt. tetramethyl methyl glucoside** (gms.)	$\%CH_3O$ (found)	$\%CH_3O$ (calc.)
1	1.0302	1.4477	.4579	56.7	56.8
2	.8972	1.4528	.1956	53.9	54.6
3	1.3354	1.4549	.1576	53.0	53.6
4	1.2095	1.4558	.0907		
5	.2324	1.4560	.0151		
6	.8403	1.4578	.0	41.6	41.9
7	.3116	1.4580	.0		
Residue	2.4900	1.4615	.0		
			.9349		

* Refractive index was determined with an Abbé refractometer.

** The percentage of tetramethyl methyl glucoside was found from the curve of Hess and Neumann (76) relating refractive index to proportion of tetra- and trimethyl methyl glucosides at 20°C.

Hydrolysis of methyl cornstarch

A sample of 10.1819 grams of the methyl cornstarch was hydrolyzed just as was the methyl limit dextrin. All but a small amount of dark flaky material was dissolved.

An attempt to separate the products of hydrolysis by the procedure of Hassid and Dore was made but again the results were unsatisfactory. Solution of the fraction containing the tetra- and the trimethyl glucoses in water

resulted in a quite cloudy solution. It is possible that the fatty acids associated with cornstarch are yet to be found in the hydrolysis products of the methyl starch and might cause considerable difficulty in the partition method of separating the tetra- and the trimethyl glucoses.

The chloroform extract which should be tetramethyl glucose amounted to .4659 grams.

CH_3O: Found, 45.6, 45.9; Calculated for $C_6O_2H_8(CH_3O)_4$, 52.5.

The water extract which amounted to 9.7725 grams had a methoxyl content in agreement with that for trimethyl glucose.

CH_3O: Found, 42.0, 41.8; Calculated for $C_6O_3H_9(CH_3O)_3$, 41.9.

The ethyl acetate extract amounted to .1048 grams or .93 per cent of the methyl glucoses expected from 10.1819 grams of methyl starch.

CH_3O: Found, 21.8, 22.0; Calculated for $C_6O_4H_{10}(CH_3O)_2$, 29.8. Certainly not more than .93 per cent of dimethyl glucose was present.

The total yield of hydrolysis products was 10.632 grams or 94.5 per cent of the methyl glucoses expected from 10.1819 grams of methyl starch.

The above chloroform extract was converted to the glucoside by treatment with two per cent hydrogen chloride in methyl alcohol. The syrup obtained was dissolved in benzene and refluxed for an hour with several pieces of sodium. The benzene was driven off by heating on the water bath and the

residue was distilled in a micro-sublimation tube. The temperature of the bath was 90°C. and the pressure was .5 millimeters of mercury. The colorless syrup which collected on the cold finger was washed off with benzene. After evaporation of the benzene .2878 grams of syrup remained which was expected to be tetramethyl methyl glucoside. CH_3O: Found 59.2, 59.5; Calculated for $C_6OH_7(CH_3O)_5$, 62.0. If the impurity is trimethyl methyl glucoside, then this syrup contains 67 per cent tetramethyl methyl glucoside.

To check this and at the same time to get some check on the method of fractional distillation for estimating the tetramethyl methyl glucoside, .2376 grams of the above syrup were placed in the Widmer distilling flask with 3.4241 grams of trimethyl methyl glucoside. The results of the distillation are shown in Table XVI.

The distillation was carried out at a pressure of .1 millimeter of mercury and distillate was collected at temperatures of the vapor of from 60° to 82°C.

The amount of .1425 grams of tetramethyl methyl glucoside represents 60 per cent of the weight of the impure tetramethyl methyl glucoside used and compares favorably with the value of 67 per cent calculated from the methoxyl analysis. Since a difference of 9.5 per cent in methoxyl content corresponds to a difference of 100 per cent in the concentration of tetramethyl methyl glucoside in trimethyl methyl glucoside, it can be seen that the methoxyl analysis is little more than a check.

TABLE XVI. Fractional distillation of a mixture of tetra- and trimethyl methyl glucosides obtained from methyl cornstarch.

Fraction	Wt. (grams)	$N^{20°}$*	Wt. tetramethyl methyl glucoside** (grams)	Wt. Trimethyl methyl glucoside (grams)
1	.3302	1.4503	.1106	.2196
2	.3753	1.4560	.0240	.3513
3	.5270	1.4570	.0079	.5191
4	.7653	1.4575	.0	.7653
5	.8253	1.4581	.0	.8253
Residue	.8103	1.4600	.0	.8103
			.1425	3.4909

* Refractive index was determined with an Abbé refractometer.

** The percentage of tetramethyl methyl glucoside was found from the curve of Hess and Neumann (76) relating refractive index to proportion of tetra- and trimethyl methyl glucosides at 20°C.

Assuming the .2878 grams of glucoside to be 60 per cent tetramethyl methyl glucoside and taking into account the losses on hydrolysis on the methyl starch and of conversion to the glucoside, the chain length of the starch is estimated to be about 67 glucose units.

Oxidation of the cornstarch and the waxy cornstarch limit dextrins with periodic acid

Rate of oxidation. Samples of .5 grams were pasted in 200 cubic centimeters of boiling water (this was not necessary with the waxy dextrin). The pastes were cooled to room temperature and made to volume in 250 cubic centimeter

volumetrics after the addition of 1.28 grams of sodium para periodate ($Na_2H_3IO_6$, 83.4 per cent pure) dissolved in 13 cubic centimeters of N sulfuric acid. The solutions were preserved in running tap water at 15°C. At intervals, ten cubic centimeter samples were removed and treated with five cubic centimeters of concentrated hydrochloric acid and one gram of potassium iodide. The liberated iodine was titrated with .1 N sodium thiosulfate solution.

In calculating the amounts of periodic acid consumed from these titrations, it must be remembered that with the oxidation of polyhydroxy compounds the periodic acid is reduced to iodic acid which likewise liberates iodine on the addition of acid and potassium iodide.

The results of the oxidation for ordinary cornstarch and its limit dextrin are shown in Figure 10. Those for waxy cornstarch and its limit dextrin are shown in Figure 11. The theoretical amount of periodic acid was calculated on the basis that each glucose unit contains free hydroxyls on carbons two and three.

In the latter stages of the oxidation of the limit dextrins, it was observed that when the iodine was liberated in the titration flasks, the oxidized dextrin was precipitated as a reddish-brown, amorphous material. This was not observed with the original starches.

Production of formaldehyde on oxidation of the cornstarch limit dextrin. Caldwell and Hixon have shown the correlation

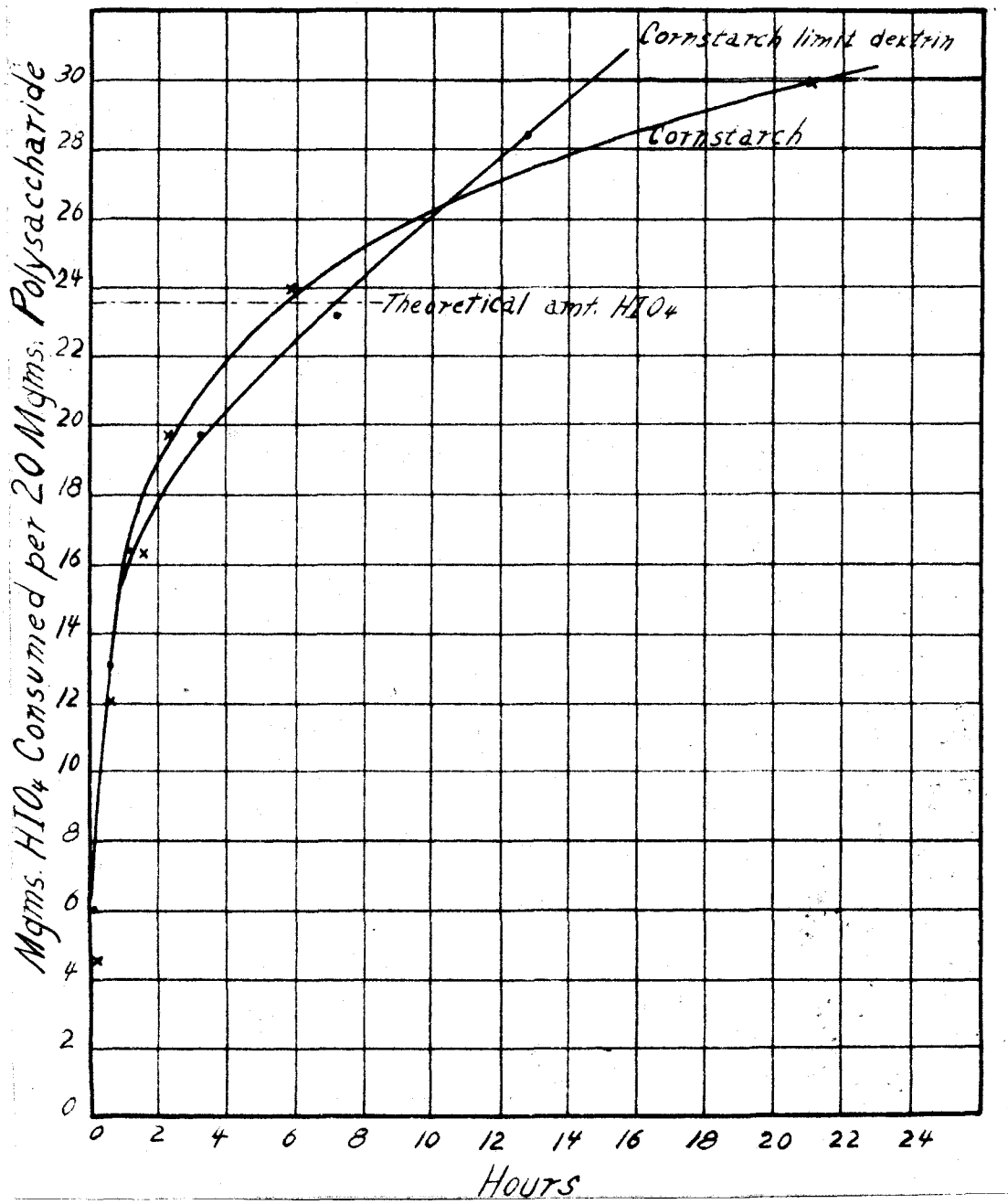

FIGURE 10. Rate of oxidation of ordinary cornstarch and of its limit dextrin with periodic acid.

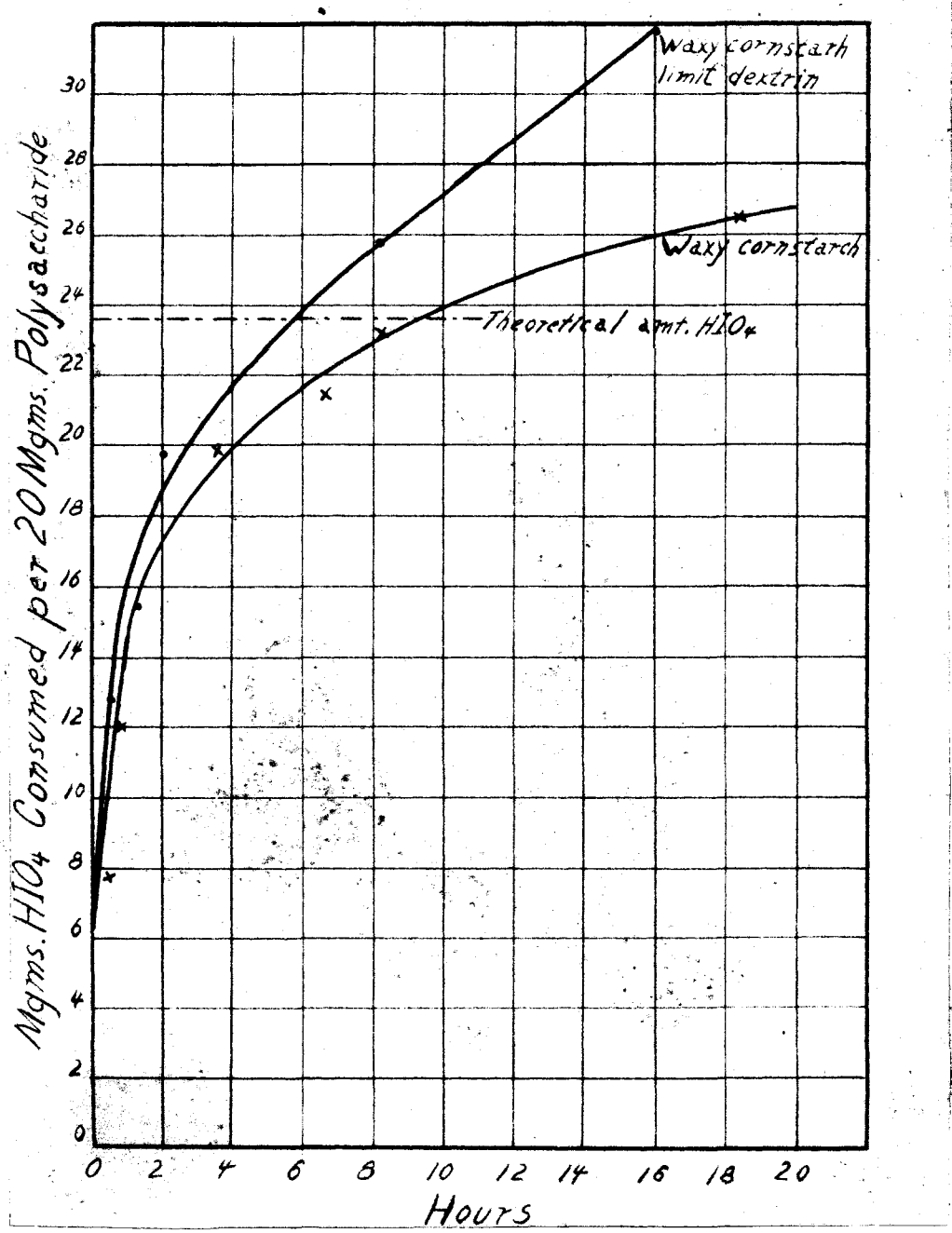

FIGURE 11. Rate of oxidation of waxy cornstarch and of its limit dextrin with periodic acid.

between the production of formaldehyde and the reducing value for a series of acid modified dextrins (Table IV, page 29) indicating that the formaldehyde arises characteristically from the primary-secondary alcohol grouping ($-CHOHCH_2OH$) found in the terminal, reducing glucose units.

Samples of the cornstarch limit dextrin were oxidized with .80 and 1.23 times the theoretical amount of periodic acid respectively. The formaldehyde produced was determined after the manner of Caldwell and Hixon (3) in the hope that the results would give some clue as to what is happening in the later part of the oxidation curves where the consumption of periodic acid is relatively slow.

With the limit dextrin sample oxidized with .80 times the theoretical amount of periodic acid, .95 milligrams of formaldehyde were produced from .8 grams of dextrin. The sample oxidized with 1.23 times the theoretical amount of periodic acid produced .38 milligrams of formaldehyde per .8 grams of dextrin. The amount of formaldehyde expected on the basis of the reducing power of the dextrin was 1.4 milligrams.

With the small amounts of formaldehyde involved, the errors in the determination are very large. About all that can be concluded from these results is that the amount of formaldehyde is in the range expected on the basis of the reducing power of the dextrin. At least no large increase in the amount of formaldehyde results in the later slow stage of the oxidation of the limit dextrin with periodic acid.

Recovery of the oxidized dextrins and catalytic reduction. The oxidized dextrins could be recovered by precipitation with iodine or, more satisfactorily, by removal of the iodic acid from the oxidation mixtures by precipitation with lead carbonate followed by evaporation of the filtrate to dryness in vacuo.

Precipitation with iodine

Two per cent pastes of the cornstarch limit dextrin (10 grams) were oxidized with from .5 to one times the theoretical amount of periodic acid for 24 hours at 15°C. Enough potassium iodide and hydrochloric acid to liberate all of the iodine was added. A precipitate of iodine and the carbohydrate material resulted. At first it was thought that this precipitate was a crystalline derivative of the carbohydrate material, but it seems more likely that the oxidized dextrin is merely incorporated in the iodine crystals. On standing in water they slowly lost iodine to leave behind the white amorphous shells, consisting of the oxidized dextrin.

Removing the iodine from the bulk of the precipitate was difficult. One method consisted of converting the iodine to sodium iodide by adding sodium thiosulfate and washing out the salts by repeated centrifugation of the oxidized dextrin in fresh volumes of water. However, after about the third washing the material began to swell and go into solution. Another method was to extract the iodine precipitate after

repeated washing with water with methyl alcohol in a Sohxlet. This removed practically all but not all of the iodine as the dried material was still slightly purple in color.

The methyl alcohol extract on slow evaporation deposited large (.2 cm. x .5 cm.) fusiform crystals with four faces. These were shiny black in appearance. On standing in air, they lost iodine and exhibited a reddish-brown color. Under the microscope the sharp outlines of the crystals were seen to have become fuzzy and looked as if amorphous, thread-like particles were sticking out of the body of the crystal. On grinding and allowing to stand in the air almost all of the iodine was lost to leave an orange-white residue. The crystals dissolved in potassium iodide solution to leave filmy, amorphous residues. Analysis of these crystals showed that only about one per cent of the amorphous carbohydrate material was present.

There is no doubt that the oxidized dextrins are partially precipitated by iodine since the isolated oxidized dextrins on solution in water and treatment with saturated iodine-potassium iodide solution always gave heavy, amorphous, reddish-brown precipitates. However, no crystals were obtained in this way. This supports the conclusion that the crystals obtained above are really iodine, since they are formed in solutions of iodate salt by the addition of potassium iodide and acid to produce iodine in greater amounts than are soluble in the concentrations used. These iodine

crystals in forming, carried down part of the oxidized dextrin as an integral part of their structure.

The yields of the oxidized dextrins obtained by precipitation with iodine amounted to 10 to 20 per cent of the original dextrin. The general observations on the iodine precipitation of the oxidized cornstarch dextrin apply to the waxy cornstarch dextrin.

A sample of the oxidized cornstarch dextrin was subjected to catalytic reduction as follows: A .05 gram sample was dissolved by warming in 10 cubic centimeters of water. After cooling, .025 grams of Adam's catalyst (PtO_2) was added and the solution was shaken under a slight pressure of hydrogen. The reaction was followed by noting the reduction in volume of hydrogen with time. The hydrogenation was complete in about three hours. Addition of .005 grams of fresh catalyst resulted in the further consumption of only enough hydrogen to saturate the added catalyst. It was found that 18.9 per cent of the theoretical amount of hydrogen was taken up on the basis that the oxidized dextrin should have suffered cleavage of each glucose unit between carbons two and three to give rise to two aldehyde groups.

Removal of the iodic acid with lead carbonate

Ten grams of the dextrins, dissolved by heating in 500 cubic centimeters of water, were treated with solutions of periodic acid (H_5IO_6) at 15°C. The oxidations were allowed to go for 16 hours. Then an excess of lead carbonate was

added and the solutions were stirred vigorously for 48 hours. The lead salts were filtered off, leaving a slight cloudiness in the filtrates which could not be removed. The filtrates were evaporated to dryness in vacuo at 45°C. to obtain the oxidized dextrins. The yields amounted to from 80 to 90 per cent of the original dextrins.

Two samples of oxidized dextrin were prepared from the cornstarch limit dextrin. One was oxidized with .63 times the theoretical amount of periodic acid and had an ash content of 5.27 per cent. This oxidized dextrin on catalytic reduction took up 43.2 per cent of the theoretical amount of hydrogen. In calculating the amount of hydrogen consumed by these oxidized dextrins, allowance was made for the salt content. This consisted of lead carbonate since solution of the samples in water and treatment with acid and potassium iodide liberated only a negligible quantity of iodine, showing practically complete absence of any iodate or periodate salts. The amount of lead carbonate was estimated from the ash content, assuming the ash to consist of lead oxide.

Another sample of cornstarch dextrin was oxidized with the theoretical amount of periodic acid. It had an ash content of 8.24 per cent. Two reductions of this oxidized dextrin showed a consumption of 69.4 and 68.6 per cent respectively of the theoretical amount of hydrogen, assuming two aldehyde groups per glucose residue.

A sample of waxy cornstarch limit dextrin was oxidized with the theoretical quantity of periodic acid. The product had an ash content of 9.15 per cent and took up 59.8 per cent of the theoretical amount of hydrogen on catalytic reduction.

Discussion

To facilitate discussion, Table XVII has been included for the purpose of summarizing some of the results obtained from the study of the samples of cornstarch limit dextrin and of waxy cornstarch limit dextrin.

The specific rotations of the limit dextrins are in the neighborhood of the values reported in the literature, if account is taken of the lowering due to the presence of sodium hydroxide. The values reported in the literature are mostly for limit dextrins from potato starch in water and range from +170° to +221°. The specific rotation of the cornstarch dextrin could not be measured in water. The values for the acetates in chloroform are in agreement with the values of from +142° to +170° reported by Haworth and his coworkers (117, 118) for potato starch limit dextrin acetates. For the methylated dextrins values of +190° to +222° were reported by these authors depending on whether the acetate or the free dextrin was taken for methylation.

Values for the molecular weights of the acetates of the potato starch dextrins by Staudinger's viscosity method were

TABLE XVII. Results of the study of the limit dextrins.

Experiment	Cornstarch Limit Dextrin	Waxy Cornstarch Limit Dextrin
Yield of dextrins (per cent)	25.9-31.1	38.4-39.5
$[\alpha]_D^{25°}$ of the dextrin in HOH*	---	+172.0°
$[\alpha]_D^{25°}$ of the dextrin in 2.5% NaOH*	+154.6°	+156.2°
$[\alpha]_D^{25°}$ of the dextrin acetate in $CHCl_3$	+159.0°	+157.2°
$[\alpha]_D^{25°}$ of the methyl dextrin in $CHCl_3$	+203.8°	---
Chain length as calculated from the value of R_{Cu}*	133	132
Chain length as calculated from the detn. of M. Wt. of the acetate in camphor	14.9	13.5
Chain length as calculated from the detn. of M. Wt. by viscosity of the acetate in m-cresol ($K=1x10^{-4}$)	263	350
Chain length as estimated from the amt. of tetra-methyl glucose resulting by hydrolysis of the methyl dextrin	9.5	---

* These data taken from Table XIII on page 111.

determined by Haworth, etc. (117, 118) using $K = 1 \times 10^{-3}$ and corresponded to chain lengths of 25 to 80 glucose units.

Haworth, Hirst and Waine (117) by the end-group method first reported a value of 16 to 17 glucose units for the chain length of the potato starch limit dextrin. Later, however, Haworth, Hirst, Kitchen and Peat (118) reported a value of 12 glucose units. In view of the fact that the dextrin is not homogeneous, the latter is in fair agreement with the value of 9.5 found here for the cornstarch dextrin. It should also be noted that these values are in fair agreement with those of 14.9 and 13.5 found for the cornstarch dextrin acetate and the waxy cornstarch dextrin acetate respectively, determined by the lowering of the melting point of camphor.

The value of 67 glucose units for the chain length of cornstarch by the method of end-group assay is not in agreement with the values of from 25 to 35 reported by Haworth, etc., for various starches, although it is in better agreement with the value of 52 reported by Hess and Lung (78). With the small amounts of tetramethyl glucose involved an error of 50 per cent in its estimation seems quite possible especially if one has not had long experience with the determination.

Beckmann and Landis (112) with their ultracentrifuge studies found molecular weights for the fractions of the limit dextrin from gelatinized potato starch corresponding to

73.2 and 178.8 glucose units. These are about the same order of magnitude as the molecular weights derived from the reducing values of the cornstarch dextrins and are a little less than the ones found by viscosity measurements using $K = 1 \times 10^{-4}$.

No attempt has been made to rigorously evaluate the various methods used for determining the molecular weights of the limit dextrins. However, it does seem probable that if the limit dextrins do not consist simply of thread-like molecules as is suggested by their resistance to further digestion by beta-amylase, then any method of end-group assay, either of the reducing or the non-reducing end groups, must give erroneous results. The values obtained from viscosity measurements would also be in error if, according to Staudinger, the relation between molecular weight and viscosity holds only for long, straight chains. In fact, Staudinger has concluded that starch has a relatively short chain length with respect to its molecular weight in contrast to cellulose which is generally accepted to consist of straight-chain molecules. He has postulated a branched-chain structure for starch.

It is interesting that the limit dextrins are produced in the same amounts (approximately 30 to 40 per cent) from different starches and have in common about the same low reducing value and the same proportion of non-reducing end groups as far as is known. This is to be contrasted with

the fact that the dextrins vary widely in solubility and that even the dextrin from one kind of starch can be separated into fractions with different solubilities and iodine colors. This fractionation is dependent on mole size as is indicated by the ultracentrifugal studies of Beckmann and Landis (112). Thus the peculiar property of the limit dextrins, i.e., their resistance to further digestion by beta-amylase appears to be bound up with the structure of the molecules rather than their size.

The low reducing value of the limit dextrins suggests that most of the terminal reducing groups are involved in the linkages of the molecule. One such picture has been presented by Hirst and Young (72) who assume the fundamental units of starch (30 glucose unit chains) to be arranged in a branched-chain structure, the terminal reducing group of one chain being joined to the hydroxyl on either carbon two, three or six of a glucose unit in a second chain by a glucosidic link. Another possibility is that the terminal reducing groups might be condensed with one another or with the intermediate glucose units of another chain in a dioxane grouping similar to that found with the simple dimer molecules, such as the methylcycloacetal of acetol, etc.

If the limit dextrins are not straight-chain molecules, then estimation of the non-reducing end groups would not give a true measure of the molecular weight. The high proportion of tetramethyl glucose obtained from the methyl

dextrin suggests that more than one non-reducing end group exists in the molecules. According to Hirst and Young, the end-group method estimates the length of the fundamental units of the dextrins, i.e., 9.5 to 12 glucose units. A logical view seems to be that the beta-amylase, starting at the non-reducing ends of the starch molecules, has eliminated the straight-chain portions (60 per cent) to leave centers which do not consist of a straight-chain structure. These might conceivably possess several branches, terminating in non-reducing end groups. Freudenberg (155) has suggested that the starch molecule consists of a Schardinger dextrin (five or six glucoses arranged in a ring) with two side chains attached by glucosidic links. At once the possibility arises that the Schardinger dextrins are to be found in the limit or residual dextrins left by beta-amylase digestion of starch. This would explain the low reducing value and the high proportion of end groups characteristic of the limit dextrins. Their heterogeneity, according to Freudenberg, would be due to a tying or knotting together of these centers with different degrees of complexity.

If the dextrins contain some type of branched chain, then appreciable amounts of dimethyl glucose might be expected on hydrolysis of the methylated dextrins since it is assumed that these structures have been concentrated in the dextrins by the action of the beta-amylase. The cornstarch methyl dextrin yielded at least no more than one per cent of

dimethyl glucose which was no greater than the amount obtained from the methyl cornstarch. The failure of any increase in the amount of dimethyl glucose with the limit dextrins may be due to the fact that structures in the dextrins giving rise to dimethyl glucose have been ruptured in the process of methylation. This would not be too surprising in view of the fact that mild autoclaving of the limit dextrins serves to remove their resistance to further digestion by beta-amylase.

The significant features of the study of the periodic acid oxidation of the limit dextrins are briefly as follows: The consumption of acid both by the dextrins and by the original starches (Figures 10 and 11) was very rapid during the first hour of oxidation at which time about 65 per cent of the theoretical amount had been consumed, based on the assumption of free hydroxyls on carbons two and three of all the glucose units. The theoretical amount was not consumed until about six or seven hours. With both the starches and their limit dextrins, the consumption of periodic acid seems to be due to two reactions. One is rapid (one to two hours) and must consist of the cleavage of the glucose units between carbons two and three. The second is quite slow, though it is seen to be faster for the dextrins than for the starches. This might explain why the consumption of acid by the dextrins did not fall off sooner than it did for the starches, as was expected. The more rapid consumption of periodic

acid by the dextrins in the secondary reaction as compared with the starches, when superimposed on the primary rapid oxidation of the dextrins, results in a curve similar to that of the starches, i.e., both begin to fall off when about 60 per cent of the theoretical amount of periodic acid is consumed. The implication of these observations is that only about 60 per cent of the glucoses in these materials contain free hydroxyls on carbons two and three.

It is interesting that from 10 to 20 per cent of the oxidized dextrins can be isolated by precipitation with iodine. This material took up only 18.9 per cent of the theoretical amount of hydrogen based on the assumption of two aldehyde groups per glucose residue. This suggests that the iodine coloring ability of the limit dextrins is involved with the glucose units which do not have free hydroxyls on carbons two and three. The failure of the oxidized starches to precipitate with iodine under the same conditions suggests that the iodine-binding linkages are more concentrated in the limit dextrins and possibly have been severed from the rest of the molecule by oxidation with periodic acid.

The oxidized dextrins (oxidized with the theoretical quantity of periodic acid) recovered in nearly 100 per cent yields all took up about 60 to 70 per cent of the theoretical amount of hydrogen on catalytic reduction. This supports the conclusion that about 40 per cent of the glucoses in the

limit dextrins and possibly more do not have free hydroxyls on carbons two and three.

In view of these observations it becomes highly significant that a Schardinger type dextrin, say with two side chains of from one to two glucoses each, would contain from 20 to 30 per cent of glucoses which might be blocked on the second or third carbons to the action of periodic acid. It would be interesting to know if the Schardinger-type dextrins could be obtained in greater yields from the limit dextrins than from the original starches.

SUMMARY

1. A method for determining the degree of fractionation of starch pastes by electrodialysis has been used which depends on the determination of the concentration of starch in the supernatant liquid after a single dialysis. The amount of soluble fraction is given by the product of this concentration and the total volume of paste used.

2. Increased temperature of preparing cornstarch pastes for electrodialysis resulted in increased amounts of the soluble fraction only in so far as it brought about greater disorganization of the starch granules. The real effect of increased temperature of preparing the pastes was to diminish the amount of the soluble fraction, as was shown by rupturing the granules of the pastes prepared at the different temperatures before electrodialysis.

3. The reducing values of the soluble and insoluble fractions of cornstarch obtained by electrodialysis indicated that the insoluble fraction has a relatively greater average mole size than that of the soluble fraction. It has been pointed out that reducing value has no significance as to molecular weight except for comparative purposes. The solutions of the so-called soluble fraction were quite cloudy indicating that this fraction is not truly soluble. In line with this observation, the conclusion was drawn that the

degree of fractionation of cornstarch pastes by electrodialysis depends for the most part on the presence of polar groups, i.e., phosphoric and fatty acids. The effect of the temperature of preparing the pastes on the degree of fractionation was attributed to a change in the colloidal state of the starch as brought about by a cleavage of the polar groups at the higher temperatures. The failure of waxy cornstarch to give a separation by electrodialysis showed that it does not contain the insoluble fraction found in ordinary cornstarch by electrodialysis. Dry-ground cornstarch gave considerably more of the soluble fraction by electrodialysis than did the original starch. This was due to the hydrolytic breakdown during grinding, as was shown by the increased reducing power of the ground starch.

4. The degree of fractionation of starch pastes by freezing was shown to vary with the kind of starch and the treatment of the paste before freezing. Cornstarch pastes gave about five per cent of a truly soluble fraction. Its solutions were water-clear and it had a relatively smaller average mole size than the original starch on the basis of reducing value. The soluble fraction of cornstarch obtained by electrodialysis was recovered in 95 per cent yields by freezing, upholding the conclusion that it is not truly soluble. Waxy cornstarch pastes could not be fractionated by freezing. A paste of dry-ground cornstarch, which had suffered considerable degradation as indicated by its reducing

value, gave more of the soluble fraction by freezing than did the original starch. The conclusion was drawn that fractionation of starch pastes by freezing is more or less dependent on the distribution of mole sizes in the pastes, and the fractions have no particular relation with the fractions obtained by electrodialysis.

5. Retrogradation of cornstarch paste was concluded to have little, if any, effect on its fractionation by electrodialysis. Under the conditions of time and temperature involved with electrodialysis, only slight aging would occur as measured by increased resistance to saliva digestion. The results of the aging experiments were in harmony with the view which pictures retrogradation as a gradual desolvation and consequent precipitation of the large, difficulty soluble molecules which are solvated at the temperatures of preparing the pastes. Pastes of waxy cornstarch and of dry-ground cornstarch did not retrograde, suggesting that their average mole sizes are small enough so that the process of desolvation does not take place appreciably on standing at low temperatures.

6. Fractionation of ordinary cornstarch and of waxy cornstarch by beta-amylase digestion to give rise to the residual or limit dextrins was carried out. The yields of the redigested dextrins amounted to from 30 to 40 per cent of the original starches; the waxy cornstarch dextrin resulted in slightly greater yields (about ten per cent) than did the

ordinary cornstarch dextrin. These dextrins characteristically showed resistance to further digestion by beta-amylase, low reducing power and retention of the ability to color with iodine. The ordinary cornstarch dextrin was heterogeneous as shown by its fractionation, by alcohol precipitation, by freezing and by electrodialysis. It consisted of a soluble, red-iodine-coloring fraction and of a difficultly soluble, blue-iodine-coloring fraction. The waxy cornstarch dextrin consisted entirely of a soluble, red-iodine-coloring material.

7. Characterization of representative samples of the cornstarch dextrin and of the waxy cornstarch dextrin by measurements of reducing value, optical rotation, molecular weight of the acetate by the Rast camphor method and by Staudinger's viscosity method and measurement of oxidation with periodic acid revealed no marked difference between these dextrins. However, on the basis of the differences in solubility and iodine colors, the view was adopted that the resistance to digestion by beta-amylase is due to some structural feature of these dextrins rather than to size or solubility. The groups responsible for impedence to beta-amylase action were considered to be concentrated in the limit dextrins.

8. Hydrolysis of the completely methylated cornstarch limit dextrin resulted in an amount of tetramethyl glucose corresponding to an estimated chain length of 9.5 glucose

units. This must be erroneous if the dextrin does not consist of straight-chain molecules as is suggested by its resistance to further digestion by beta-amylase. On the same grounds, estimation of the reducing end groups and determination of molecular weight by viscosity measurements would give false values. An estimated chain length of 67 glucose units was found for cornstarch by the method of end-group assay. This value is subject to the reservation that considerable error in the determination of such small amounts of tetramethyl glucose is probable.

9. Failure to find evidence for any increase in the amount of dimethyl glucose from the methylated cornstarch limit dextrin over that from the methyl cornstarch lead to the conclusion that structures which might give rise to dimethyl glucose, i.e., branched chains, etc., are either absent or are ruptured in the process of methylation.

10. Evidence that the limit dextrins do not consist of straight-chain molecules and that either one or both of the hydroxyl groups on carbons two and three of the glucose units are involved, was found from a study of the periodic acid oxidation of the limit dextrins. The rate of oxidation was shown to slow up when about 60 per cent of the theoretical amount of periodic acid was consumed. The theoretical value is based on the presumption of a glycol grouping on carbons two and three of all the glucose units. The dextrins oxidized with the theoretical quantity of periodic acid and

recovered in nearly 100 per cent yields took up only 60 to 70 per cent of the theoretical amount of hydrogen on catalytic reduction. This supports the conclusion that from 30 to 40 per cent of the glucose units in the limit dextrins do not have a free hydroxyl group on both carbons two and three. The fact that the oxidized dextrins can be precipitated with iodine suggests that it is the structures associated with these blocked glucoses which are responsible for the iodine coloration of the limit dextrins and of starch. The interesting possibility is suggested for further investigation that the crystalline Schardinger dextrins can be obtained from the limit dextrins in greater yields than from the starches.

ACKNOWLEDGMENTS

The writer wishes to express his sincere appreciation to Dr. R. M. Hixon for suggesting this problem and for his continued interest and guidance.

Mr. L. F. Remmert assisted with some of the experiments on electrodialysis.

Mr. H. D. Kaslow prepared the sample of waxy cornstarch.

The sample of ordinary cornstarch was donated by the Clinton Company of Clinton, Iowa.

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