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CHANGES OF STARCH DURING OXIDATION

by

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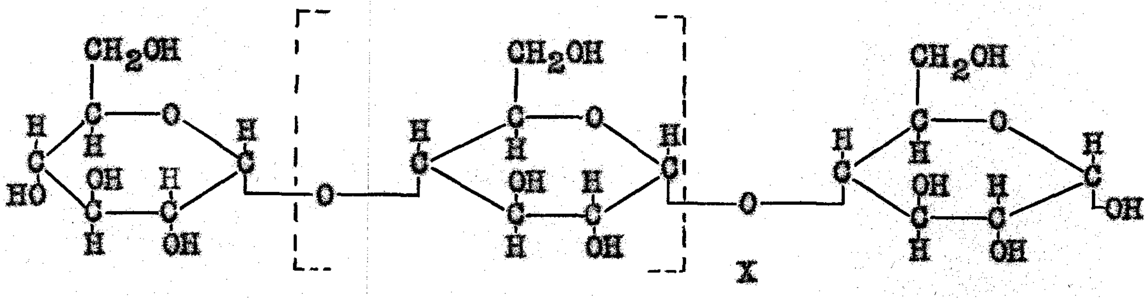
I. INTRODUCTION

The starch industry manufactures numerous types of modified starch. These modifications are produced by such processes as alkaline, acidic or enzymic treatment, swelling in hot water, in steam or in salt solutions, heating, oxidation, or by any combination of these factors. Both wet and dry methods have application. The various modified starches find their greatest use in the textile, paper, laundry and food industries.

Although oxidation has been employed commercially for at least forty years as a means of producing modified starch, the mechanism by which the modification is effected has not received extensive study nor clearcut elucidation. Some of the difficulties in determining the mechanism of starch oxidation have been due to incomplete formulation of the structure of starch. Although the degree of polymerization, the question of branching, the state of the terminal groups and other aspects of the structure of starch are still in dispute it is generally agreed that starch consists largely of chains of alpha-glucopyranose units joined by 1-4 linkages:



Starch

It is apparent from the above formulation that the site of attack of an oxidizing agent is not very limited. There are available for oxidation terminal aldehydic groups, primary alcoholic groups and secondary alcoholic groups. The priority which one or the other of these groups may enjoy in the initial stage of starch oxidation has been the subject of much speculation.

A survey of the literature on the oxidation of starch disclosed that among the large number of papers only a few contributed substantially to the study of the mechanism of the oxidation. This lack of data on the oxidative mechanism is due in part to the fact that the oxidations were on the one hand too mild and on the other hand too extensive to throw light on the intermediate stages. Many investigations have been concerned with the physical properties of the slightly oxidized starches and with the end products of extensive oxidation to sugar fragments but very little work has been done on the hydrolysis and investigation of intermediates. However, the mechanism of one type of oxidation, periodic acid oxidation, has been greatly clarified by Jackson and Hudson (169,170) and Caldwell and Hixon (36).

This thesis concerns two phases of the oxidation of starch by halogens in alkaline and neutral media. One of these is a study of the oxidation of raw starch[1] by an easily controlled process, viz., by an electrolytic sodium hypochlorite treatment in alkaline solution. This study seeks information regarding the factors which influence the properties of the oxidized starches and the changes in properties resulting from an increasing amount of oxidation. The other phase is a study of the mechanism of the more extensive oxidation of gelatinized starch by bromine in neutral solution. This involves stabilization of the labile oxidized starch, cautious hydrolysis to oxidized glucose units, fractionation of the hydrolysate and identification of the resulting carbohydrate acids.

A review of the literature on all types of oxidation of starch has been made as an orientation.

Oxidizing agents can not be compared on a weight basis, e.g., one pound of chlorine is equivalent to eight pounds of activin. In order that satisfactory comparisons may be made among the oxidizing agents used on starch, in this thesis the

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1. The term "raw starch" refers to native ungelatinized starch granules and is used in this thesis in preference to the longer unwieldy term.

weight of each oxidant has been converted to equivalents and reported as the number of equivalents for each glucose anhydride ($C_6H_{10}O_5$) unit. For example, two equivalents of chlorine per glucose unit would mean 70 grams of chlorine per 162 grams of starch. This system shows the error in many comparisons which have been made in the literature on a weight basis and makes possible a rational comparison of all oxidizing agents.

II. HISTORICAL

A. The Oxidation of Starch

Although the fact that starch can be hydrolyzed by acid has been far more important to commerce and research than has the oxidation of starch, the hydrolysis of starch was not discovered until twenty years after starch had been oxidized. Fourcroy (82) in 1792 had oxidized starch by means of concentrated nitric acid, later by chlorine gas, both before Kirchhoff (186) in 1811 discovered the important conversion of starch to glucose by means of acid.

The course of the oxidation of starch depends to a large extent on the pH of the medium. Starch has been oxidized in acid, alkaline and neutral solutions.

1. Oxidation of starch in acid media.

In an acid medium hydrolysis of starch occurs along with oxidation. Oxidation in acid solution gives, therefore, oxidized forms of the hydrolytic products of starch.

a. Oxidation by nitric acid. Doroschewski and Rakowski (56) observed during quantitative studies of starch hydrolysis by very dilute nitric acid that concentrations over 0.6N nitric acid resulted in oxidation. Stronger nitric acid solutions oxidized starch to saccharic, tartaric, and oxalic acids and carbon dioxide (90,114,122,184,197,287). Hachihama and co-workers (90,122) reported a 49% yield of saccharic acid and a 10% yield of oxalic acid.

By using concentrated nitric acid Bechamp (8) produced oxidized starches of varying solubility in cold water. Oxidized starch containing nitrogen was obtained by many of the following when the oxidation was carried out by concentrated nitric acid. Petit's (243) product contained 6% nitric acid. The nitric acid, however, was liberated by treatment with water. The purified resulting product had acidic and reducing properties. Further oxidation produced the same acids as obtained with dilute nitric acid, viz., saccharic, tartaric and oxalic. This last acid, oxalic, was also obtained by Fourcroy (82) and Pelouze (239) when they used concentrated nitric acid on starch. Braconnot (20) made a product soluble in cold water. By using a large excess (117 equivalents) of concentrated nitric acid, Will and Lenze (327) produced a trinitrate (14% N_2) of starch which exploded at 194°C. Brown and Millar (30) used 4.6 equivalents of strong nitric acid to produce a sesquinitrate (9.1% N_2) from soluble starch, limit dextrin, maltodextrin and Nägeli's amylodextrin. Although the regenerated starch was little altered by nitration and still had a blue iodine color, the distinct acid character of the nitrated dextrins together with their loss of reducing power was a proof that oxidation had occurred. The starch nitrates of Berl and Butler (14) containing 12.86 to 13.44% nitrogen were prepared from corn, potato, rice and soluble starches by 8 equivalents of nitric acid mixed with sulfuric acid.

Lieben (197) obtained a mononitrostarch (6.76% N_2) by the action of fuming nitric acid. The nitrostarch of Berl and Smith (15) contained 14.08% N_2 and gave a strongly reducing acid when the nitrogen was removed by alcoholic sodium hydroxide. Pelouze (240) suggested, because of its combustibility and its resemblance to guncotton, that nitrostarch should be used as an explosive.

Some of the patents on nitric acid oxidation of starch are listed in this paper (66,152,165,210,217,222,227,252,293).

b. Oxidation by ammonium nitrate. The decomposition of ammonium nitrate was accelerated by the presence of starch (75). Evidence of oxidation was the carbon dioxide and carbon monoxide evolution. Ammonium nitrate was also used in a patented process (256).

c. Oxidation by chromic acid. Several investigators allowed chromic acid to act on starch and dextrin under mild conditions to produce a small amount of oxidation. Starch thus treated was converted to soluble starch (55,136,258,270, 311) or to dextrins (62,144,197). The amount of chromic acid employed by Harz (144) was one equivalent per glucose unit. Reychler's (258) oxidized starch, though insoluble in hot water, dissolved readily in very dilute alkali. It was produced in the cold by 2.5 equivalents of chromate. Jambuserwala (171) used dilute chromic acid for the oxidative hydrolysis of starch. His data on the reducing value and viscosity of the products do not allow the conclusion that oxidation has taken place even though it is practically certain on theoretical grounds that the starch has been oxidized. A later publication by Jambuserwala and Kanitkar (173) did offer evidence that oxidation of the starch occurred, viz., the gradual increase in acidity during oxidation. Several earlier workers had observed the acidic properties of these chromic acid products (258,329).

Cross, Bevan and Beadle (44) reported a yield of about 10 to 11% furfural when starch was oxidized in the cold by six equivalents of chromic acid, the furfural being distilled from a solution of the oxidized starch in 12% hydrochloric acid. Mann, Krüger and Tollens (206) repeated the experiment of Cross, Bevan and Beadle but heated the oxidation mixture. They obtained no test for uronic acids. Alkaline permanganate, however, gave an oxidized starch which showed the presence of uronic acid by furfural evolution and by a color test. It seems probable that heating of the chromic acid solution caused destruction of the polyglucuronic acids.

Semichon and Flanzy (278) obtained large amounts of formaldehyde. When starch was heated with Hehner's mixture containing 7.4% potassium dichromate and 75% sulfuric acid, Lieben and Molnar (198) were able to oxidize starch completely. By that time chromic acid had already been used to determine cellulose quantitatively by complete oxidation. Richardson, Higginbotham and Farrow (259) applied the method to starch successfully. They oxidized the starch completely by boiling in a mixture of sulfuric acid and potassium dichromate, then titrated the excess dichromate with ferrous ammonium sulfate. The amount of starch was calculated from the equivalents of dichromate used for oxidation.

The chromic acid treatment of starch has also been patented (221,257).

d. Oxidation by permanganates. Langbein (193) found that one gram of starch would reduce 1.5 grams of potassium permanganate in acid solution. By less drastic treatment with permanganate, Lieben (197) obtained a brown product that was not studied. Very mild oxidation, such as that obtained by Wolff (329) on using 1% potassium permanganate in the cold, or by Reychler (258) or Nakamura (220), yielded soluble starches.

Patents have been registered for the use of acidic permanganates on starch (175,257).

e. Oxidation by hydrogen peroxide. The action of hydrogen peroxide on starch has attracted more workers than that of any other oxidizing agent (5,28,38,61,73,95,96,99,100,111,112,113, 136,137,138,225,228,229,263,288,298,332). Hydrogen peroxide alone, according to Wurster (332), hardly attacks starch at ordinary temperatures but in boiling acid solution produces dextrins and glucose. Gruzewska (95,113) identified dextrins, maltose and oxalic acid but no glucose among the oxidation products when the reaction was carried out at 37°C. As the oxidation proceeded a precipitate of retrograded amylose formed, the iodine color and material precipitable by alcohol disappeared, the reducing power showed a slight decrease, and the acidity increased to a constant value in twice the time required for the iodine color to be dissipated (96,111,112). Soluble starch behaved like a mixture of amylopectin and amylose, on each of which control oxidations had been run. Neuberg and Miura (225), contrary to Gruzewska's findings claimed that 1.3 to 2.7% glucose was among the products when two equivalents of peroxide were used in the presence of ferrous sulfate. Their claim was based on the rotation and reducing power of the resulting solution and on the melting point and nitrogen analysis of the glucosazone. Gerber (99,100) claimed to have simulated an amylolytic hydrolysis of starch by using very dilute hydrogen peroxide (0.1% and less). Dextrins and maltose were the products; the latter was oxidized when larger amounts of peroxide were used. Omori (228) states that the action of hydrogen peroxide on starch is governed by a mechanism different from that of diastasis.

Fernbach and Wolff (73) sought accelerators for solubilizing starch by means of hydrogen peroxide. They, too, measured the production of acid during the oxidation. Durieux (61) used ferric chloride as an accelerator of the oxidation after finding that colloidal iron had no effect on the rate. Biedermann and Jernakoff (17) obtained considerable catalysis in the peroxide oxidation by using iron or copper salts. Omori (228) claimed that hydroxyphenol was a better catalyst than the heavy metal ions. According to Omori (229), the combination of iron salt and hydrogen peroxide on starch was made more effective in its modifying power by the addition of cystine, tyrosine, glutathione or thioglycollic acid.

With increasing amounts of hydrogen peroxide for a definite time of oxidation, Durieux (61) found that the reducing power soon reached a maximum value, then fell to zero. The maximum in acidity was passed while the reducing power was decreasing. When this catalyzed oxidation was carried out with a constant amount of hydrogen peroxide the acidity reached a constant value after a certain time. Samec (262,263) noted the large increase in acidity and stated that there were a considerable number of carboxylic acid groups produced in a sample of starch oxidized by hydrogen peroxide. The distillates from peroxide oxidized starch contained substances which absorbed iodine (38). Brown (28) obtained formic acid in the distillate. He stated, however, that neither glucose nor maltose was found in the residue although aldehyde and reducing tests were positive.

Palit and Dhar (236) found that 69.6% of the original starch was oxidized to carbon dioxide in two hours at 50°C by hydrogen peroxide and ferric sulfate. In the oxidation of starch at 100°C (137,138) hydrogen peroxide was more effective than activin or sodium perborate; at 137°C activin was the most effective. Patents (39,46,135,151,301) have been registered for the peroxide oxidation.

f. Oxidation by halogens.

(i) Fluorine. Carbon was deposited when Moissan (213) allowed fluorine to react with starch. The carbon burned in the fluorine and produced enough heat to make the decomposition very rapid.

(ii) Chlorine. Many early investigators used chlorine to modify starch. De Gassicourt (48) gives Fourcroy and Parmentier credit for converting starch to sugar by chlorine treatment before Kirchhoff (186) discovered the conversion of starch to sugar by means of acid. The product obtained by Fourcroy and Parmentier had both a bitter and a sweet taste. Sometime later, however, Liebig (199) reported that starch was very difficultly decomposed by the prolonged action of chlorine, only 1/20 of the starch in solution being decomposed by chlorine gas in eight hours. When Städeler (290) distilled starch with manganese dioxide and hydrochloric acid, carbon dioxide was evolved, chloral, formic acid and an oil were formed. Lieben (197) did not study the colored product which he obtained.

Wolff (329) obtained a slightly modified starch with acid properties. Gerber (101) reported that a very small amount of chlorine added to starch paste before the addition of an amylase accelerated hydrolysis by the enzyme. A larger amount of chlorine inhibited the digestion by amylase. Similarly, in the bleaching of wheat flour by chlorine, enzymatic activity was increased (4). The amount of chlorine used was very small, 300 to 500 parts per million, but it nevertheless increased acidity and diastatic activity and improved baking qualities. These changes were not encountered to any noticeable extent when nitrogen peroxide, nitrogen trichloride or benzoyl peroxide were used for flour bleaching.

Samec (262,263) oxidized moist starch with chlorine gas according to Kindscher's patent (185), but at room temperature rather than at 100°C. Samec's chlorinated starch was oxidized to a greater extent than the starches oxidized by any of the other agents or methods which he used. The gel phase of this chlorinated starch had the lowest molecular weight, the highest titratable acidity, the highest hydrogen ion concentration and the highest conductivity of all his oxidized products.

When chlorine water was allowed to act on amylose (beta-amylose), Fletcher and Taylor (77) noted that the pH dropped rapidly from 7 to 1, while the reducing value underwent a sharp rise and the viscosity a simultaneous drop a few hours after the inception of oxidation. The reducing value and viscosity soon reached a constant value. This was not true of the reducing value when a phosphate buffer was used to keep the pH of the medium above 2. In the latter instance the sharp changes of viscosity and reducing value came in two minutes.

Numerous patents have been registered for the modification of starch by chlorine: (11,12,13,16,93,94,141,166,180, 185,204,217,282,289,320).

It is interesting to note in connection with modification of starch by chlorine that Hirst, Plant and Wilkinson (160) used chlorine as a catalyst in acetylation. When amylopectin (alpha-amylose) is acetylated by this procedure the acetate obtained varies with the method of preparation, being more soluble and less viscous the more chlorine is used; amylose (beta-amylose), however, always yields the same acetylated product (139). It is possible that the catalyst chlorine, is oxidizing or modifying in some other way the amylopectin before it is acetylated.

(iii) <u>Bromine</u>. Habermann (120,121) found that treatment of dextrin with five equivalents of bromine per glucose anhydride unit evolved carbon dioxide and bromoform and gave gluconic acid as a product. He prepared the calcium, barium, lead and cadmium salts and ethyl ester of the gluconic acid, the first two of which were crystalline. Further oxidation converted the gluconic acid to bromoform, bromoacetic acid and oxalic acid. Lieben (197) also obtained gluconic acid. Franchimont (83), in order to exclude oxidation, treated dried potato starch with dry bromine and with bromine in chloroform. He reported the starch to be absolutely unchanged if all moisture was excluded. If moist air was present, hydrobromic acid was formed and acted on the starch. When dry hydrobromic acid and dry bromine in chloroform were added to dry starch, an orange product resulted. Lintner (201) and Syniewski (304) prepared a soluble starch by using bromine. Gerber (101) found that bromine, like chlorine, made starch more susceptible to amylolytic hydrolysis if small amounts of bromine were added to starch paste before digestion.

Bergmann and Ludewig (10) shook iodine-potassium iodide solution and bromine-potassium bromide solution with starch and with acetylated starch of 46-48% acetyl content to determine the affinity of the starch for these halogens. The presence of acetyl groups had very little effect on the absorption of the halogens. The authors stated that this agreed with the view that bridge oxygen atoms were responsible for halogen absorption of starch. While starch and starch triacetate absorbed both iodine and potassium iodide from I-KI solution, only the bromine was absorbed from bromine-potassium bromide solution. Bergmann and Ludewig, because of the short time that the starch was in contact with the bromine (four minutes), regarded it as improbable that the bromine absorption (0.08 equivs Br/glucose) could be due only to oxidation and substitution, and considered some of the bromine held by absorption.

Everett and Sheppard (65) measured the optical rotations, various color reactions, titratable acidities, Sumner reducing values, Folin-Wu reducing values, and the ratio between the last two, of solutions of polysaccharides treated with bromine water. Rotations showed that maltose and maltobionic acid were not intermediates in the hydrolysis and oxidation of starch, soluble starch, dextrin and glycogen. The oxidations resembled those of alpha-glycosides. Color tests and Sumner/Folin-Wu ratios gave evidence of the formation of keturonic acid derivatives of the anhydroglucose polymers. These higher keturonic acids reduced analytical sugar reagents at room temperature.

One patent mentioned the use of bromine (180).

(iv) Iodine. When the colored starch-iodine complex was decolorized by heating to 100°C, Personne (242) claimed that some of the iodine was lost by volatilization and some of the iodine converted the colored complex partly to a colorless starch-iodine compound and partly to sugar. These last two processes undoubtedly involved hydrolysis and oxidation. Rodewald and Kattein (260) however, solubilized starch by heating with iodine at 130°, recovered the starch by precipitation and iodine removal and reported that the product resembled pure starch grains. Andrews and Goettsch (2) were able to restore the original intensity of the starch-iodine color if the heating to 100° and subsequent cooling were carried out quickly. Otherwise the returning color became progressively weaker depending on the length of the heating period. The iodine color vanished after heating for 65 hours with 2.3 equivalents of iodine. Hale (127) claimed that loss of iodine in starch indicator was due to an oxidation of the impurities in the starch. In the titration of arsenite with iodine more iodine was used in the presence of starch than in its absence. The impurities causing this loss of iodine were soluble dextrins which gave a blue color with iodine.

The degradation of dextrin and glycogen by heating with iodine at 100° until decolorized was investigated by Vintilescu and Faltis (314). Hydrolysis and oxidation took place, the acidity being greater than that corresponding to the hydriodic acid. Formaldehyde and formic acid could be distilled from the liquid. The residue contained a non-reducing humic oxidation product which contained no combined iodine.

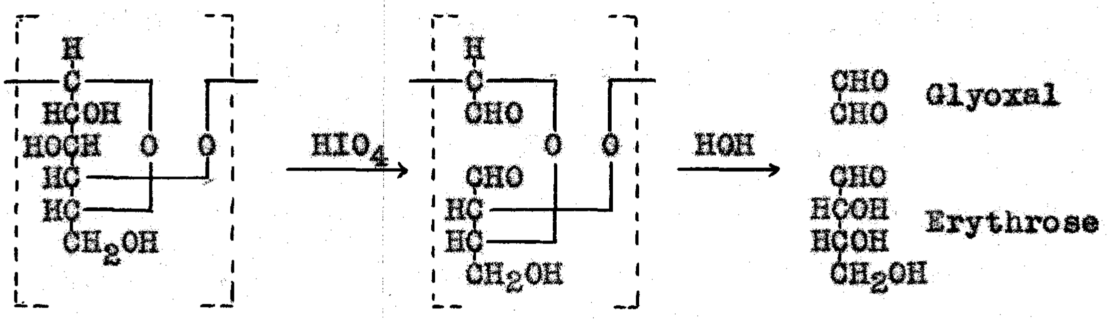
According to Angelescu and Mircescu (3) the decolorization of the starch-iodine complex on heating is due to diminution of adsorption, alteration of the degree of dispersion of the starch, and to irreversible changes of the starch or the iodine. This last would include oxidation of the starch. They further state that the temperature of decolorization does not correspond to the temperature of recoloration nor is it reproducible a second time with the same solution.

g. Oxidation by oxy-halogen acids.

(i) Hypochlorous acid. Bräutigam (22) found that hydrolysis of starch to glucose preceded oxidation by hypochlorous acid. The final products were oxalic acid and carbon dioxide. Craik (43) obtained an inconclusive test for maltose among the soluble products and reported only slight acidity at the end of the reaction. The rotation of the solution increased. On the basis of his experiments with beta-glucosan and disaccharides, he postulated an addition of the hypochlorous acid to the ring or bridge oxygen atoms. Fletcher and Taylor's (77) experiments with chlorine water on amylose (beta-amylose) have been discussed in the section on oxidation by chlorine. Jambuserwala and Kanitkar (172) used 0.25, 1, 2 and 3% acidified hypochlorite on corn, wheat, sago and farina starches. They measured the R_{cu} reducing value (mg. Cu/per gram of starch), the carboxyl content, the alkali-labile value, viscosity, and a few properties of value in the textile industry, e.g., the adhesive value. With increasing amount of oxidant the R_{cu} curves showed a slight decrease then a rapid increase along a straight line. The carboxyl content of each starch gradually increased during oxidation, the viscosity and adhesive value showed a sudden, initial drop to a low value with only a small amount of oxidant. Thereafter the decrease of these last two properties was very slight upon further oxidation. Seck (277) patented the oxidation by hypochlorous acid.

(ii) *Chloric acid.* Schmerber (270) prepared a soluble starch by using chloric acid prepared from a chlorate and sulfuric acid. Haake and Haake (116) as well as Mirow (211) registered the process in patents.

(iii) *Periodic acid.* Periodic acid reacted quantitatively with corn starch according to Jackson and Hudson (169) and oxidized the glycol grouping of the non-terminal glucose units to a dialdehyde structure. One mole of oxidant reacted per $C_6H_{10}O_5$ unit to split each anhydrohexose unit between the second and third carbon atoms into a two- and a four-carbon fragment. Hydrolysis of the oxidized starch would be expected to yield glyoxal from the two-carbon fragment and d-erythrose from the four-carbon fragment.



After hydrolysis Jackson and Hudson observed that the rotation was near the $[\alpha]_D$ of -14.5° reported for d-erythrose. Caldwell and Hixon (36) isolated the glyoxal as the phenylosazone and as the benzylphenylosazone. Further studies of this reaction, Malaprade's reaction (205), on a series of dextrin fractions were employed by Caldwell and Hixon to determine the molecular size of the dextrins. The quantity of formaldehyde liberated from the aldehydic end of the dextrin chain gave values for the chain lengths which correlated with the values determined by reducing value. These dextrins had chain lengths between 25 and 134 glucose units, indicating a much higher value for starch than the 25 to 30 units proposed by Haworth, Hirst and Woolgar (148).

Jackson and Hudson (170) later isolated the same two derivatives of glyoxal from the hydrolysis products of oxidized starch. They were further successful in identifying d-erythrose in the hydrolysate by bromine oxidation of the solution to d-erythronic acid. The erythronic acid was characterized as the brucine salt and as the d-erythronic lactone. Jackson and Hudson stated that the presence of other types of units in the oxidized starch in minor quantities was not excluded. Another product was, indeed, soon discovered. Grangaard, Michell and Purves (109) isolated a crystalline compound in yields of 0.7 - 0.9% after they had hydrolyzed the oxidized starch in methyl alcohol containing hydrogen chloride. This crystalline substance had the formula, $C_{13}H_{16}O_8(OCH_3)_4$, was stable to further oxidation by periodic acid or Fehling's solution. However, after mild acid hydrolysis it reduced Fehling's solution, pointing to a methyl acetal of an aldehyde or ketone. Corn, wheat, potato, arrowroot and soluble potato starches all yielded the same product.

h. Oxidation by other per compounds. In his studies on soluble starches produced by oxidation Samec (262,263) included sodium perborate and ammonium persulfate among the oxidants. The same measurements were made on these oxidized starches as were reported above in discussing Samec's work on starches oxidized by hydrogen peroxide and chlorine. Neither ammonium persulfate nor sodium perborate gave an extensively oxidized product. There was a slight increase in acidity over that calculated from the phosphoric acid content, but in general these two oxidants gave less modification than the other five oxidants, excluding air, which Samec used. Nakamura (220) likewise found about the same order of oxidizing power since the phosphorus content was decreased more by ammonium persulfate than by sodium perborate and more by potassium permanganate than by either of the other two agents. Sodium perborate and ammonium persulfate have been used to produce slight physical modification of starch granules (136).

Various patents described the use of persulfates and perborates (27,37,78,79,88,89,130,135,151,162,175,182,192,273, 286,294,295,296).

i. Oxidation by oxides in acid solution. Döbereiner (54) distilled a solution of starch in sulfuric acid and manganese dioxide with the result that carbon dioxide was evolved and formic acid was found in the distillate.

According to Schmidt and Graumann (271) chlorine dioxide, an agent for removing incrustations from plant skeletal substances, did not attack starch in 24 hours. Practically none of the chlorine dioxide was used in this time. Samec and Ulm (268) concurred in the above opinion to the extent that the microscopic appearance of potato and wheat starches was unchanged but found chemical changes in the treated starches. Chlorine dioxide removed amounts of phosphorus from wheat and potato starches which varied with the length of treatment, 0.2N chlorine dioxide on wheat starch for 8 days removing 84% of the phosphorus. When the starches were treated with chlorine dioxide and pyridine instead of chlorine dioxide alone, noticeable acidity was developed beyond that demanded by the phosphoric acid content. Samec and Ulm concluded that oxidation had occurred.

j. Oxidation by irradiation. Massol (208) and Bielecki and Wurmser (18) stated that irradiation of soluble starch solutions for 10 days at 45° gave splitting and oxidation. The products were dextrins, pentoses, glucose, formaldehyde and acidic substances. Both the conductivity and hydrogen-ion concentration rose to constant values in 100 hours. Ono (230) disintegrated starch paste by irradiation with ultrasonic waves and reported that oxidation may have been responsible for a small amount of depolymerization.

k. Oxidation by air in acid solution. This method was patented (33).

1. Oxidation by ozone. Gorup-Besanez (107) reported that potato starch in water was indifferent to ozone, showing no change in several days. König (189,190) bleached and deodorized amylaceous materials by passing ozone through solutions of starch or dextrin until the desired effect was obtained. Siemens and Halske (281) patented the preparation of an "ozone starch" by double treatment with chlorine and ozone. According to Schaeffer and Scheurer (269) the Siemens and Halske products varied in iodine color from blue to violet and from insolubility in cold water to complete solubility, the latter giving strong reduction of Fehling's solution. Pieper's patent (244) used ozone to prepare clear dextrins and gums free from odor and repulsive taste. The ozone was passed over the starch during roasting. Friedenthal (87) mentioned that a commercial soluble starch known as "ozone starch" had a molecular weight of 9450. Löb (202) passed a silent electric discharge through 1% solutions of starch, noted the formation of ozone, the gradual disappearance of the starch-iodine color, the reduction of Fehling's solution, the formation of osazones, but no true criterion of oxidation.

2. Oxidation of starch in alkaline media.

In alkaline media the various enolization reactions of Nef (224) can occur previous and concurrent to oxidation. Evans (34) and co-workers have studied the mechanism of the oxidation of sugars in alkali and have found evidence for enolization. The action of alkaline oxidizing solutions would, therefore, be expected to produce oxidized forms of hydrolyzed and enolized starch. Theoretically, no hydrolysis should occur, but there exists good evidence to the contrary. This is mentioned in the Discussion.

a. Oxidation by hypohalites.

(i) Alkaline hypochlorite. Schmerber (270) and Dollfus and Scheurer (55) solubilized starch with bleaching powder. Haller (134) noted that the temperature rise depended on the amount of sodium hypochlorite he used on potato starch. Trotman (311) reported an oxidation procedure for the modification of starches for sizing of cotton which used hypochlorite. Rassow (253) also measured the temperature rise as well as the decrease in viscosity and the increase in reducing power. Lobenstein's (203) thesis concerned the preparation of soluble starch by means of alkaline sodium hypochlorite.

The last two authors published a comprehensive treatise in collaboration (254) in which they traced the temperature rise during oxidation partly to heat of adsorption, partly to heat of decomposition of the sodium hypochlorite, and partly to heat of oxidation. The extent of the temperature rise depended on the amount of the hypochlorite solution. Completely dry starch gave an even more vigorous reaction, probably due to heat of hydration or heat of swelling. When large amounts of hypochlorite were used in 0.19N alkali, the solutions soon became acidic and reduced Fehling's solution in the cold. The reduction increased with the amount of hypochlorite used, while the viscosity decreased. The viscosities of all oxidized starches were considerably less than that of native starch. Barium starches were prepared by adding saturated barium hydroxide to the oxidized starches, and precipitating the salt with alcohol. Barium analyses of three fractions showed one barium atom per two, four and six glucose units respectively. No sharp differentations between the abilities of oxidized and native starch to be stained by dyes were observed although many acidic, basic and direct dyes were tried. When a 5% paste of one of these oxidized starches was aged, the viscosity dropped from 184 to 40 seconds in less than ten days. On long standing the oxidized starch powders increased their titratable acidity considerably during the first three months and reached a constant acidity only after twelve to eighteen months.

Samec and Blinc (264) allowed alkaline hypochlorite to act until a test sample of the starch went into solution at 95°C. The microscopic appearance including the shape and birefringence was very little changed although some granules showed cracks and stratification. The specific rotation was 198°, the iodine color blue to violet, the molecular weight by osmometry somewhat lower than that of native starch, but the viscosity at 55°C was greatly increased (by 64%).

Although there was a decrease in conductivity in the solution and in the dialyzed sol of the oxidized starch from that of native corn starch, the purified gel (by dialysis) showed an eight-fold increase in conductivity on oxidation. Oxidation had increased the hydrogen-ion concentration of the sol from 0.8×10^{-5} to 51×10^{-5}. The reducing power of the sol was high, the rotation lower than that from native starch. Samec and Blinc explained the increase in viscosity in spite of the decrease in particle size as being due to the production of hydrophilic groups which augmented hydration. Because a decrease in molecular size always accompanies oxidation of starch, the authors tended to believe that glucosidic bonds were broken which liberated aldehydic groups for oxidation to carboxylic groups.

Fletcher and Taylor (77) treated corn beta-amylose with alkaline hypochlorite at pH 10 and 13. The viscosity remained practically constant for thirty days but the alkali-labile value and initial reducing value fell practically to zero. Fletcher and Taylor likewise surmised that the reaction was primarily an oxidation of aldehydic groups at the end of the chains, but that some disintegration of the carbohydrate accompanied this change.

Ammonium hypochlorite was used by Jambuserwala and Kanitkar (173) to oxidize corn starch. The milliequivalents of COOH, the R_{cu} values and the alkali-labile values were directly proportional to the amount of available chlorine used (1,2 and 3%). The copper numbers of these alkaline hypochlorite treated starches were smaller than those of the starches oxidized in neutral solution (actually acidic: pH 5.2 to 6.8) by the same authors (172). They contended that this confirmed an earlier conclusion that the copper numbers of cellulose increased with decreasing alkalinity of the hypochlorite solution.

Some of the patents on the modification of starch by alkaline hypochlorite are included in the bibliography (19,32, 49,51,59,91,92,102,115,116,117,118,119,151,157,164,182,214, 223,256,274,289,301,312,316). This is one of the most common agents used in the commercial oxidation of starch (177). It produces a wide variety of products depending upon the concentration of the hypochlorite, the temperature of the mixture and the length of time the oxidation is allowed to proceed (279). In the surface sizing of paper ordinary starch forms gels which are too viscous. The gels of "chlorinated" starches, i.e. those produced by chlorine treatment, have a greater fluidity. The property of high grade tapioca starch paste of "setting up" to only a limited degree on standing is transferred to other kinds of starch by hypochlorite oxidation. Hypochlorite starches are also finding use in the mill sizing of textile fibers.

Oxidation beyond the thin-boiling stage produces cheap, rapid drying and efficient adhesives (60). These adhesives may contain as little as 8% or as much as 50% starch.

(ii) Alkaline hypobromite. When De Chalmot (47) oxidized starch with two equivalents of bromine per glucose unit in the presence of sodium carbonate, part of the starch was liquefied. The starch mixture reduced Fehling's solution in the cold and gave a small amount of a phenylosazone. De Chalmot concluded that oxidation had taken place because neither bromine nor sodium carbonate alone would act on starch perceptibly in a week. His proposed mechanism for the oxidation included conversion of the primary alcohol groups (6th carbon atom) through aldehydic to carboxyl groups. The carboxyl groups were then eliminated as carbon dioxide without affecting the rest of the hexose unit.

Hönig and Ruziczka (163) used 1/3 and 1/2 equivalent of bromine per glucose unit and obtained maltobionic acid as the calcium and brucine salts. The solution was kept alkaline with barium hydroxide. A patent (256) was secured on the process.

(iii) Alkaline hypoiodite. Myrbäck (218) oxidized the reducing end groups of starch with 0.054 equivalent of iodine per glucose unit. The oxidized starch gave the same yield of maltose by alpha- or beta-amylase as raw starch.

b. Oxidation by alkaline chlorite. Liebig (199) noted that starch was attacked only with difficulty by salts of chlorous acid. The textile industry (57) has found an application for sodium chlorite in the kiering operation to remove starch in strong alkali and at a high temperature.

c. Oxidation by alkaline activin. Feibelmann (68,69,70, 71), Frère (85), Haller (129,133,137,138), Ekhard (63), Trotman (311) and Walker (317) reported on the production of soluble starch by means of "activin", sodium-p-toluenesulfochloroamide, $CH_3C_6H_4SO_2N(Cl)Na$. This compound liberates chlorine at a definite rate when heated with water. By heating with activin and water the starch is gelatinized and thinned. About 1% of activin, which amounts to 0.006 equivalent of chlorine, is used to produce soluble starch. Copper salts catalyze the oxidation and bleaching. The efficiency of activin (71) in comparison with calcium hypochlorite and perborates is claimed to be in the ratio of 2.4:1.3:1.0. Haller, Hackl and Frankfurt (137,138) titrated several oxidized starches with iodine and alkali to determine the extent of oxidation. The starch from activin oxidation at 137°C consumed more iodine than that from hydrogen peroxide or sodium perborate. Hydrogen peroxide was the most effective of the three at 100°C.

Several patents were registered for the activin oxidation (130,131,132,151,256,274).

d. Oxidation by alkaline permanganates. Lieben (197) obtained a brown product by permanganate oxidation. Lintner (201) used increasing amounts (1.5 to 6 equivalents) of potassium permanganate on soluble starch at 60-70°C to obtain gummy products whose iodine colors ranged from blue through red to brown or colorless. The optical rotatory power decreased in parallel fashion with the iodine color; a violet iodine color corresponded to $[\alpha]_D$ of 182.4 to 170°, a red brown color to 153.1° while rotations of the achroic solutions were 154.3 to 128.4°. The respective amounts of permanganate at each of these three stages were 1.5, 4 and 6 equivalents. Only a small reduction of Fehling's solution and no color reactions with phloroglucinol or orcinol were given. All of the gums were acidic, which led Lintner to call them "dextrinic acids". With lead acetate or barium hydroxide, solutions of these dextrinic acids gave a precipitate. Schmerber (270) reported a decrease in viscosity and increase in transparency upon oxidation of starch with 0.015 equivalent of potassium permanganate. Mann, Krüger and Tollens (206) on distillation of an oxidized starch with 12% hydrochloric acid reported that considerable furfural was collected. This oxidized starch had been prepared with ten equivalents of permanganate on starch paste for one half hour. The product also gave the naphthoresorcinol color test for uronic acid. Syniewski (304) isolated a crystalline osazone melting at 195° from starch oxidized by permanganate.

Dextrin treated with a large amount of calcium permanganate in the presence of ammonia yielded 0.57% of urea at room temperature and 2.85% at 95°C according to Fosse (80). The addition of cupric carbonate increased the yield to 2.57% urea from dextrin and gave 2.0% urea from starch. Heating the last two mixtures at 95°C increased the yields to 21.85 and 24.28% respectively. The urea was isolated as the xanthydrol derivative. Later experiments by Fosse (81) measured the yield of hydrocyanic acid from carbohydrates treated with a solution of calcium permanganate, ammonia and silver nitrate. Sugars, dextrin and starch gave about 1% HCN. The addition of cupric carbonate increased the yield from dextrin and starch only to 1.6%.

Samec (262,263) observed an increased conductivity and an increased acidity of the dialyzed gel phase of a permanganate-oxidized starch. The equivalents of titratable acidity of this gel phase were greatly in excess of the gram atoms of phosphorus. Samec interpreted this to mean that carboxyl groups had been added by oxidation. The acidity was due in part to phosphoric acid groups and in part to carboxyl groups. For the dialyzed sol phase of this product, however, the gram atoms of phosphorus and the equivalents of titratable acidity were practically identical.

Nakamura (220) found that potassium permanganate decreased the phosphorus content of starch to a greater extent than did ammonium persulfate or sodium perborate. The viscosity was decreased over that of the original starch and the products were more readily acetylated. Permanganate was also used by Haller (136) for very mild modification.

Randall, Benger and Groocock (251) oxidized numerous organic substances with alkaline permanganate. The ease of oxidation of some carbohydrates diminished in the order: glucose, maltose, starch, methyl glucoside, cellulose. These authors measured the time required for complete oxidation at reflux temperatures and the percent of the carbon appearing as carbon dioxide, oxalic acid and acetic acid. For starch the time was three hours and the respective percentages were 66.0, 24.1 and 7.5. Symons and Buswell (302) found that the biochemical oxygen demand (BOD) of starch under the conditions of the common test was 79 to 88% of the theoretical.

A few of the patents on permanganate oxidation have been included (23,45,179,221,257,273,284).

e. *Oxidation by alkaline peroxides.* At ordinary temperatures alkaline peroxide hardly attacks starch, according to Wurster (332), but does modify starch in boiling solution to dextrin and glucose. Von Asboth (315) added hydrogen peroxide, made alkaline with ammonia, to a boiling starch paste. On concentration of the solution a precipitate of "starch cellulose" formed. The filtrate on treatment with several volumes of alcohol gave as a precipitate a tough substance which constituted 80% of the original starch. This precipitate gave a blue-violet iodine color, was non-reducing, had an optical rotation of 178°. On standing the alcoholic filtrate gave a further precipitate constituting 15% yield which was non-reducing, gave a violet-red iodine color and an optical rotatory power of 170°. Hot concentrated barium hydroxide gave a fourth precipitate, not colored by iodine, reducing silver solutions but not Fehling's solution and analyzing to be $C_5H_8O_4 \cdot H_2O$. This loss of one carbon atom from the hexose units agreed with the observed evolution of carbon dioxide. The filtrate from these four precipitates still contained some sugars.

A cold solution of sodium peroxide gave in one hour none of the above drastic changes, when Syniewski (303) used it for oxidation. Although the product was soluble to 12.5% in cold water and completely soluble in hot water, it nevertheless gave a blue iodine color, no reduction of Fehling's solution, showed retrogradation on standing, and had a rotation of 183 to 189° depending on the concentration of the starch in solution. The precipitate from barium hydroxide treatment contained one barium atom per three glucose units.

Another preparation (304) gave the same barium analysis but higher rotatory power, 195°. Acetyl and benzoyl derivatives corresponded to seven free OH groups for every three glucose units. Molecular weights according to Raoult's method were 773 and 880 for the acetyl and benzoyl derivatives respectively, somewhat lower than the 798 and 1148 calculated for hepta-derivatives of a trisaccharide. Since the iodine color, solubility and lack of reducing power are incompatible with such a small molecular weight, it is probable that decrease in molecular size occurred during the drastic conditions of derivatization. Syniewski's third paper (305) stated that sodium peroxide did not oxidize the starch but merely solubilized it. A molecular weight determination of the soluble starch corresponded to a chain of nine glucose units.

Samec and Jencic (265) oxidized starch according to Syniewski's method using a time of two hours instead of one. The product was easily soluble in water, showed some reducing power and a lowered viscosity. Later, Samec (262,263) extended the time of oxidation to six hours but still obtained only mild oxidation as evidenced by the acidity, iodine color, viscosity and osmometric molecular weight.

Two patents used barium peroxide for oxidation (143,245); other peroxides were also used (50,102,116,241).

f. Oxidation by air in alkaline solution. Dhar and coworkers (52,212,233,234,235,236,237) published a series of papers on the induced oxidation of carbohydrates by air. Air was passed through alkaline solutions containing carbohydrates and an oxidizable substance such as sodium sulfite, ferrous hydroxide or cerous hydroxide. This latter substance underwent oxidation at room temperature and induced the oxidation of the carbohydrate. The carbon dioxide produced from the carbohydrate was liberated after oxidation and absorbed in calcium hydroxide solution for measurement. The amount of oxidation in a given time increased with the alkaline concentration of the solution except when sodium sulfite was the inductor. One tenth gram of starch in 100 cc. of solution containing cerous hydroxide or ferrous hydroxide was completely oxidized by 36.5 liters of air in 5.5 hours when the concentration of sodium hydroxide was 0.5 to 0.7%. Starch was found to be more easily oxidized than any of the several sugars used. This order does not agree with that determined by permanganate (251). Sodium sulfite induced the oxidation of 26% of the starch even when no alkali had been added to the solution but did not give complete oxidation at any alkaline concentration up to 0.7%. When air was passed through an alkaline starch solution containing no inductor, 23 to 35% of the starch was oxidized at different concentrations of sodium hydroxide. When the concentration of sodium hydroxide was kept constant 31.7% of the starch was oxidized in 5.5 hours while 45.3% was oxidized in 9 hours. But when sodium bicarbonate was substituted for the sodium hydroxide, the values were 35.9% and 31.9% respectively, showing a decrease on longer oxidation.

In the presence of sodium bicarbonate or various inductors (236) glycogen showed the following percentage oxidation: sodium bicarbonate present, 12.0%; sodium sulfite present, 21.8%; ferrous hydroxide, 64.5%; cerous hydroxide, 87.5%. However, in the presence of ferric hydroxide which is certainly not a reducing agent like the other inductors, there was 67.9% oxidation, more than in the presence of ferrous hydroxide. This weak point in the theory is emphasized by the fact that cupric hydroxide also gave a large amount of oxidation, 69.8%.

Further experiments by Dhar and associates (235,237) studied the effect of sunlight on the oxidation of carbohydrates by air in neutral solution. 36.5 liters of air passed through starch solution in 5.5 hours oxidized 35.6% of the starch in tropical sunlight, 38.8% in sunlight of greater intensity, 66.9% in the latter case if zinc oxide (a photosensitizer) was present, 92.9% if the photocatalyst was ferric nitrate, and 100% if the photocatalyst was uranium nitrate. The respective values for glycogen oxidation were 15.9, 19.7, 38.7, 82.3 and 71.0%. In the photochemical oxidations as in the induced oxidations starch was oxidized more easily than any of the sugars or glycogen.

Samec (262,263) passed air for three days through a 15% starch suspension which was 0.05N in sodium hydroxide and obtained very little evidence of oxidation. The osmometric molecular weight was high, the iodine color blue to lilac, the titratable acidity and the per cent of dialyzable substance very low.

In the production of dextrins by dry heating in air at 200°C, Katz and Weidinger (178) found a measurable acidity due to weak oxidation. The amount of acidity developed was always less than 0.01 equivalent per glucose unit. In an atmosphere of nitrogen no acidity was developed.

Baudisch and Deuel (7) presented a new test for carbohydrates in which a carbohydrate solution containing sodium bicarbonate is distilled and the distillate treated with o-aminobenzaldehyde to produce a blue fluorescence due to an acetol derivative, 3-hydroxyquinaldine. The derivative may be extracted and crystallized. Although all of the sugars tried and dextrin gave acetol on distillation, starch and glycogen gave no acetol. Starch was oxidized for 48 hours with sodium pentacyanoaquaferroate and oxygen but failed to give any acetol.

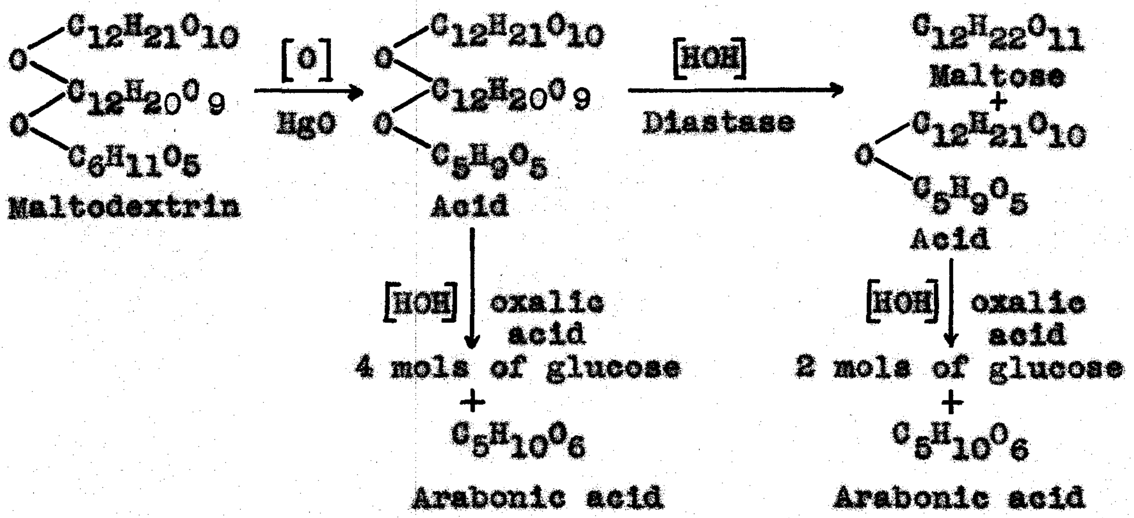
The oxidation of starch by air has likewise been patented (232).

g. Electrolytic oxidation. Leconte (194) used electrolysis as one step in the preparation of a very white grade of rice starch. An alkaline suspension of the starch was electrolyzed between aluminum, zinc or other metallic electrodes. Harvey (142) and White (326) patented a process for the Perkins Glue Company which was designed to convert starch to a glue. An aqueous suspension of the starch was made conducting by the addition of hydrochloric or sulfuric acid, a salt or alkali. Iron or carbon electrodes were used with a direct current of 3.5 amperes at 110 volts. The electrolysis was stopped before the starch reached the soluble stage. It is apparent that some oxidation occurred due to the products of electrolysis of the conducting substances. The authors were apparently ignorant of the starch modifying mechanism, because they do not mention that any one electrolyte is more effective than another. The electrolytes were added "merely to make the bath sufficiently conducting". Actually one might expect hydrolysis and oxidation from the acid electrolytes but only oxidation from the alkaline or saline electrolytes in the concentrations used.

Fink and Summers (76) attempted to electrolyze starch between graphite electrodes in 2% potassium bromide solution at 40°C kept neutral by means of calcium carbonate. They reported no reaction, stating that electrolytic oxidation in the bromide bath seemed specific for aldoses. Very probably they lacked the methods to detect oxidation in starch.

h. Oxidation by alkaline mercuric oxide. Brown and Millar (29) oxidized a maltodextrin (of about five or six glucose units) by heating at 100°C with mercuric oxide in barium hydroxide solution. Under these conditions oxidation proceeds further than the conversion of the aldehydic group of the terminal glucose unit to a carboxyl group. Malt amylase (alpha- and beta-amylase) hydrolyzed the oxidized maltodextrinic acid to maltose and a smaller acid. The original maltodextrin was only slowly attacked by alpha-amylase, indicating that beta-amylase splits maltose units off the non-aldehydic end of the starch chain.

During the oxidation, after three atoms of oxygen were consumed per mol of maltodextrin, the reduction of Fehling's solution ceased. The barium salt at this point gave chiefly an acidic fraction of $[\alpha]_D$ 189-192°. Analysis of the calcium salt corresponded to the formula $(C_{29}H_{49}O_{26})_2Ca$. Diastatic hydrolysis of the maltodextrinic acid gave maltose and a smaller acid whose calcium salt analyzed as $(C_{17}H_{29}O_{16})_2Ca$. Hydrolysis of these two calcium salts by oxalic acid gave 85.55 and 67.7% glucose respectively and an acid whose calcium salt analyzed as $(C_5H_9O_6)_2Ca$, presumably calcium arabonate. These data fit the following scheme of oxidation and hydrolysis:



Brown and Millar (31) prepared another dextrin from hydrolysis of starch by malt extract to 80% maltose and also oxidized this dextrin with mercuric oxide in barium hydroxide solution. The resulting acid was converted to the calcium salt, whose analysis and hydrolysis to glucose and arabonic acid suggested this formula for the acid: $(C_6H_{10}O_5)_{39}\text{-}C_5H_9O_5$. This meant that the original dextrin contained 40 glucose units, a value which agreed exactly with the reducing power of the dextrin. The usual equation used for malt digestion at that time (1899) was:

$$20\ C_6H_{10}O_5 + 8\ H_2O \rightarrow 8\ C_{12}H_{22}O_{11} + 4\ C_6H_{10}O_5$$

Starch 80% Maltose Stable Dextrin

On the basis of their findings, Brown and Millar proposed a new equation:

$$200\ C_6H_{10}O_5 + 81\ H_2O \rightarrow 80\ C_{12}H_{22}O_{11} + (C_6H_{10}O_5)_{39}\text{-}C_6H_{11}O_6$$

They stated further that the molecular weight of starch could not be less than five times that of the stable dextrin. Since the molecular weight of the dextrin was 6498, starch would have a minimum molecular weight of 32,400 or possess 200 glucose units. The molecular weight of this same dextrin, determined cryoscopically, was 6221 and the molecular weight of a soluble starch by the same procedure was between 20,000 and 30,000. Brown and Millar then proposed a ring structure for starch.

i. Oxidation by alkaline persulfates. Persulfate made alkaline by ammonia modifies starch for cotton sizing according to Trotman (311). 0.036 equivalent is used. Supf (300) has a patent on alkaline persulfate oxidation.

3. Oxidation of starch in neutral media.

In a neutral solution acid or alkaline hydrolysis and enolization would be expected to be eliminated. Oxidation, then, should not be complicated by these other reactions; the best studies of oxidation mechanisms should be possible in neutral solution.

a. Oxidation by bromine. Franchimont (83) found no reaction when dry bromine and dry starch were brought together in chloroform. Syniewski (306,307) oxidized amylodextrin by means of bromine in the presence of barium carbonate. The amylodextrin was prepared by autoclaving starch paste for 12 hours at 125-138°. When the iodine color disappeared, the oxidation was stopped by removal of the excess bromine. The barium salt was precipitated with alcohol, had $[\alpha]_D = 191^\circ$, reduced Fehling's solution in the cold to the equivalent of 23% maltose, gave a red solution with hydroxylamine and an orange-red precipitate with phenylhydrazine. A naphthoresorcinol test indicated glucuronic or malturonic acid units. Distillation of the amylodextrinic acid in 12% hydrochloric acid gave furfural equivalent to 33.9 and 34.5% glucuronic anhydride. This was in agreement with the assumed formula for amylodextrinic acid which contained 24 glucose units and 12 carboxyl groups. Syniewski assumed that the carboxyl groups were formed from the sixth carbon atom of the glucose units by the oxidation of primary alcohol groups. His naphthoresorcinol test lends support to this mechanism but might also indicate keturonic acids or aldehydic acids, as he stated. Syniewski concluded that no keto or aldehydic acids were present in this case. It is, indeed, very improbable that any aldehydic groups would remain unoxidized in the presence of bromine. On the other hand there is strong evidence that Syniewski's amylodextrinic acid contained keto groups, viz., the reduction of Fehling's solution in the cold, the colored compound with hydroxylamine, the precipitate with phenylhydrazine, and the reducing power of 23% maltose equivalent.

Felton, Farley and Hixon (72) oxidized starch paste with increasing amounts of bromine in the presence of calcium carbonate and recovered the calcium salts of the oxidized starch. Uronic acid determinations showed that the oxidation of primary alcohol groups to uronic carboxyl groups passed through a maximum of 50.7% glucuronic anhydride when six equivalents of bromine were used per glucose unit. The calcium content of the oxidized starch underwent a steady increase proportional to the amount of bromine used. By means of the curve for uronic acid content the authors were able to calculate the amount of calcium necessary for the uronic carboxyl groups. When these values of uronic acid calcium were subtracted from the values for total calcium, the difference would indicate the calcium values of the non-uronic carboxyl groups. These latter, the non-uronic carboxyl groups, could result from the oxidation of terminal aldehydic groups or from the oxidative splitting of hexose units at the site of a ketone group. No evidence was at hand to decide which of the two types of oxidation was responsible for the non-uronic acids. However, some indication that oxidative splitting of ketone groups was the chief source came from the reducing power of the products, a measure of the production of ketone groups from secondary alcohol groups. The reducing power of the oxidized starches passed through a maximum at two equivalents of bromine, then decreased proportional to the amount of bromine used. Just beyond this maximum, at three equivalents of bromine, the non-uronic acid groups began to appear and were produced in proportion to the amount of bromine used. The logical conclusion was that most of the non-uronic carboxyl groups resulted from oxidative decomposition of the reducing units.

Felton, Farley and Hixon also oxidized, by the same procedure, amylose (beta-amylose) and amylopectin (alpha-amylose) prepared by electrodialysis from corn starch paste. Practically the same product was obtained from each fraction, although the oxidation of the amylose required one-fourth as much time as that of the amylopectin.

Fink and Summers (76) electrolyzed starch in a solution containing potassium bromide and calcium carbonate but observed no reaction.

Kihara (183) oxidized starch paste according to a procedure substantially the same as that employed by Felton, Farley and Hixon (72). His further observations were that the oxidized starch was precipitated by calcium or barium hydroxides but not by copper sulfate or Fehling's solution, and that takadiastase scarcely hydrolyzed the product. Kihara prepared an acetyl derivative which melted at 145°C.

b. Oxidation by iodine. When Andrews and Goettsch (2) heated starch at 100°C with 2.3 equivalents of iodine in the presence of calcium carbonate, 110 hours were required to reach the achroic point. This same stage was reached in 65 hours in the absence of calcium carbonate.

B. Characterization of Oxidized Starch

After starch has been subjected to the action of an oxidant it is not a simple matter to determine that oxidation has taken place. Depending upon the extent of oxidation, the methods used to detect and to measure the changes due to oxidation include color reactions, microscopic observations, enzymic digestion, measurements of physical properties of starch pastes, observations of gelatinization phenomena, action of swelling agents; determinations of acidity, of alkali-labile value, of reducing power; hydrolysis and isolation of the oxidized glucose units.

1. General differentiation of starches.

For a mildly oxidized starch the methods for detecting oxidation resemble very closely those used in the characterization and differentiation of various raw starches.

a. Reichert's analyses. By far the best systematic procedure for identifying a given starch sample botanically is that of McNair (209). This summary of methods taken from Reichert's (255) comprehensive treatise on starch differentiation used eleven processes of analysis such as histological examination, color reactions, swelling reactions, gelatinization temperatures, etc., to distinguish between starches of different families and species and even between starches from different varieties of one species, e.g., the numerous corn starches.

b. Iodine color. A limited differentiation of raw starches may be made by use of the color which starches give with iodine solution. This phenomenon, that starch is colored an intense blue by iodine solution, was discovered by Colin and De Claubry (41) in 1814. Until 1885 it was believed that all raw starches gave a blue color with iodine. In that year Dafert and Kreusler (250) reported that the starch of glutinous rice gave a red color with iodine. More recently (321) the starch from waxy corn has also been found to give a red iodine color. Dr. R. S. Bear of this laboratory suggests the name "erythrostarch" for all raw starches which give a red color with iodine. The iodine color of starches, therefore, gives an immediate differentiation of erythrostarches and those giving a blue color. In differentiation work the method of Giri (103) is claimed to give a different result with wheat and corn starches from that with rice and barley starches. Starch-agar plates treated with amylase for 24 hours give characteristic zones of color when flooded with iodine solution. Photometric studies of the starch-iodine color by Simerl and Browning (283) showed a different percentage of transmission of light for each starch. Reichert (255) and

McNair (209) used iodine colors as well as swelling phenomena in iodine-chloral hydrate and in iodine-lactic acid solutions for differentiation.

c. Iodine vapor. Iodine vapor was reported by Gobley (104) and Dubosc (58) to give quite different colors with the various starches. However, we have found that the color depended on the moisture content (all the starches studied giving a blue color in a humid atmosphere) and on impurities in the starches (167). No further differentiation could be made than was possible with iodine solution.

d. Gelatinization temperature. The gelatinization temperature of starches has been in use for many years as a means of differentiation. McNair's (209) summary of Reichert's (255) volumes lists the gelatinization temperatures of hundreds of starches. From the values recorded there one is led to believe that the measurements are accurate to at least one tenth of a degree. This is undoubtedly an average value for the temperature of maximum gelatinization. There is, for any starch, no true gelatinization temperature analogous to a melting point; there is only a gelatinization range of some five to ten degrees (226). The gelatinization temperature is the temperature at which the largest number of granules are gelatinizing and is only an estimate. Despite this discrepancy, the usefulness of this physical constant depends on the fact that gelatinization temperatures differ considerably and range from 50 to 90 degrees Centigrade. The gelatinization temperature is usually measured visually on a heated microscope stage. Cook and Axtmayer (42) measured photoelectrically the light passing through a starch suspension while it was being heated. When the light transmission in microamperes was plotted against the temperature of the starch paste, a characteristic curve was obtained for each starch. The tropical starches gave an entirely different type of curve from that of the temperate-zone starches. Morgan (216) extended the use of the above method.

e. Amylolytic digestion. Many workers, among them Stone (297), O'Sullivan (231), Nagao (219), Sherman, Walker and Caldwell (280), Amberger (1), Hermano and Rask (153), Kashiwaya (176) and Iwanoff, Kurgatnikov and Kirsanova (168), noted that differences occur in the digestibility by amylases of starches from different sources. When the amount of amylolytic digestion of a starch was plotted against the temperature of substrate preparation, Martin and Newton (207) obtained a very characteristic curve for each type of starch thus indicating a mathematical differentiation of starches by data from enzymic digestion.

f. Dyes. Dyestuffs are important in starch differentiation as is evidenced by the important role they play in Reichert's analyses (255). Schulz and Steinhoff (275) also used dyes.

2. Following the modification of a starch.

At times it is very desirable to follow the changes which take place as a starch is modified, whether the modification is effected by hydrolysis, digestion, oxidation, pyrolysis or other such treatment. Among the properties of starches which lend themselves to measurement during the course of a starch treatment are iodine color, gelatinization temperature, optical rotation, viscosity, rigidity, gel strength, acidity, alkali-labile value, alkali number, reducing power and digestion by soybean beta-amylase. Each of these is reviewed here.

a. Iodine color. The initial stages of the hydrolysis of starch may be followed by the change in iodine color. This color test has been in use from early times on acid- or enzyme-catalyzed hydrolyses. As the hydrolysis proceeds the blue starch-iodine color changes progressively to violet, red, brown, yellow and then disappears entirely, the last stage being called the achroic point. Saare (261) used the iodine color to estimate the percentage of soluble dextrin in a dextrin sample. Numerous workers have reported on methods of following enzymic digestion of starch by means of iodine colors. Some measured the time needed for conversion to the achroic stage; others measured the amount of enzyme needed in a constant time. Samec and Mayer (266) compared the intensity of blue at each stage with the initial intensity. The values were incorporated into characteristic curves on a graph. Hanes and Cattle (140) used the spectrophotometer to follow the absorption spectrum of the starch-iodine complex during hydrolysis. At the moment when the iodine color disappeared during hydrolysis the dextrin present had an estimated chain length of 6 or 7 glucose units (139).

b. Gelatinization temperature. Photoelectric methods for following the modification of a starch have been developed by Cook and Axtmayer (42) and Morgan (216). These methods measure the light which a starch suspension transmits as it is heated through the gelatinizing range. The current from the photoelectric cell plotted against the temperature of the starch paste gives a gelatinization curve for each starch sample. Newton, Farley and Naylor (226) employed the loss of birefringence at the gelatinization point as a measure of the temperature coefficient of gelatinization. The effect of oxidation upon the gelatinization temperature was followed in this manner. Morgan (216) also followed the oxidation of corn and tapioca starches by its effect in decreasing the pasting temperature. In addition he gave curves to show the effect of dextrinization of corn and tapioca starches. Radley (247) described a method for following the course of gelatinization by means of sedimentation volume. In the experimental section data are shown for change in volume of sediment at a given temperature of gelatinization as the starch is oxidized.

c. Optical rotatory power. When a starch has been modified far enough to be soluble in water or some other solvent, the decrease in optical rotation may be used to show the course of modification beyond that stage. This has been done by Lintner (201), Craik (43), Felton, Farley and Hixon (72) and others.

d. Viscosity. One of the most used tools in the control laboratory of a starch plant is the viscosimeter. By way of explanation it need only be mentioned here that the viscosity of a starch paste is very important to the textile and paper trade. The usual procedure for viscosity measurements consists in a series of determinations while the starch is being modified. The numerical values are used for comparisons in the manufacture of such products as oxidized and thin-boiling starches, dextrins, British gums and adhesives. Caesar's apparatus (35) measures the viscosity during the pasting of the starch and the cooling thereof. The amount of work required to operate the electrical stirrer is taken as a measure of the viscosity. The records obtained by plotting current against temperature vary greatly from one starch to another and have been used to follow various modifications.

e. Rigidity. The rigidity (reciprocal of elasticity) of a starch paste does not depend on its viscosity, but is an entirely different property. Brimhall and Hixon (25) reviewed the earlier methods for rigidity determinations and improved the Schwedoff technique. By means of a newly designed rigidometer, Brimhall and Hixon measured the decrease in rigidity resulting from such treatments as homogenization, oxidation, cold acid treatment, grinding in a ball mill and autoclaving. The experimental section of this thesis shows the successive changes in rigidity during the oxidation of raw starch.

f. Gel strength. Hixon and Brimhall (161) constructed a gelometer to measure both the deformation and the breaking strength of starch gels. They used suction to deform the gel and measured the volume of deformation hydrostatically. It will be shown below how gel strength decreases during electrolytic oxidation.

g. Acidity. A characteristic property of oxidized starches is their acidity due to carboxyl groups. Titration with alkali is usually the procedure employed for measuring acidity of starches. The end-point is determined with phenolphthalein or electrometrically. pH determinations are also used. During alkaline oxidations a metallic salt is formed from each carboxyl group produced; an analysis for the metal then replaces the titration of acid. Among those who followed the change in titratable acidity during oxidation were Gruzewska (95,113), Fernbach and Wolff (73), Massol (208), Bielecki and Wurmser (18), Durieux (61), Vintilescu and Faltis (314), Samec (262,263), Rassow and Lobenstein (254), Samec and Blinc (264), Everett and Sheppard (65), Samec and Ulm (268), Fletcher and Taylor (77), Katz and Weidinger (178) and Jambuserwala and Kanitkar (172,173). Fletcher and Taylor (77) followed the change in pH during oxidation. Mann, Krüger and Tollens (206), Syniewski (306,307), Everett and Sheppard (65) and

and Pauli and St. Szper (238) presented evidence to show that uronic acids were responsible for a portion of the acidity. Felton, Farley and Hixon (72) measured the production of uronic acids during the successive stages of oxidation. Metal analyses as an index to the production of carboxyl groups in the oxidation of starch were used by Habermann (120,121), Syniewski (303,304), Brown and Millar (29,31), Rassow and Lobenstein (254) and Felton, Farley and Hixon (72).

h. Alkali-labile value. Taylor and co-workers (308,309, 310) discovered that when starch is heated with 0.1 N alkali for one hour at 100°C some of the alkali is neutralized by the starch. The iodine-reducing substances were then measured by means of iodine solution and reported as the alkali-labile value of the starch. This value undergoes large variations during such treatments as solubilization by cold acid, dextrinization, grinding or conversion to a thin-boiling starch. Further applications of the alkali-labile value have been made by Jambuserwala and Kanitkar (172,173) Fletcher and Taylor (77) and Samec and Skerl (267).

i. Alkali number. Schoch and Jensen (272) simplified the alkali-lability procedure by omitting the iodine titration. Their method measures, by titration with acid, the alkali consumed during the one hour digestion. The authors term this value the "alkali number" because it is somewhat different from the alkali-labile value. The alkali number determination is sensitive enough to detect slight modifications caused by acid, oxidation, dextrinization, etc.

j. Reducing power. The aldehydic groups of starches, modified starches and dextrins impart to these products a certain reducing power. In the case of oxidized starches, reduction may also be due to ketone groups. Many of the methods for determining reducing power have been taken directly from cellulose chemistry while only a few methods have been reported specifically for starch. The methods used on cellulose are, therefore, included as a part of the literature review.

Vignon (313) in 1897 used Fehling's solution to measure the reducing power of cellulose. In 1907 Schwalbe (276) reported the "copper numbers" of several cellulosic products determined by a modified Fehling's test. The cuprous oxide precipitated by reduction was dissolved in nitric acid and determined electrolytically. Many subsequent workers modified Schwalbe's method, viz., Freiberger (84), Hägglund (124), and the following. Knecht and Thompson (187) dissolved the cuprous oxide in ferric alum and titrated with permanganate. Sunderland (299) presented a rapid electrolytic method as well as an iodide-thiosulfate determination of the cuprous oxide. Braidy (21) replaced the alkaline tartrate of Fehling's solution with a carbonate-bicarbonate mixture. Braidy's method received wide application; it is also known as the Schwalbe-Braidy method. In the same year Koehler and Marqueyrol (188) also published a modification using a carbonate-bicarbonate mixture. Clibbens and Geake (40) compared the various modified forms of the Schwalbe procedure, concluding that the Braidy method was the best. Heyes (156) developed a Braidy micro-method. Gault and Mukerji (97) dissolved the cuprous oxide from Fehling's solution in molybdophosphoric acid reagent and titrated with permanganate. Benesch (9), Staud and Gray (110,291), Hägglund (125,126), Brissaud (26) and Wenzl (323) made only slight changes in the Schwalbe method, while Hess and associates (155) studied the numerous factors affecting the determination of copper numbers. Barthélémy (6) conducted the test in an atmosphere of nitrogen. About this time Hall (128) stated that the Hägglund modification was the most rapid and accurate. A novel modification was advanced by Krüger and Tschirch (191) in which the unreduced copper in the solution was treated with thiocyanate and iodide, then titrated with thiosulfate.

Silver solutions for determining the reducing power of cellulose were substituted for copper solutions in Götze's (108) method. Geiger and Müller (98) described three micro methods using, respectively, copper, ferricyanide and iodine solutions. The micro-iodine method was unreliable whereas the micro-ferricyanide procedure was not as accurate as the colorimetric micro-copper determination.

In order to follow the hydrolysis of starch, Fargher and Probert (67) used the Braidy (21) copper number determination which had been devised for cellulose. After a lag period the reducing power was proportional to the time. Lintner soluble starch and autoclaved starch had low copper numbers while the copper numbers of raw starches were zero. Taylor and co-workers (77,308,310) measured the "initial reducing value" of a number of modified starches by the Willstätter and Schudel (328) iodine titration for aldoses. For various starch fractions, ground starches, thin-boiling starches, soluble starches and white dextrins the initial reducing value ranged from 0.6 to 6.0 mg. iodine per 100 mg. of material. Taylor and his associates used the initial reducing value for following modifications of starch by chlorine and alkaline hypochlorite but claimed that their alkali-labile value was more sensitive and more widely applicable.

In 1936 Richardson, Higginbotham and Farrow (259) made a noteworthy advance in the determination of copper numbers. They showed that the lag period when the hydrolysis of starch was followed by reducing power was due to loss of a small amount of cuprous oxide by virtue of its solubility in the reagents. To each sample measured by the Fargher and Probert (67) technique they added a measured amount of glucose solution, then subtracted the value obtained for glucose in a blank determination. By this scheme all samples produced enough cuprous oxide to exceed its solubility, and the lag period of hydrolysis disappeared. Raw starches were shown to have a definite reducing power which ranged from 2.8 to 8.9 mg. of copper per gram of starch. This value was termed the R_{cu} value; it was used by these workers as well as by Caldwell and Hixon (36), Jambuserwala and Kanitkar (172,173), Newton, Farley and Naylor (226) and others to follow the modification of starch. There will be presented below a new method for determining R_{cu} values which shortens the time for a determination from three hours to twenty minutes.

Recently Kerr (181) measured the reducing potential of corn starch and beta-amylose by means of a Coleman electrometer, the respective values for these two carbohydrates being 59 and 68 millivolts in 1% solutions as compared with 111 for maltose and 183 for glucose.

k. Enzymic digestion. The amylase digestion curves for several starches examined by Martin and Newton (207) showed characteristic differences. These differences were significant enough to suggest the use of soybean beta-amylase to follow the industrial modification of starches. Newton, Farley and Naylor (226) employed this amylase digestion to measure the changes in susceptibility of the starches during various treatments, such as grinding starch in a ball mill for varying lengths of time, oxidation by different amounts of chlorine, dextrinization, acid hydrolysis (thin-boiling starch) and dry milling. For any given starch each of a series of suspensions was heated at a different temperature for thirty minutes, the resulting starch paste being subsequently digested for three hours at 40°C with soybean beta-amylase. The final reducing value of each sample was plotted against the temperature of pretreatment of the starch. The resulting digestion curves differed in their extrapolated intercept on the temperature axis, in their slope during the gelatinizing range, and in their value for maximum digestion. Physical modification of starch by grinding in a ball mill for 150 to 600 hours greatly increased the amount of starch digestible at low pretreatment temperatures (below 60°C). This increase amounted to 45 or 50% maltose equivalent. Acid hydrolysis had very little effect on starch digestibility at temperatures below the gelatinization point. Slight acid hydrolysis of starch caused a small increase in digestibility (5% or so) above the gelatinizing range. Progressive dextrinization effected progressive increase in susceptibility of starch to beta-amylase digestion at low pretreatment temperatures (40,50 and 60°C); above the gelatinizing range the opposite effect, a progressive decrease in maltose production, was noticed. Corn flours, paste flakes and brewers' flakes likewise gave curves following one another in the exact order of the extent of modification. The use of amylolytic digestion to follow the electrolytic oxidation of starch is presented below in the experimental section.

1. Loss of birefringence. An investigation by Newton, Farley and Naylor (226) to determine the meaning of the intercept of the enzyme digestion curves showed a definite correlation between amylolytic digestion and loss of birefringence of

the starch granules. The curves presented by Newton, Farley and Naylor indicated that soybean beta-amylase begins to digest starch at approximately the same pretreatment temperature at which the starch begins to lose its birefringence and begins to gelatinize. This temperature is spoken of as the intercept of the digestion curve. As more and more of the starch granules lose their double refraction (polarization cross) and gelatinize the rate of digestion increases as a straight-line function, giving the steepest portion of the digestion curve. When the majority of the more heat-labile starch granules have been gelatinized the digestion and birefringence curves bend toward zero slope as the more heat-resistant granules gelatinize. Radley (248) presents the same type of curve for the gelatinization of starch. His curve is likewise based on microscopic observations and is accompanied by substantially the same explanation as the curves of Newton, Farley and Naylor. These birefringence curves may be used to follow the modification of starch by oxidation as is shown below in the experimental section.

III. EXPERIMENTAL

A. Oxidation of Raw Starch by Electrolysis in Sodium Chloride

The sodium hypochlorite used for the industrial production of oxidized starch is made by passing chlorine into alkali. The electrolytic method described below prepares the hypochlorite in solution by electrolysis of sodium chloride.

1. Electrolytic oxidation process.

a. Apparatus. The D.C. electricity was produced by a motor-generator assembly which delivered about 10 volts. Depending on the conditions of each oxidation the current strength was between 0.1 and 7.0 amperes. The total current was computed as accurately as feasible for determining the ampere-hours and faradays of electricity consumed and therefore equivalents of chemical change produced.

Electrolyses were carried out in a crock or beaker of 4 liters capacity. Into this was lowered the electrode assembly and stirrer. Attempts to use carbon or graphite electrodes resulted in carbon particles flaking off the electrodes, especially from the anode. The flaking was decreased only slightly by soaking the anode in paraffin. Every filtering device which was constructed operated too slowly when filters were used which were fine enough to remove the carbon. A portion of the carbon came off in colloidal form and could never be removed by filtration. Carbon and graphite electrodes were abandoned.

An electrode assembly of four iron cathodes and one platinum anode was used for the electrolytic data of this thesis. The platinum cylinder anode was mounted in the center of the assembly and equidistant from each of the four iron cathodes. A large blade stirrer passed through the platinum cylinder to stir the solution from the bottom of the vessel.

b. Method. One pound (454 grams) of cornstarch was suspended in 3000 ml. of distilled water to which had been added 60 grams (2%) of sodium chloride and 12 grams (0.4%) of sodium hydroxide. The electrode assembly and stirrer were lowered into the suspension. The stirring was adjusted at a rate which kept the starch suspended, then the D.C. generator was turned on. Since the area of the platinum anode was 37.5 square centimeters, the average current of about 4 amperes gave a current density of approximately 0.1 ampere per square centimeter. When the requisite number of ampere-hours had been generated for the desired amount of oxidation, the generator was stopped and the electrodes removed from the container. Stirring was then resumed until a test with potassium iodide solution or a titration showed the absence or near absence of hypochlorite ion. The titrations were made with thiosulfate on an aliquot of the oxidation liquor after addition of potassium iodide.

During certain oxidations the electrolytic container was thermostated; in all others the temperature rose gradually during oxidation and fell as gradually during the stirring period. This temperature rise was usually five to ten degrees centigrade. In concentrated starch suspensions as large a rise in temperature as twenty-five degrees was recorded.

After the hypochlorite had been depleted, the starch suspension was neutralized to phenolphthalein with hydrochloric acid, centrifuged and washed alternately until the washings showed the absence or only a trace of chloride ion. The starch samples were finally filtered on a Büchner funnel, sucked dry, then dried at 50°C in a hot air dryer.

c. <u>Recovery</u>. Figure 1 shows the recovery of oxidized starch by the electrolytic process together with that of a commercial oxidation. The recovery decreased proportional to the extent of oxidation. The electrolytic recovery is much higher than the commercial recovery because milder conditions were used in the former process. Whether or not this is a desireable feature depends upon the type of product sought. The electrolytic products did not approach the high transparency and low viscosity of the commercial products until the concentration of starch in suspension was greatly increased and the oxidation carried on more rapidly, both of which contributed to the development of a higher temperature during

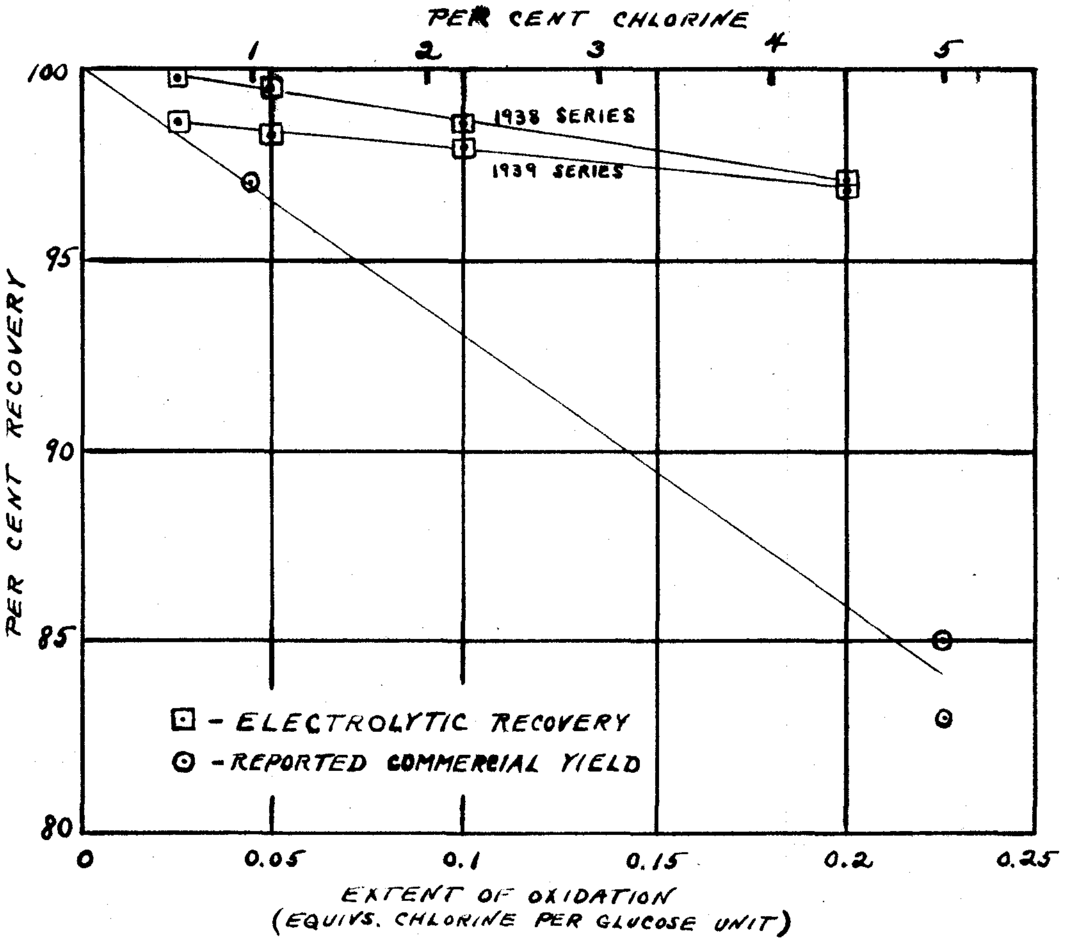


Fig. 1. PERCENTAGE RECOVERY OF OXIDIZED STARCHES.

oxidation. Even under these conditions which produced an oxidized starch approaching the physical properties of the commercial starch oxidized with the same amount of chlorine, the electrolytic recovery was 95% as compared with an 85% recovery in industry. Better recovery is in part due to greater ease of washing the products free from the small amount of salt used.

Treatments which gelatinized some of the starch resulted in much lower recovery. Partial gelatinization by 0.72% sodium hydroxide gave a 76% recovery; for the same reason a constant temperature oxidation at 55°C showed an 80% recovery, whereas the normal recovery was 98%. Each recovery was calculated on a dry basis from the moisture content of the starches.

d. Cost. Cost comparisons were made between the chlorine and caustic soda oxidation and the electrolytic oxidation based on the use of 2% chlorine (0.1 equivalent of chlorine per glucose anhydride unit) on 100 lbs. of starch.

The cost of the chlorine and caustic soda method involves (1) the cost of chlorine, (2) the cost of sodium hydroxide to produce hypochlorite and (3) the cost of sodium hydroxide to neutralize the acid groups produced by oxidation.

(1) Cost of chlorine - 0.1 equivalent of chlorine requires $\frac{(0.1)(35.5)}{162} = 0.0219$ g. chlorine per gram of starch or 2.19 lbs. chlorine per 100 lbs. of starch.

At \$2.15 per 100 lbs., the cost of chlorine is

$\frac{(2.15)(2.19)}{100}$ = \$0.0471 per 100 lbs. of starch.

(2) Cost of sodium hydroxide to produce hypochlorite:

$Cl_2 + 2NaOH = NaCl + NaOCl + H_2O$
70 80

$\frac{(2.19)(80)}{70}$ = 2.51 lbs. NaOH per 100 lbs. starch.

Caustic soda (76% Na_2O; 98% NaOH) costs \$2.60 per 100 lbs.

$\frac{(2.60)(2.51)}{(100)(0.98)}$ = \$0.0665 per 100 lbs. starch.

(3) Cost of sodium hydroxide to neutralize acid groups:

$C_6H_{10}O_5 + NaOH + 2\ O = C_5H_7O_4COONa + 2H_2O$
162 40

$\frac{(100)(40)}{(162)(4)(10)}$ = 0.62 lbs. NaOH per 100 lbs. of starch.

$\frac{(2.60)(0.62)}{(100)(0.98)}$ = \$0.0165

Total Cost = 0.0471 + 0.0665 + 0.0165 = \$0.1301 or

13.0 cents per 100 lbs. starch.

The cost of the electrolytic oxidation involves (1) the cost of sodium chloride, (2) the cost of sodium hydroxide to neutralize the acid groups formed by oxidation and (3) the cost of electricity.

(1) Cost of sodium chloride:

(0.0219) $\frac{2NaCl}{Cl_2}$ (100) = 3.66 lbs. NaCl per 100 lbs. starch. At \$14.80 per ton, the salt costs

$\frac{(14.80)(3.66)}{2000}$ = \$0.0271

(2) Cost of sodium hydroxide to keep the medium alkaline is the same as for the commercial process: \$0.0165.

(3) Cost of electricity, using 10 volts:

1.655 ampere hours are required for 0.1 equivalent chlorine per glucose unit per 100 grams of starch.

$$\frac{(1.655)(453.6)(100)(10)}{(100)(1000)} = 7.5 \text{ kilowatt hours}$$

With 1.4 cents per kilowatt hour as the basic rate,

$(7.5)(1.4) = 10.5$ cents

Total cost $= 0.0271 + 0.0165 + 0.105 = 0.1486$ or

14.9 cents per 100 lbs. starch.

For 100 lbs. of starch oxidized by 2% chlorine the cost of the chlorine and caustic soda method would be 13.0 cents while the electrolytic cost would be 14.9 cents. In the electrolytic method the fact that the liquors may be used repeatedly with an occasional addition of alkali and sodium chloride makes the cost of the chemicals insignificant. This would reduce the electrolytic cost considerably below 13 cents and approaching 11 cents per 100 lbs. of starch.

Most of the cost of the electrolytic method is that of the current, which is dependent upon securing low cost electric power.

The cost of using platinum anodes may be estimated as follows. The usual anodic current density was approximately 0.1 ampere per square centimeter on a platinum sheet anode. By using a platinum wire anode the current density was easily raised to 0.8 and later 1.6 ampere per square centimeter. When the salt concentration in the electrolytic cell was increased a current density of 2.3 amperes per square centimeter could be attained.

Using the highest current density (1.6) obtained with normal salt concentrations (2% sodium chloride) the following calculations may be made. For 0.1 equivalent of chlorine per glucose unit (2.2% chlorine) 100 lbs. of starch require 750 ampere-hours. Allowing one hour to generate the hypochlorite, there are required 750/1.6 or 468 square centimeters of platinum surface to oxidize 100 lbs. of starch by 2.2% chlorine. Using only one side of a platinum sheet one thousandth inch thick the cost would be approximately $70. If 12 hours are used to generate the hypochlorite the cost would be about $6 for the platinum electrode to oxidize 100 lbs. of starch by 0.1 equivalent (2.2%) of chlorine.

2. Properties of the products of electrolytic oxidation.

a. **Viscosity.** In Figure 2 is shown the change in hot viscosity during electrolytic oxidation. This series of oxidations used increasing amounts of hypochlorite from 1/2% up to 11% active chlorine (0.025 to 0.5 equivalent). The relative viscosities were measured at 90°C in an Ostwald viscosimeter using a 3% paste of the starch. The values represent seconds of flow under a pressure of 5 cm. of water.

The curve shows that only a small amount of oxidation (1/2% chlorine) was sufficient to cause a large drop in hot viscosity, so large a drop that it is not explainable on the

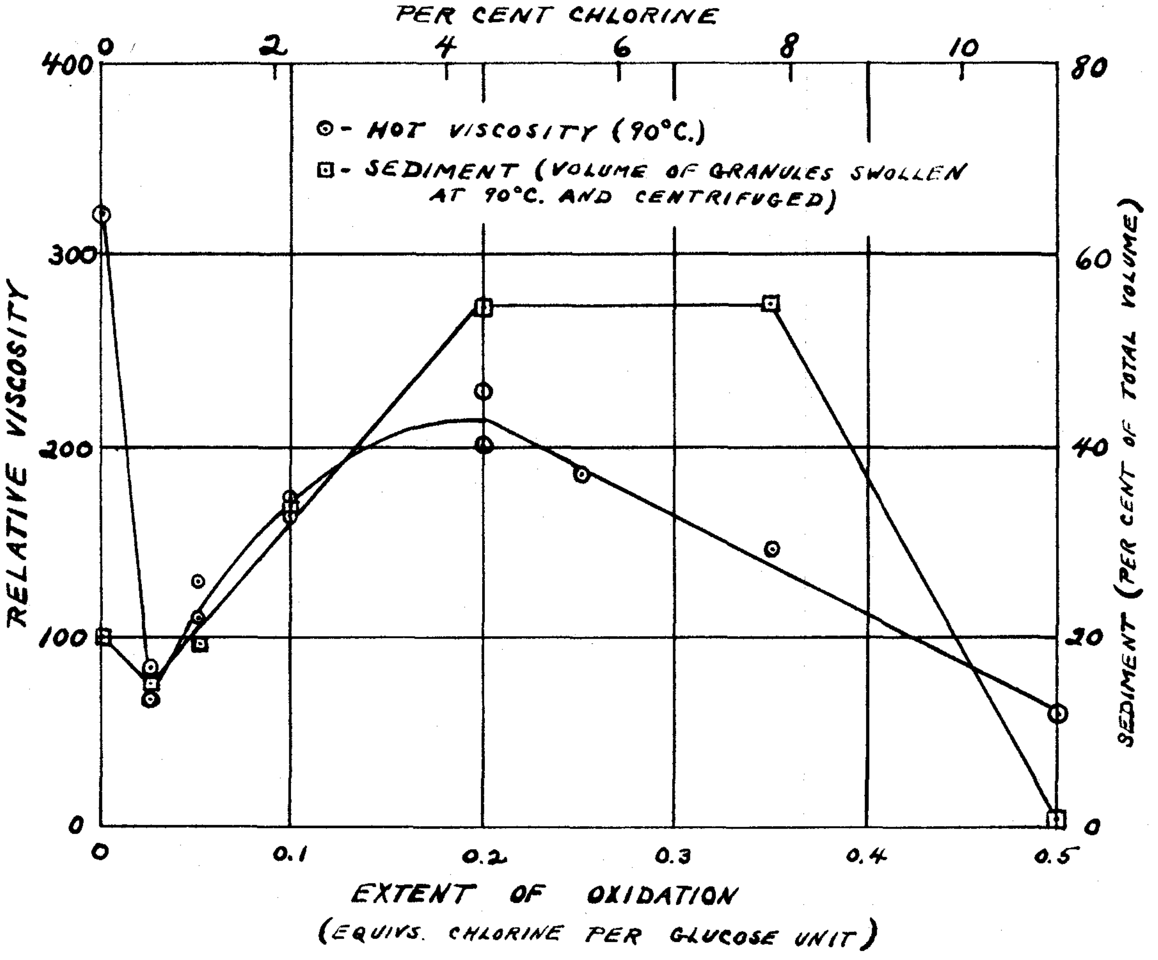
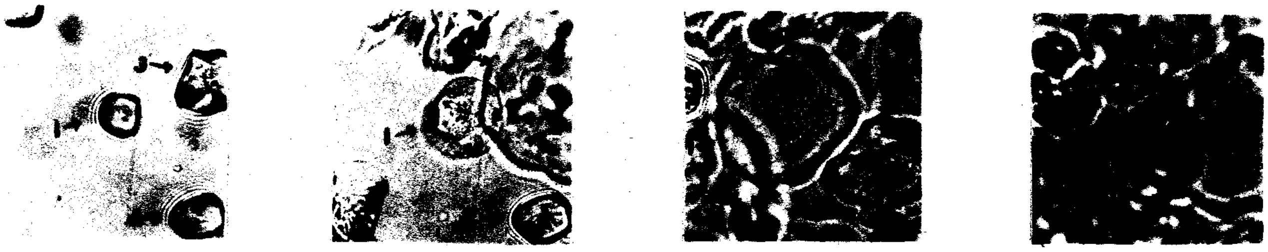


Fig. 2. CHANGE OF VISCOSITY AND VOLUME OF SWOLLEN GRANULES DURING OXIDATION.

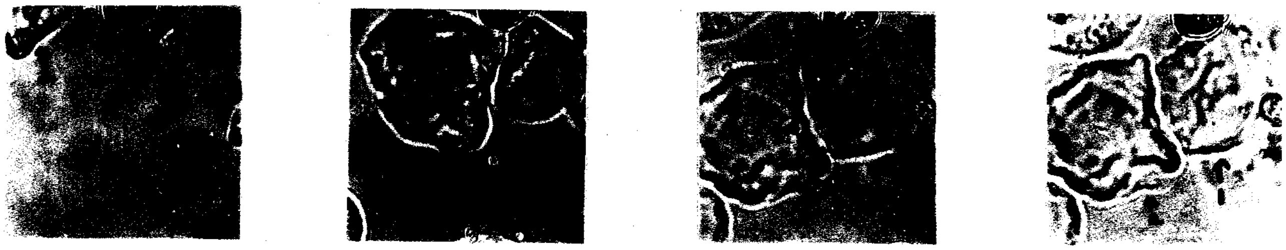
basis of a chemical change. It is difficult to conceive that such a small amount of active chlorine could destroy three-fourths of the viscosity. This large change is probably a physical change due to a very small chemical change of the starch substance. The ensuing viscosity increase (1/2 to 4.5% chlorine) is possibly due to the larger amount of the starch of each granule which has become susceptible to hydration. A maximum in this tendency was reached at 4.5% chlorine beyond which it is believed that degradation by oxidation became extensive enough to weaken the granular structure as explained in the next section. A steady decrease in hot viscosity followed the use of more oxidant.

b. Volume of swollen granules. 1% pastes of raw starch and of each of the oxidized starches prepared at 90°C were centrifuged and the volume of sediment measured. The sediment volume which represents the total volume of the swollen granules was plotted against the extent of oxidation in Figure 2 where it could be compared with the viscosity curve. It is apparent that the two curves are parallel, that the minima coincide at 0.025 equivalent (1/2%) of chlorine and that the maxima correspond very closely. It is believed that viscosity and the volume of the swollen starch granules follow very closely the hydration capacity.

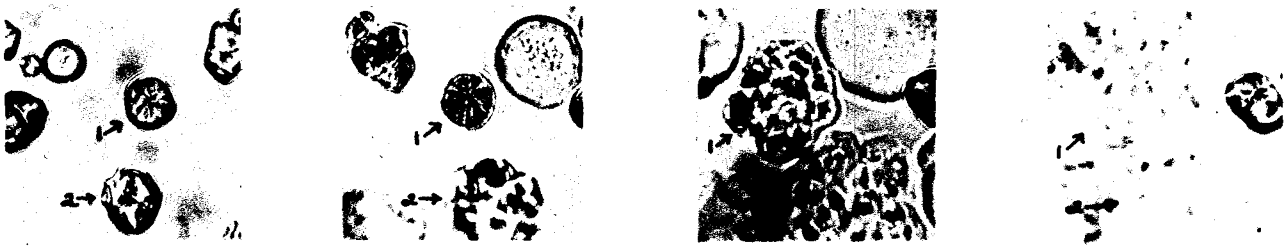
Microscopic observations substantiated this opinion. These observations are shown pictorially on the following page. In 0.70% sodium hydroxide solution cornstarch granules



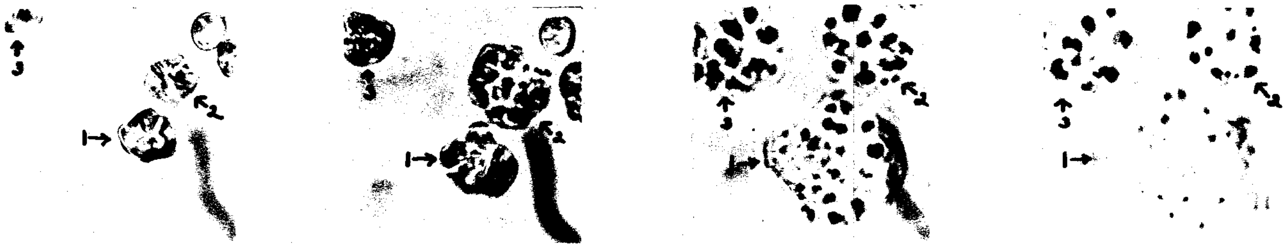
SWELLING OF RAW CORNSTARCH IN 0.70% SODIUM HYDROXIDE
(X450)



CORNSTARCH OXIDIZED WITH 0.025 EQUIVALENT (0.5%) OF CHLORINE
AND SWOLLEN IN 0.70% SODIUM HYDROXIDE



CORNSTARCH OXIDIZED WITH 0.1 EQUIVALENT (2.2%) OF CHLORINE
AND SWOLLEN IN 0.70% SODIUM HYDROXIDE



CORNSTARCH OXIDIZED WITH 0.2 EQUIVALENT (4.5%) OF CHLORINE
AND SWOLLEN IN 0.7% SODIUM HYDROXIDE

CORNSTARCH OXIDIZED WITH 0.5 EQUIVALENT (11%) OF CHLORINE
AND SWOLLEN IN 0.70% SODIUM HYDROXIDE

Plate I. Swelling of Raw and Oxidized Cornstarch swell slowly enough for observation. They can be seen to increase in size and become internally homogeneous as the granular contents become hydrated and gelatinize. Cornstarch oxidized with 0.5 equivalent (11%) of chlorine and subjected to the same conditions of alkali treatment shows practically no swelling but only disintegration of the granules. This occurs along the visible radial fissures of the granule first into a few pieces, each of which in turn gives rise to many more pieces by the same crumbling process. Numerous small particles result from each granule and begin to dissolve in the alkaline solution. The granular sack or membrane is conspicuously absent. This last sample showed a very low viscosity; its paste was so clear that the absence of sediment on centrifugation was as expected (See Figure 2).

Intermediate samples, oxidized by 0.1 or 0.2 equivalent (2.2 or 4.5%) of chlorine, showed a combination of the above effects. The swelling of the granules to a large size was observed along with simultaneous disintegration of the internal structure. The swollen granules contained some of the unswollen small particles so characteristic of the highly oxidized starch. The granules of starches oxidized by 0.1 or 0.2 equivalent of chlorine swelled to a larger volume than did raw starch granules. Although the viscosity of these same samples was increased beyond the viscosity of the less extensively oxidized starches, it did not reach the

the viscosity of raw starch. A sample of starch oxidized by 0.35 equivalent (8%) of chlorine showed a large number of granules which had swollen irregularly or had broken. This weakening of the granular membrane and its almost complete destruction in the 0.5 (11%) chlorine sample agrees with the steady decrease of viscosity resulting from the use of more than 0.2 equivalent (4.5%) of chlorine per glucose anhydride unit.

The sample of starch oxidized by 0.025 equivalent (1/2%) of chlorine showed a minimum viscosity and a minimum volume of centrifuged sediment. Microscopic examination disclosed that the granules were swollen somewhat less than raw starch granules and much less than the 0.1 or 0.2 chlorine samples. No other visible features could be discerned which would explain the low viscosity of the 0.025 chlorine sample. The granules during all stages of swelling acted very much like raw starch granules.

c. Rigidity. Measurements of rigidity (resistance to being deformed) were made on 6% pastes of the starches at room temperature in the rigidometer designed by Brimhall and Hixon (25). The values are reported in dynes per square centimeter. Figure 3 shows the effect of oxidation on the rigidity of "pearl" starch. Whereas 1/2% of chlorine caused a large decrease in hot viscosity, no such effect was noticed on the rigidity. The rigidity remained practically unchanged

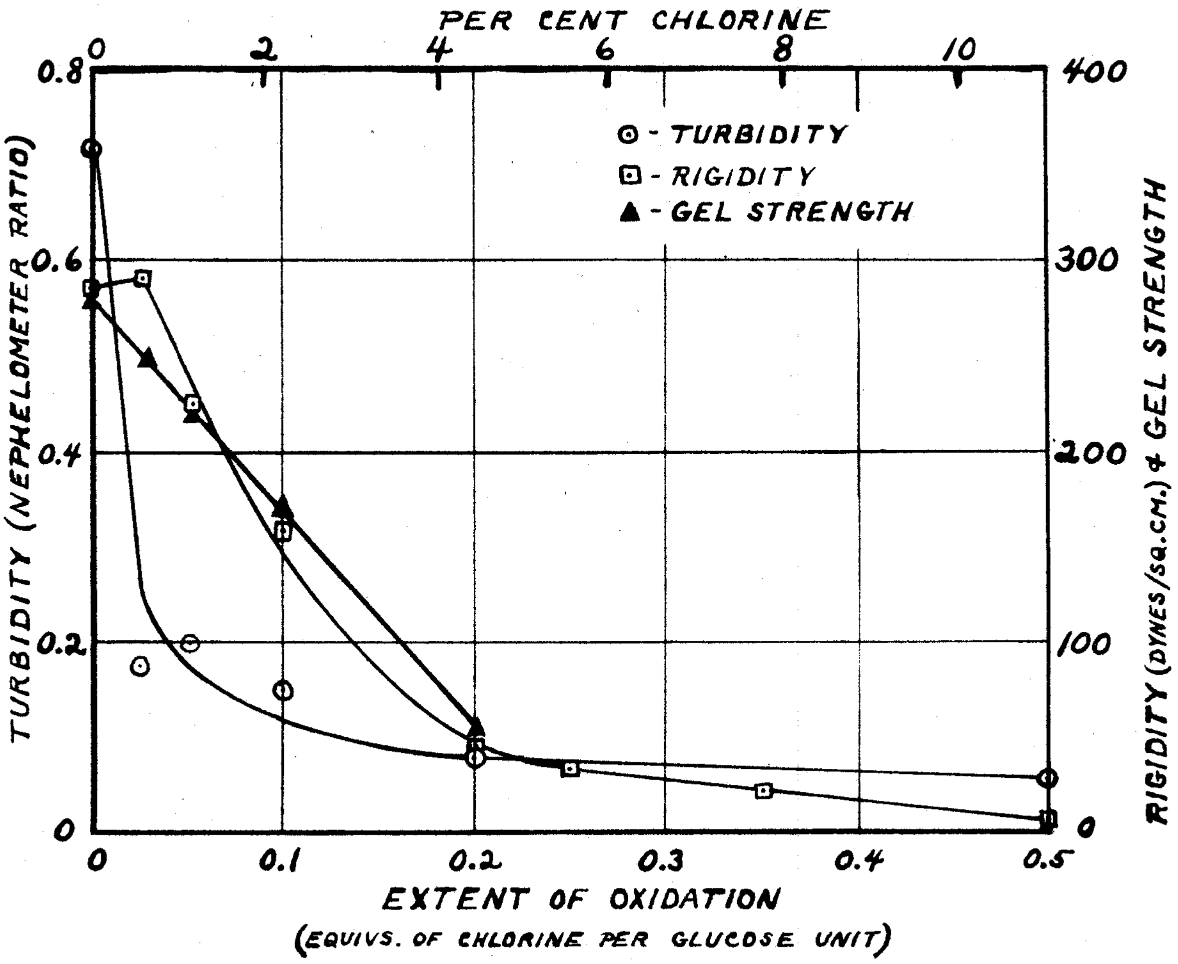


Fig. 3. CHANGE OF TURBIDITY, RIGIDITY AND GEL STRENGTH DURING OXIDATION.

until more than 1/2% of chlorine was used. Then a rapid and continued decrease in rigidity was evidenced up to the samples oxidized by 4.5% chlorine. This point, which represented a maximum in the hot viscosity curve, also represented a change in slope of the rigidity curve. Further increments of oxidizing agent did not decrease the rigidity at the former rapid rate but only slowly. 11% chlorine (0.5 equivalent) was nearly sufficient to completely destroy all rigidity. The disintegration of the internal structure observed microscopically on the highly oxidized starches may explain the rapid decrease in rigidity and gel strength while the starch still has a high viscosity. Such internal disintegration is apparently opposed to the subsequent formation of a strong and rigid gel.

d. Gel strength. When cornstarch was oxidized by electrolytic hypochlorite to the extent of 4.5% chlorine (0.2 equivalent) the gel strength decrease was a straight line function of the amount of hypochlorite used for oxidation. This is shown in Figure 3. Since the measurement of these samples, Hixon and Brimhall (161) have developed a different type of gelometer. Only a change in the slope of the curve would result if the values were measured on the newer instrument. Relative values are quite sufficient to follow these modifications of starch. The decrease in gel strength is very probably related to disintegration of the internal granular structure as mentioned above.

e. Turbidity. The relative turbidity of 1% pastes of the oxidized starches was measured in a nephelometer. Because of the great difference in color between a starch paste and a silver chloride suspension comparisons were almost impossible. In the absence of an absolute standard, relative turbidities were obtained by using a "pearl" starch paste as the standard. Figure 3 contains the turbidity curve from one series of electrolytically oxidized starches. It is apparent that only a small amount of oxidant (1/2% chlorine) is necessary to reduce the turbidity of the starch paste by three-fourths of its original value. This, too, should be considered as chiefly a physical change of the starch granule. Beyond this sudden dissipation of the turbidity further increments of oxidation have very little effect on the small residual turbidity.

f. Reducing power. The copper number has been used extensively for reporting the reducing power of cellulosic materials and recently by Richardson, Higginbotham and Farrow (259) for starch as was outlined in the historical section. There is herein described a much simpler method for determining the reducing power or copper number of starches and dextrins which shortens the time required from three hours to less than twenty-five minutes.

During the measurement of the reducing power of a series of electrolytically oxidized starches by the Gore and Steele

(106) modification of the Hagedorn and Jensen (123) method, it was noticed that the apparent maltose equivalent of the more soluble products when converted to milligrams of copper gave values equal to the copper numbers determined by the Richardson, Higginbotham and Farrow method. For raw starch and very slightly oxidized starch high values were obtained by the Gore and Steele method. These high values were attributed to the visible entrapment in the starch of iodine which was very difficultly released for measurement in the thiosulfate titration. Figure 4 compares the Gore and Steele values with the Richardson, Higginbotham and Farrow values.

In the determination of the maltose equivalent of starch-maltose mixtures Martin and Newton (207) avoided the difficulty due to iodine entrapment by using Hassid's (145) ceric sulfate titration. It seemed probable that the same ceric sulfate titration could be used to advantage here to measure the reducing power, not of a starch-maltose mixture, but of starch or dextrin alone. This proved to be true after the following modifications were incorporated. The potentiometric measurement used by Martin and Newton was eliminated because the color change of the solution from green to yellow just before the large voltage change was a satisfactory indication of the end-point. However, starch products with very little reducing power produced only a slight green color and made accurate determination of the end-point quite difficult. The addition

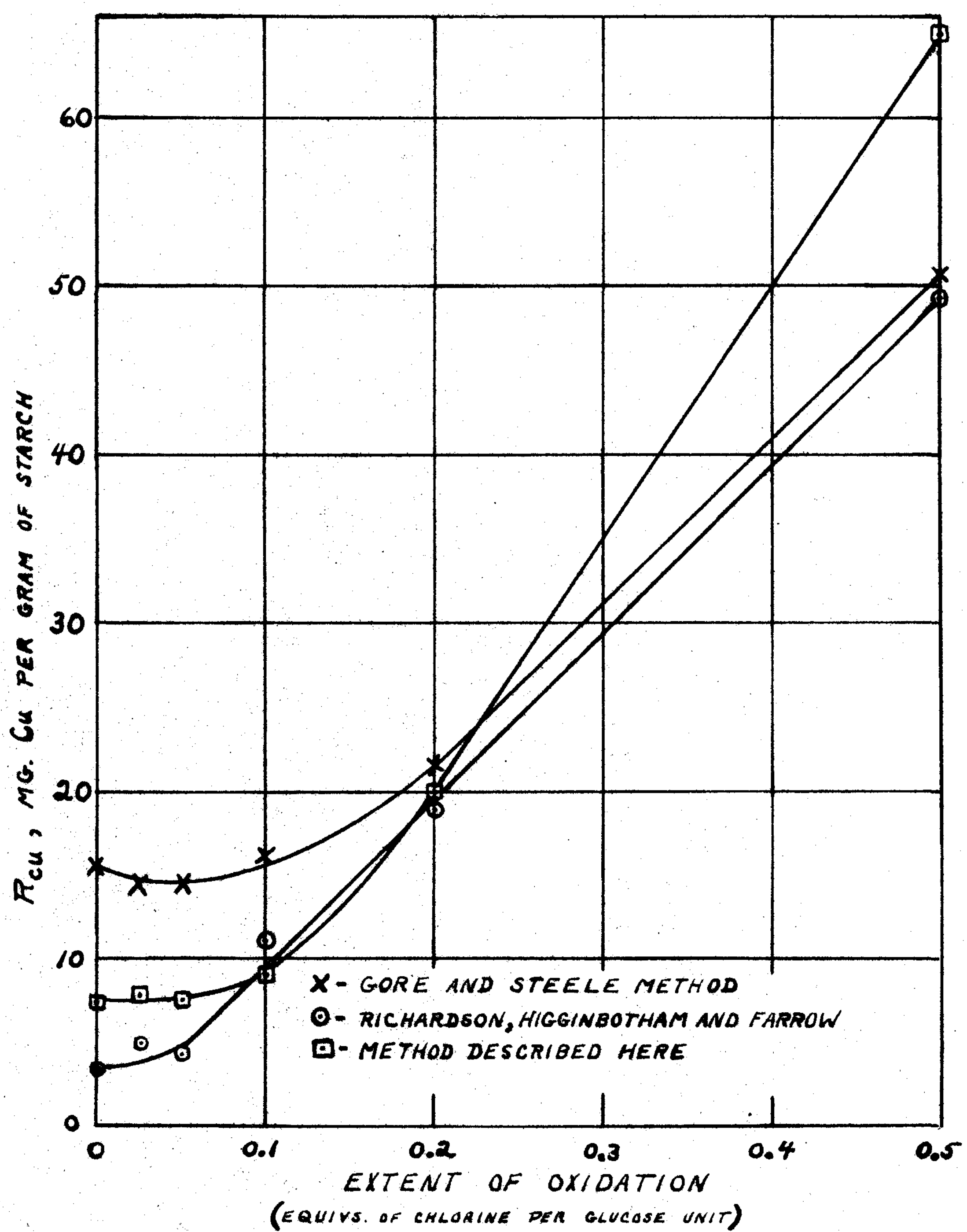


Fig. 4. CHANGE OF REDUCING POWER DURING OXIDATION AND COMPARISON OF REDUCING POWER METHODS.

of a measured amount of glucose solution to each starch sample obviated this difficulty. A correction for the reducing power of this added glucose was made by a blank determination. Figure 4 also shows the reducing power curve after these changes in procedure had been made.

Figure 5 compares the new method with that of Richardson, Higginbotham and Farrow during the hot and cold acid hydrolysis of starch. When the R_{cu} values (milligrams of copper per gram of starch) were plotted against the time of hydrolysis in hot acid, there resulted a straight line for R_{cu} values up to 1000 by both methods. The two methods gave the same results in the starch and dextrin range but not in the range of the sugars. This shorter method for copper numbers uses iron salts instead of copper salts. The results, therefore, might be reported as R_{Fe} units but it seems better not to introduce another such unit but to report the results as R_{cu} units to allow comparisons with copper numbers. When the maltose, glucose or iron equivalent is desired the following conversion factors are used:

$$\text{Per cent maltose equivalent} = \frac{R_{cu} \times 100}{1900}$$

$$\text{Per cent glucose equivalent} = \frac{R_{cu} \times 100}{2800}$$

$$R_{Fe} \text{ (mg. Fe per gram of starch)} = R_{cu} \times \frac{55.84}{63.57}$$

The utility of this method in the relative characterization of starches and dextrins is shown by Table I. The

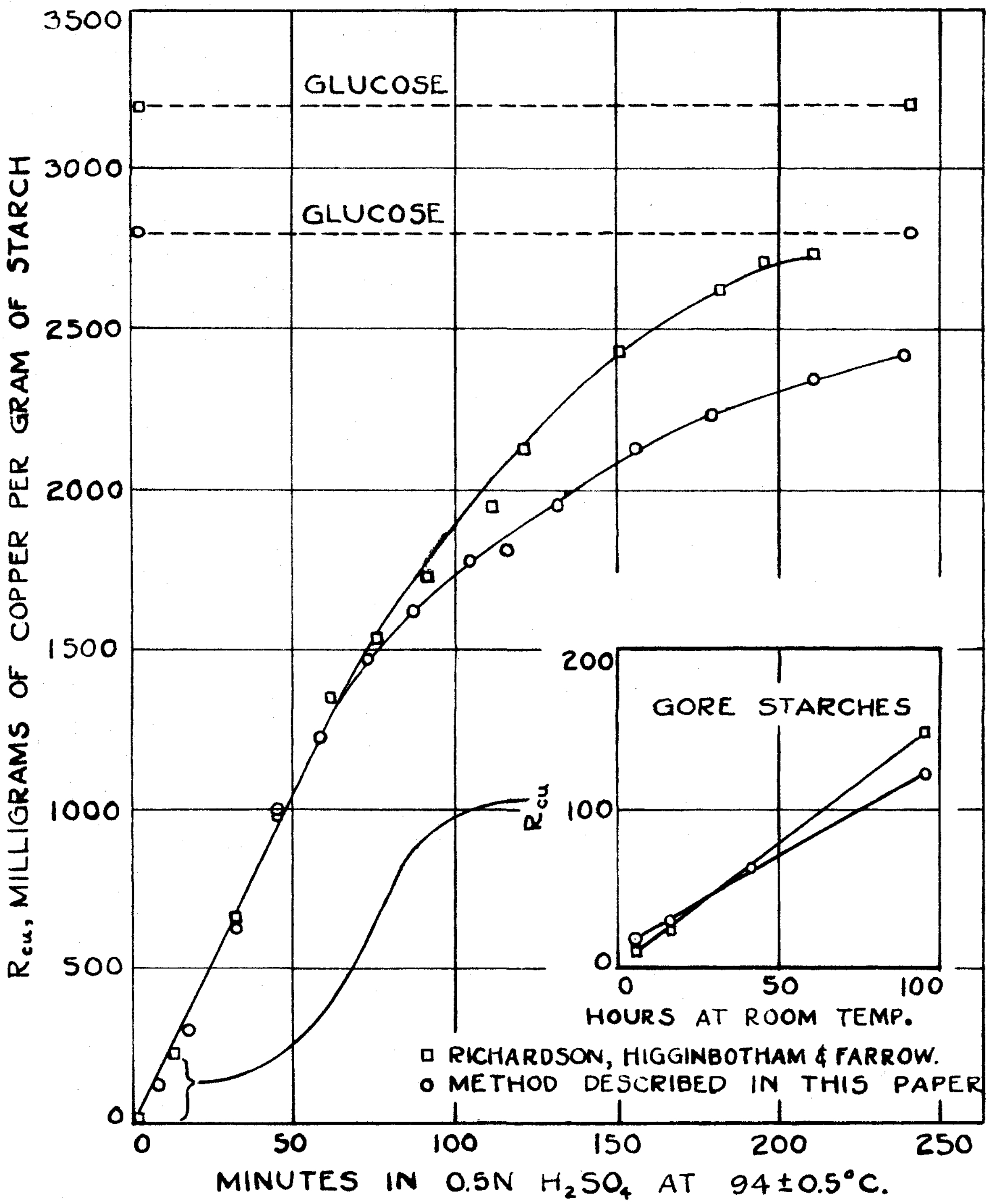


Fig. 5. Comparative determinations of reducing power during the hydrolysis of starch.

Table I. Reducing Power of Various Starches and Dextrins

Sample	R_{Cu} (mg. Cu per gram of starch)
"Pearl" starch (control)	6.8-7.9
Other commercial cornstarches	7.7, 9.4, 10.1, 11.6
Waxy maize starch	9.0
Thin-boiling starch, 40-fluidity	6.5
Thin-boiling starch, 90-fluidity	27.2
"Chlorinated" starch, 2.5% chlorine	14.9
"Chlorinated" starch, 5% chlorine	32.9
Electrolytically oxidized starch	6.8, 3.0, 6.3, 4.5
Alkali dextrin, A	10.5
Alkali dextrin, B	33.0
Alkali dextrin, C	71.0
Acid dextrin, A	12.0
Acid dextrin, B	25.5
Acid dextrin, C	39.0
Gore (105) starch, 5 hrs. conversion	19.6
Gore (105) starch, 42 hrs. conversion	61.6
Gore (105) starch, 96 hrs. conversion	120.0
Maltose	1900.
Glucose	2800.

reducing power increases with increasing conversion of starch products except for the electrolytically oxidized starches during the early stages of oxidation. These latter, because of the method of oxidation and washing, have values equal to or lower than those of raw starches. Table I shows how this simplified determination of reducing power has been used to follow the acid and alkaline conversion of starch, e.g., on the "thin-boiling" starches, on the Gore (105) starches, and on the dextrins formed by acid or alkaline catalyst.

The reagents for this reducing power determination are as follows:

Alkaline ferricyanide. 32.9 grams of potassium ferricyanide and 50 grams of anhydrous sodium carbonate are dissolved in water and made up to one liter. The solution is approximately 0.1N with respect to potassium ferricyanide.

Glucose solution. This is a 0.2% glucose solution containing a small amount of phenol.

Sulfuric acid solution. (5N) 139 ml. of concentrated sulfuric acid are made up to one liter of solution.

Ceric sulfate. A 0.05N ceric sulfate solution is made as follows: 26.5 grams of reagent ceric sulfate (G. Frederick Smith Chemical Company) are added to about 900 ml. of a solution containing 100 ml. of concentrated sulfuric acid. The mixture is digested until the solid has dissolved, is then cooled and made up to one liter. The ceric sulfate solution is standardized against a standard ferrous ammonium sulfate solution potentiometrically or in the presence of orthophenanthroline indicator.

The procedure for the determination is the following: Five hundred milligrams of the starch sample are weighed into a 300 ml. Erlenmeyer flask. 25 ml. of alkaline ferricyanide reagent and 5 ml. of 0.2% glucose solution are added. The flask is placed in a boiling water bath for exactly 15 minutes and is rotated while the starch is gelatinizing in order to give a uniform mixture. At the end of 15 minutes the flask is cooled under the tap to room temperature, 25 ml. of sulfuric acid solution are added, and the resulting green solution is titrated dropwise with standard ceric sulfate. The color change is from green to yellow. A blank determination is run on 5 ml. of the glucose solution. The equivalents of ceric sulfate are converted directly to milligrams of copper and reported as R_{cu} units (mg. Cu per gram of starch). Milliliters of ceric sulfate X normality of ceric sulfate X 63.57 / grams in sample = R_{cu}. For starch products which have an R_{cu} greater than 100 a smaller sample should be used.

g. <u>Characterization by enzymic digestion</u>. Soybean beta-amylase was used to follow the changes in susceptibility of starch during electrolytic oxidation. The method is that of Newton, Farley and Naylor (226). For each oxidized starch each of a series of 2% suspensions was heated at a different temperature (40 to 100°C) for thirty minutes, the resulting starch paste being subsequently digested for three hours at $40^\circ C$ with soybean beta-amylase. The final reducing value of each sample in terms of maltose equivalent was plotted against the temperature of pretreatment of the starch. The digestion curves of progressively oxidized starches are given in Figure 6. Oxidation with samll amounts of chlorine (up to 0.05 equivalent of chlorine per glucose unit) caused only slight retardation of enzyme digestion at all pretreatment temperatures. Oxidation with 0.1 equivalent or more of chlorine impeded digestion by soybean beta-amylase at higher pretreatment temperatures and reduced the total maltose equivalent producible. With the use of larger amounts of chlorine (0.5 equivalent) a marked increase in the digestion at lower pretreatment temperatures was observed in addition to the above-mentioned effect. This was further demonstrated by the digestion curves of two commercially chlorinated starches. These two curves were very similar to the curve of the starch oxidized with 0.5 equivalent of chlorine. The logical conclusion of the study of enzyme digestion of oxidized starch is that oxidation changes some of the glucose units of starch to structures which are not attacked by beta-amylase under the conditions of these experiments. In support of this observation, experiments described later in this thesis showed that gelatinized cornstarch oxidized more drastically than the above failed to show any digestion by malt extract.

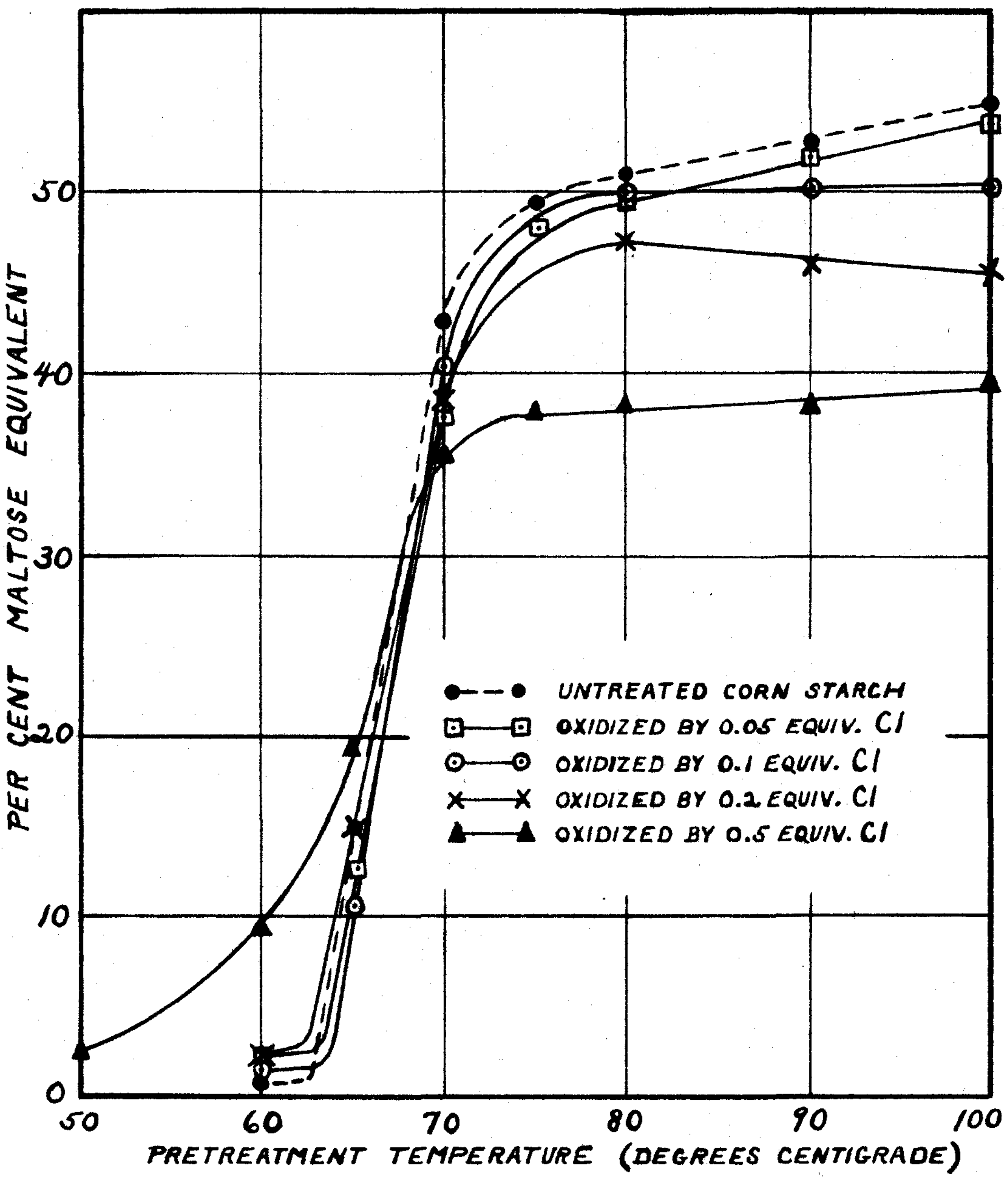


FIG. 6. EFFECT OF OXIDATION ON SUSCEPTIBILITY TO DIGESTION BY BETA-AMYLASE.

h. Microscopic differentiation by birefringence. As was shown by Newton, Farley and Naylor (226) the gelatinizing characteristics of a starch may be followed microscopically by counting the per cent of granules which lose the polarization cross as the temperature of the paste is raised.

Figure 7 shows the curves for the loss of birefringence of raw starch and of a starch oxidized with 0.5 equivalent (11%) of chlorine. The corresponding digestion curves are given for comparison. The effect of oxidation is at once apparent.

When the amount of birefringence of the granules rather than the loss of birefringence was measured quantitatively by optical methods (to be published soon by Dr. R. S. Bear) no change in the amount of birefringence could be detected after oxidation. Table II presents the values obtained. The absence of a change in birefringence indicates that, although some of the starch substance between radial starch crystallites has been modified by oxidation, the crystalline regions of the granule are not greatly altered by this type of oxidation.

3. Effect of various factors on the products of electrolytic oxidation.

a. Effect of heat. A temperature rise accompanies the electrolytic oxidation of starch and is directly proportional to the amount of oxidizing agent used, amounting to 7 or 9°C

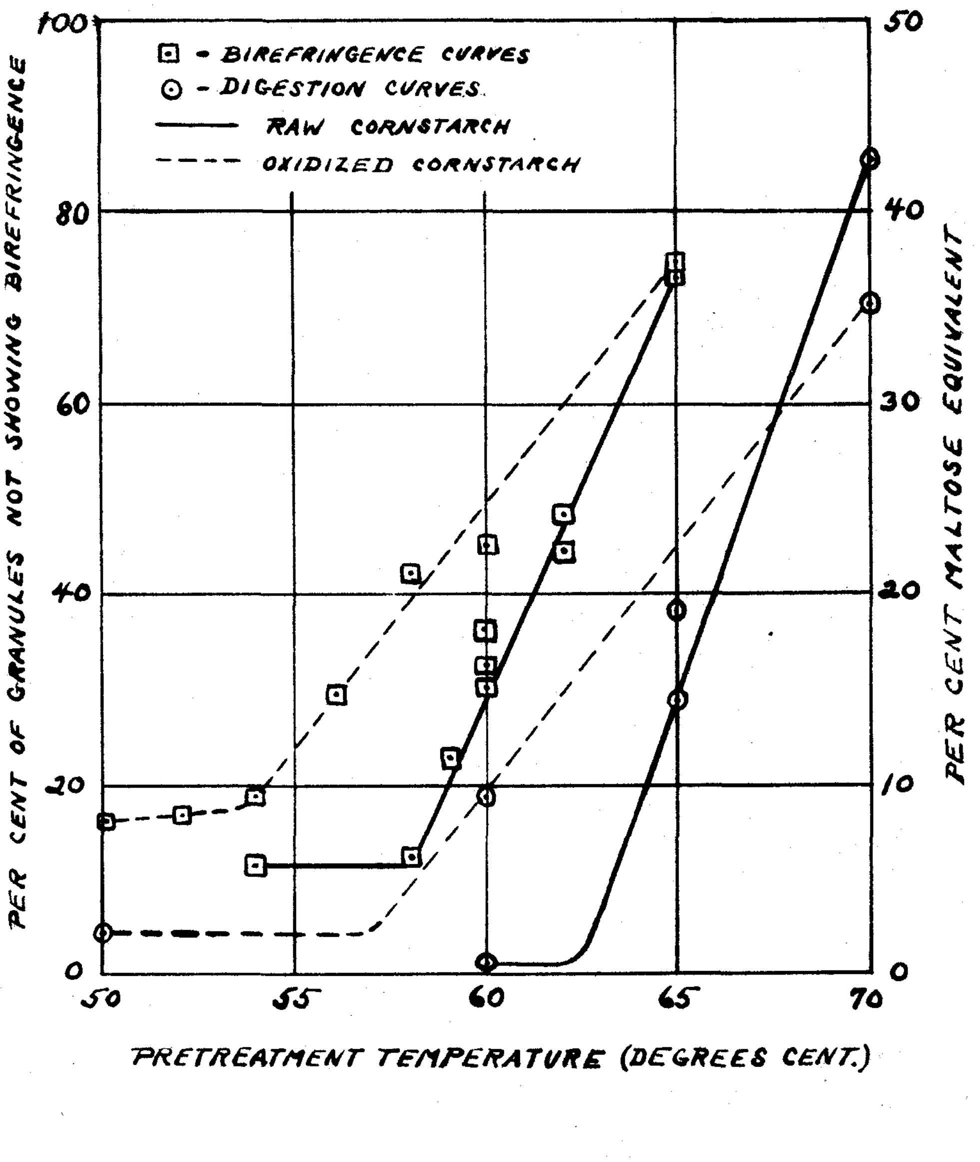


Fig. 7. BIREFRINGENCE CURVES OF RAW AND OXIDIZED STARCH.

Table II. Quantitative Estimation of the Birefringence of Raw and Oxidized Starch Granules.

Birefringence, $n_a - n_o = 0.00546 \frac{\Phi \text{ (in degrees)}}{d \text{ (in micra)}}$

Raw cornstarch	Cornstarch oxidized by 0.5 equivalents of chlorine per glucose unit
0.00546	0.0104
0.00638	0.0083
0.00925	0.0044
0.0093	0.0062
0.00754	0.0100
0.0078	0.0067
0.00993	0.0076
0.00785	0.0108
-	0.0092
-	0.0091
0.00794 (Average, $n_a - n_o$)	0.00827

when 0.1 equivalent of hypochlorite is used. Samples of starch oxidized by 0.25 equivalent of hypochlorite showed a temperature rise of 15 to 25 degrees depending on the density of the starch milk. In this latter case not only the temperature rise but the final temperature attained is important. This is especially true if the final temperature attained is near the gelatinization temperature of the starch.

Two oxidations were carried out with all conditions identical except the total volume of solution. The electrolysis was allowed to generate 0.25 equivalent (5%) of chlorine. Table III shows the results.

Table III. Effect of Heat on Oxidation of Raw Starch

Chlorine used		Total vol. during oxidation ml.	Temp. rise °C	Final temp. °C	Recovery %	Relative viscosity	Rigid. dynes/sq.cm.
Equiv. per glucose unit	%						
0.25	5	3000	15	42	96.9	186	38
0.25	5	1220	25	53	94.6	24	0

The effect of the higher temperature on the recovery, viscosity and rigidity is very apparent. The second product gave a very thin and clear paste.

In order to further determine the effect of heat, several electrolytic oxidations were made in a constant-temperature bath at the temperatures of 45,50 and 55°C. This last temperature, 55°C, caused some gelatinization of the starch to occur. This showed up very noticeably in the centrifuging and washing of the gummy product and in the measurement of viscosity and recovery. In Table IV it is shown that, as the temperature of oxidation is increased, the viscosity and recovery are decreased. The change in R_{cu} is a small increase but is not large enough to be significant.

b. Effect of concentration of alkali. A concentration of 0.4% sodium hydroxide prevailed in the standard procedure for electrolytic oxidation. To discover the effect of more alkali on the oxidation, a series of starches was oxidized in solutions whose sodium hydroxide concentrations were respec-

Table IV. Constant Temperature Oxidations of Raw Starch

Chlorine used		Constant temperature	Recovery	Relative viscosity,	Reducing power,
Equiv. per glucose unit	%	°C	%	seconds	R_{cu}
0.1	2.2	45	97.8	145	3.0
0.1	2.2	50	92.1	115	7.7
0.1	2.2	55	80.3	131	7.7

tively, 0.5, 0.6 and 0.72%. The last of these caused gelatinization of the starch and defined the upper limit of the series.

Additional sodium hydroxide beyond 0.4% rapidly decreased the viscosity and rigidity of the oxidized starches but had no effect on the reducing power or the recovery as Figure 8 shows. The values at 0.72% sodium hydroxide are irregular because of gelatinization of the starch by such a high concentration of alkali.

c. Effect of anodic current density. The usual anodic current density for these electrolytic oxidations did not vary much from 0.1 ampere per square centimeter. The platinum sheet anode had an area of 37.5 square centimeters, while the average current was about 4 amperes. One pound of starch was oxidized at a much higher current density by using a platinum wire anode of 3.14 square centimeters area. With the current at 2.5 amperes the current density was 0.8 ampere per square centimeter or eight times as high as usual. This

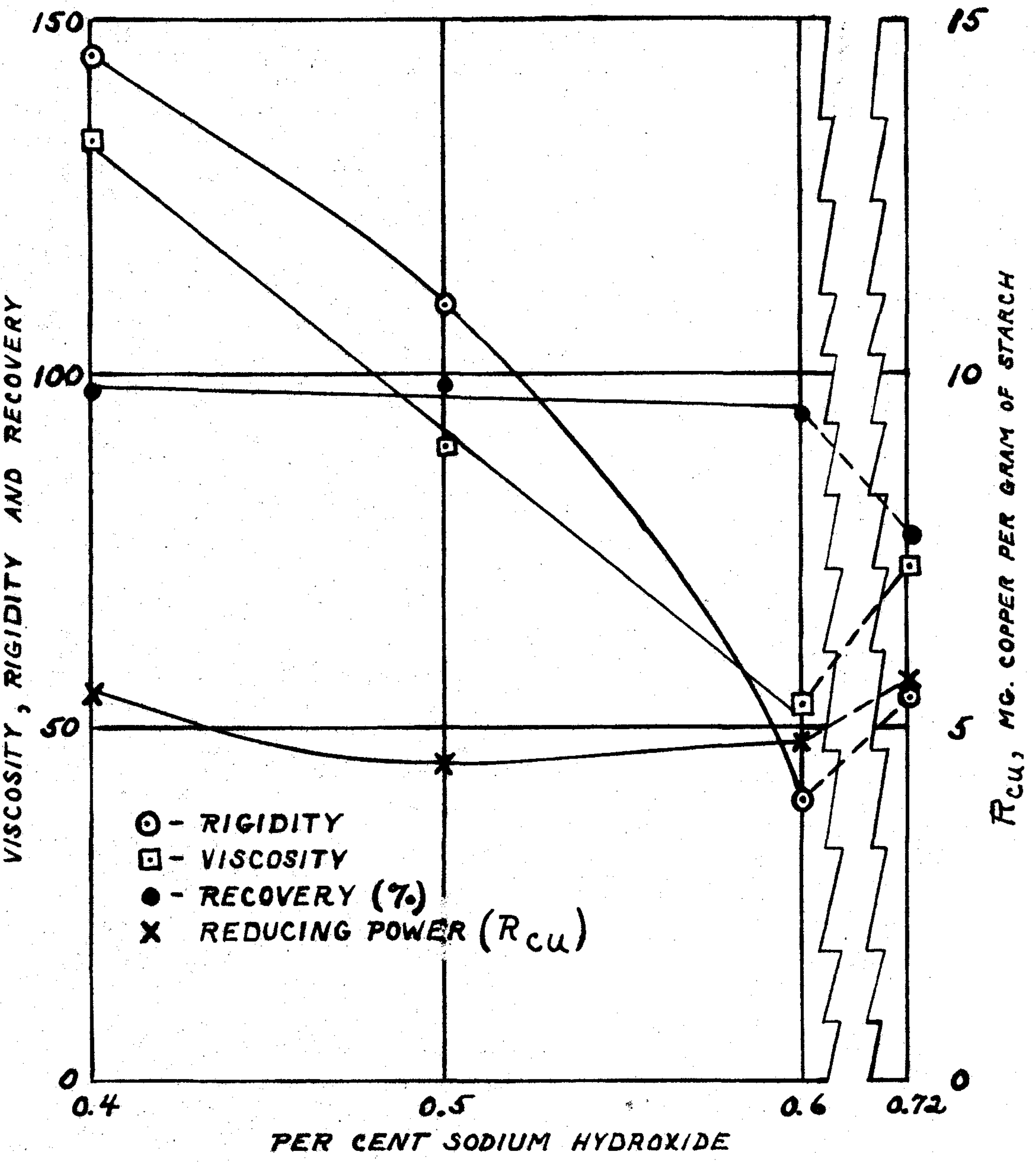


FIG. 8. EFFECT OF SODIUM HYDROXIDE CONCENTRATION ON THE OXIDATION OF RAW CORNSTARCH BY 0.1 EQUIVALENT (2.2%) OF CHLORINE.

large increase in anodic current density had no effect at all on the oxidation product as Table V shows.

Table V. Oxidations at Different Anodic Current Densities

Chlorine used						
Equiv. per glucose unit	%	Current density amp./sq.cm.	Recovery %	Relative viscosity seconds	Rigid. dynes/sq.cm.	Reducing power R_{cu}
0.1	2.2	0.133	96.2	133	145	6.3
0.1	2.2	0.795	94.8	150	145	6.8

d. <u>Effect of concentration of sodium chloride</u>. One sample of starch was oxidized in a solution containing only 0.5% sodium chloride instead of the ordinary 2.0% of sodium chloride. This lowering of the salt concentration had no effect whatever on the properties of the product. See Table VI.

Table VI. Oxidations at Different Salt Concentrations

Chlorine used						
Equiv. per glucose unit	%	Concentration of sodium chloride %	Recovery %	Relative viscosity seconds	Rigid. dynes/sq.cm.	Reducing power R_{cu}
0.1	2.2	2.0	96.2	133	145	6.3
0.1	2.2	0.5	97.6	125	145	6.8

e. <u>Effect of starch concentration</u>. Two samples of starch were oxidized under identical conditions except for the volume of solution, the volumes being 3000 and 1220 ml. The respective concentrations of starch were 13% and 27%. The only effect of the change in concentration of starch was a temperature effect, due to the smaller volume of solution absorbing the heat. The properties of the two products have been presented above in Table III and discussed in the section concerning heat effects.

4. Enzymic digestion products from electrolytically oxidized starches.

It was shown above that oxidation of raw starch progressively increases its resistance to enzymic digestion. The amount of maltose producible by soybean beta-amylase is progressively decreased by oxidation of the raw starch as Figure 6 shows. Samples of several oxidized starches were digested by beta-amylase to equilibrium (76 hours). The insoluble residue formed during digestion was filtered off, washed well with water, dried and weighed. The digestion liquors were treated with two volumes of 95% alcohol to precipitate the limit dextrin. The digestion data and properties of the digestion products are given in Table VII.

The decreasing yield of maltose with increasing extent of oxidation substantiates former data (226). This is just as true for starches oxidized by the commercial hypochlorite process as it is for the electrolytic oxidation products and shows that oxidation changes a portion of the starch to structures which are non-digestible. The yield of insoluble residue

Table VII. Products from Amylolytic Digestion of Oxidized Starches

Product	Amount of chlorine used for oxidation		Extent of digestion:				
	equiv. per glucose unit	%	% maltose produced	Yield %	Iodine color	$[\alpha]_D^{25}$	Reducing power R_{Cu}
Limit dextrin pptd. by alcohol	0.5	11	43.4	19	Red	+149°	47.8
Water - insoluble residue	0.5	11	43.4	8	Blue	+141°	53.6
Water - insoluble residue	0.25	5	57.2	14	Blue	-	-
Water - insoluble residue	Commercial	7	41.7	1	Blue	-	-
Water - insoluble residue	Commercial	5	39.0	2	Blue	-	-
Water - insoluble residue	Commercial	2.5	51.0	6	Blue	-	-

likewise decreases as oxidation proceeds. This means that the oxidation has decreased the amount of starch which can retrograde or precipitate on aging. The textile industry is interested in soluble starches which give a permanently clear solution. Starches whose solutions become cloudy during storage are undesireable. The starches used in textile work whose solutions are claimed to be permanently clear are either produced by oxidation or contain substances added to inhibit retrogradation. Likewise in the manufacture of "clear gum" confectionery a starch is demanded which will not become opaque on storage (249). The commercial samples oxidized with 5 or 7% chlorine and shown in Table VIIwould fulfill either of the above technical demands since the amount of retrograded material in these two samples was 2 and 1% respectively.

B. Oxidation of Gelatinized Starch by Bromine in Neutral Solution

For the more extensive oxidations gelatinized starch was used. The solution was maintained at neutrality by means of calcium carbonate in order to minimize enolization and hydrolytic changes. Bromine was chosen as the oxidizing agent since it would simulate the commercial sodium hypochlorite oxidation and yet be conveniently handled in the laboratory. The procedure for oxidation, recovery, purification and analysis is that of Felton, Farley and Hixon (72) with certain modifications.

1. Preparation of the oxidized starches.

A suspension of 40 grams of pearl starch was prepared in a minimum amount of distilled water. This was poured slowly into 1000 ml. of boiling distilled water in a 3-neck round bottom flask of two liters capacity. The resulting starch paste was boiled gently with stirring for thirty minutes. An excess of calcium carbonate was then stirred into the paste, the amount of calcium carbonate being the same as the weight of bromine used, viz., 10 grams for each equivalent of bromine per glucose anhydride unit. The flask was brought to room temperature in a cold water bath while the stirring was continued. The necessary quantity of bromine for the desired amount of oxidation was added dropwise with stirring. After the bromine had been added stirring was continued until disappearance of the yellow color showed that practically all the bromine had been used. The time required for the bromine to oxidize the starch varied from 1/2 to 6 days depending on the amount of bromine used. The excess bromine was removed by sucking air through the suspension for about one hour. The residual calcium carbonate was filtered off, washed with a small amount of distilled water, and the filtrate poured slowly with stirring into twice its volume of 95% alcohol. A white flocculent precipitate formed which was allowed to settle for a few hours, then filtered with suction, washed with alcohol and ether and dried in a vacuum oven at 60°C. For purification from halides it was necessary to dissolve the sample in water and pour into three volumes of alcohol and repeat the solution and precipitation until the precipitate showed the absence of halogen.

2. Analytical comparison of the oxidized starches.

The oxidized starch samples were analyzed for calcium and uronic acid content. The reducing power and optical rotatory power were measured. The results are given in the above-mentioned paper (72) and are plotted in Figures 9 and 10. The analyses indicated that oxidation of primary alcohol groups to produce uronic acids, of secondary alcohol groups to produce ketone groups, and of ketone and aldehyde groups to produce non-uronic acids, had taken place.

To test this theory further the sample oxidized by 4 equivalents of bromine per glucose anhydride unit was chosen for hydrolysis to the oxidized glucose units. (This sample will be referred to hereafter as the 4Br oxidized starch). The 4Br oxidized starch should contain a fair amount of each of the different oxidized types since its analysis showed 47.5% uronic acid anhydride, 1.45% non-uronic calcium (excess calcium over that calculated for the uronic acid present) and a reducing value of 373 mg. Cu per gram of sample (just beyond the maximum reducing value attained, as Figure 10 shows). This sample should contain, therefore, uronic acids, keto-acids or keto-sugars and non-uronic acids.

3. Hydrolysis of the oxidized starch.

In attempting to hydrolyze the 4Br oxidized starch without

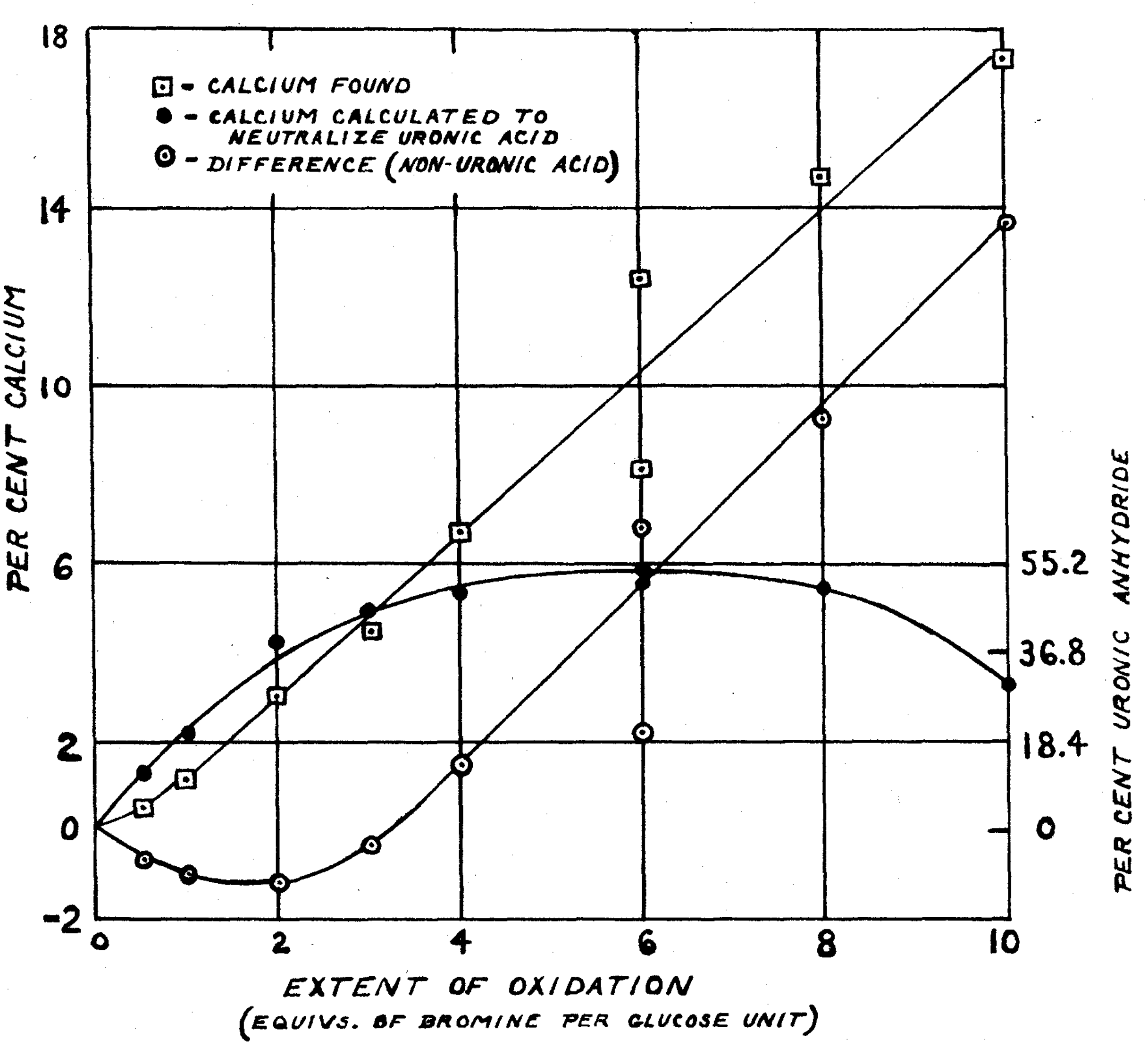


FIG. 9. VARIATION OF CALCIUM CONTENT AND PRODUCTION OF URONIC ACID DURING THE OXIDATION OF GELATINIZED STARCH.

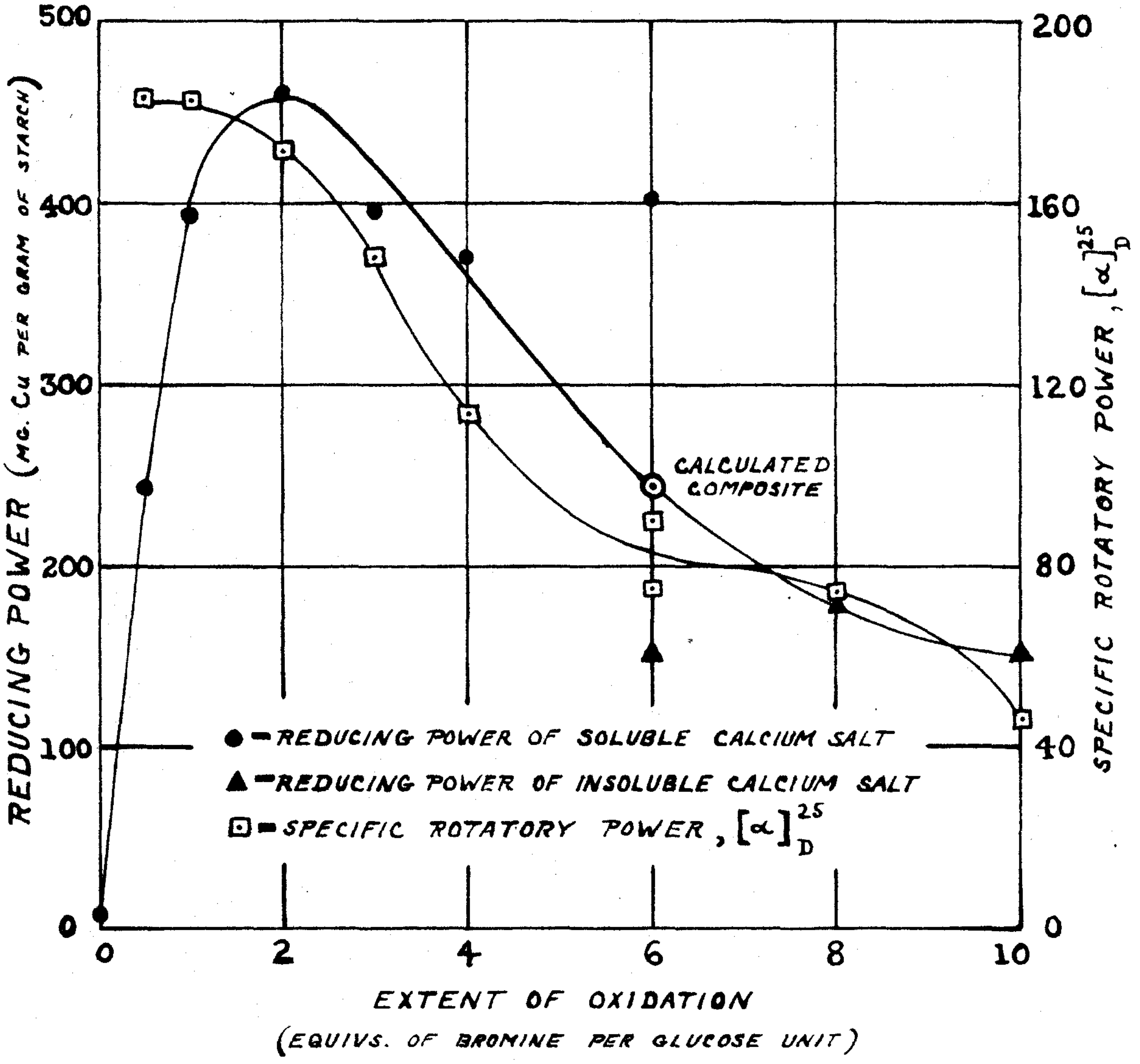


FIG. 10. CHANGE OF REDUCING POWER AND SPECIFIC ROTATORY POWER DURING THE OXIDATION OF GELATINIZED STARCH.

extensive decomposition of the constituent oxidized glucose units, both enzymic and acid hydrolyses were given trial.

a. Enzymic hydrolyses. Digestion of the calcium salts of various oxidized starches by malt diastase at 55°C. for two one-hour periods showed that samples of gelatinized starch oxidized by more than 2 equivalents of bromine gave no maltose. Table VIII shows the extent to which the various starches were hydrolyzed by diastase.

Table VIII. Hydrolyzable Fraction of Oxidized Starch

Extent of oxidation, Equivs. Br per glucose unit	Per cent maltose equivalent from 2-hour digestion by malt diastase
0	75.2
0.5	49.5
1	20.6
2	1.7
3 et seq.	0

To make certain that the above non-digestibility of 4Br oxidized starch was not due to the large amount of calcium present the calcium was removed by means of oxalic acid. The carbohydrate acid thus obtained was found to contain no material susceptible to beta-amylase digestion in 5 hours.

Three further attempts at enzymic digestion of this acid were made using Pectinase 46 AP and Diastase C from Röhm and

Haas Company, and beta-amylase, each for ten days. Diastase C showed no production of maltose; Pectinase 46 AP and beta-amylase gave 3% maltose equivalent which was probably reducing sugar produced by slight acid hydrolysis (the solutions were buffered at pH 4.7).

b. Acid hydrolyses.

(i) Hydrogen chloride in methanol. The procedure of Morrell and Link (215) for the hydrolysis of pectins was adapted to oxidized starch. 9.2 grams of 4Br oxidized starch were mixed with 100 ml. of anhydrous methyl alcohol containing 3% dry hydrogen chloride and refluxed for 12 hours in a soil flask fitted with a ground glass condenser terminated by a calcium chloride tube. The concentration of hydrogen chloride was increased to 5% by the addition of 50 ml. of methyl alcohol containing about 10% hydrogen chloride. Refluxing was continued for a total of 47 hours. The methyl alcohol, as well as two successive additions of methyl alcohol, was removed in vacuo at 40°C. On conversion to the barium salts and precipitation with alcohol, tests indicated incomplete hydrolysis such as is the case with gum arabic in weakly acid solution (322).

The barium salts were treated with enough sulfuric acid to remove the barium and leave the solution 1N. This solution was refluxed for 12 hours, the sulfuric acid being then removed by means of barium hydroxide leaving just a trace of barium. The solution of the carbohydrate acids was concentrated in vacuo at 45° to a volume of 50 ml.

(ii) Identification of glucuronic acid. 10 ml. of this solution were heated for one hour on the water bath with excess cinchonine. The alkaline solution was cooled, filtered, extracted with chloroform and evaporated almost to dryness in vacuo. The cinchonine derivative crystallized and had a melting point of 172-175°C. After five or six recrystallizations from water the cinchonine salt melted at 199-200° sharply. A mixed melting point with the cinchonine salt of glucuronic acid prepared from gum arabic was undepressed. (The reported M.P. varies from 198 to 204°.) The cinchonine derivative of glucuronic acid from oxidized starch gave a positive naphthoresorcinol test for uronic acid.

This method of hydrolysis produced considerable coloration both in anhydrous methyl alcohol and in the aqueous sulfuric acid. The extremely low yield of the glucuronic acid derivative attests to the suspected decomposition of the uronic acids during hydrolysis. A better method was sought in which uronic and keturonic acids would not be decomposed.

(iii) Mercaptalation and hydrolysis. Wolfrom's (330) method of hydrolysis in fuming hydrochloric acid at 0°C with simultaneous mercaptalation of the liberated aldehydic groups by ethyl mercaptan was tried on the 4Br oxidized starch.

The solid mercaptals were acetylated in pyridine and acetic anhydride and recovered with chloroform. On following

Wolfrom's procedure only an oil separated which could not be crystallized from any of the solvents or solvent mixtures tried.

The oil was acetylated a second time but did not crystallize. An acetyl analysis showed 18.4% acetyl which corresponds to about two acetyl groups per glucose unit.

(iv) Acetylation and hydrolysis. Samples of the 4Br oxidized starch were acetylated according to the procedure of Brigl and Schinle (24) with slight modifications due to the great solubility of the oxidized starch. The acetylated product was hydrolyzed in glacial acetic acid containing 30-32% hydrobromic acid. Every sample thus treated turned black shortly, making it impossible to follow the hydrolysis by rotation. The decomposition was too extensive to warrant further study.

(v) Tests with hydrochloric acid. The possibility of hydrolyzing the 4Br starch directly in hydrochloric acid was investigated. Because of the ease with which uronic acids lose carbon dioxide when heated in acid solution, heating was eliminated. At room temperature the 4Br starch in concentrated hydrochloric acid became too dark for rotatory measurements in less than 18 hours; at 0°C the solution was dark in three days. The reduction product of 4Br starch at room temperature and at zero was somewhat more stable. The oxime of 4Br starch (described below) at room temperature was dark in ten or twelve hours, while at 0°C the solution remained clear for three or four days even though it developed a yellow to red color. These tests indicated some possibility of hydrolyzing the oxidized starch by using the oxime in concentrated hydrochloric acid.

4. Some derivatives of the oxidized starch.

Because of the great instability of the 4Br oxidized starch an attempt was made to prepare stable derivatives which could be hydrolyzed in acid without extensive decomposition. It is known that uronic acids and keturonic acids are very unstable in acid solution, especially when the solutions are heated (200). The decomposition is one of decarboxylation and is used for the quantitative determination of uronic acids (53,195,206).

a. Reduction product. Reduction of the oxidized starch was attempted in order to stabilize the ketone groups in the form of secondary alcohol groups. A Walden inversion would be involved, producing derivatives of mannose, allose and altrose as well as derivatives of glucose.

One gram of 4Br oxidized starch dissolved in 10 ml. of water was shaken in the hydrogenation apparatus with 5 mg. of platinum oxide catalyst. After 35 minutes shaking of the solution in an atmosphere of hydrogen it was noticed that hydrogen absorption was becoming very slow. Another 10 mg.

of catalyst were added and shaking resumed. After 130 minutes hydrogen absorption ceased, 14.75 ml. of hydrogen (at standard conditions) having been absorbed. From the nitrogen content of an oxime of 4Br oxidized starch it was calculated that complete reduction of the ketone groups in a one gram sample would require 91.1 ml. of hydrogen. 14.75 ml. represents only 16.2% of 91.1 and, therefore, 16.2% of complete hydrogenation. Even if several more additions of catalyst had been made it is doubtful that any approach to complete hydrogenation could have been obtained. Hydrogenation probably ceased because the catalyst was poisoned or because of some other colloidal effect.

The R_{cu} of the 4Br oxidized starch was 701 and that of the reduction product 587. The loss in reducing power calculated from these values was exactly the same as the calculated percentage hydrogenation, viz., 16.2%.

b. Methyl ester of 4Br oxidized starch. An attempt to stabilize the uronic acid units of the oxidized starch consisted in conversion to the methyl ester. Five grams of the calcium salt of 4Br oxidized starch were converted to a silver salt in aqueous solution by adding silver nitrate.

Two grams of the dry silver salt were shaken occasionally over a period of two days with 10 ml. of methyl iodide in a glass stoppered bottle. The methyl iodide filtrate and the methyl alcohol washings from this suspension gave a glassy film of the methyl ester of 4Br oxidized starch on evaporation of the solvents. The yield of methyl ester was too small to offer any useful possibilities.

c. Acid of 4Br oxidized starch. The properties of the acid obtained from the calcium salt of 4Br oxidized starch by means of oxalic acid explain some of the difficulties encountered during hydrolysis, fractionation and derivatization. This carbohydrate acid was recovered from solution by alcohol precipitation as a gum which on standing filled with gas bubbles from auto-decomposition (presumably carbon dioxide from decarboxylation) and darkened progressively. The uronic acid equivalent of the calcium salt was 47.5% on a calcium-free basis while that of the acid was 43.8%.

d. Oxime of 4Br oxidized starch. To protect the ketone groups in the oxidized starch an oxime was formed. To a saturated solution of 5 grams of the oxidized starch on a hot water bath was added a solution of 2.5 grams of hydroxyl amine hydrochloride and 1.9 grams of sodium carbonate. The resulting pink solution was heated for 45 minutes whereupon an additional solution of 0.5 gram of hydroxyl amine hydrochloride and 0.4 gram of sodium carbonate was added. After a 15 minute heating period the solution was filtered hot and allowed to cool. Three volumes of alcohol were added to precipitate the pink colored oxime. This was filtered off, dried in vacuo at 50°C, dissolved in water containing a small amount of acetic acid, neutralized with calcium hydroxide solution, reprecipitated with alcohol, filtered off and again dried in vacuo at 50°C. The yield was 4.7 grams of oxime. This oxime was used for hydrolyses and methylations described below.

Found; N_a: 5.22, 5.33, 5.40, 4.80% (two preparations)
Calculated for monooxime per glucuronic anhydride unit,
N_a: 6.73%

e. <u>Hydrolysis of the oxime of 4Br oxidized starch</u>. At 0°C the hydrolysis of the oxime of 4Br starch in concentrated hydrochloric acid appeared complete in less than five hours when followed polarimetrically and by reducing power. Alcohol fractionation separated the hydrolytic products into four fractions, two of which gave color tests for uronic acids and gave crystalline osazones. This work was discontinued because a successful methylation procedure was developed meanwhile.

5. <u>Methylation of the oxidized starch.</u>

a. <u>Dimethyl sulfate and alkali</u>. Attempts to methylate the 4Br oxidized starch by the first step of the Hess and Lung (154) procedure for starch gave no methylated material which was insoluble in water. Considerable coloration was noticed. On the next trial the Haworth (146,147,149) procedure for di- and tri-saccharides was used, paying special attention to the suggestions of Levene and Tipson (196) and West and Holden (324) that rapid stirring was necessary, and to the precaution of Haworth (146) and Hendricks and Rundle (150) that strong alkali, e.g., 60%, caused sugar decomposition. The product after three methylations was still soluble in water, could not be extracted from the aqueous solution by chloroform, but was extracted from the sodium sulfate by methyl alcohol after the liquors were evaporated to dryness in vacuo at 50°C. 15% of the dry product was soluble in chloroform.

Found, MeO: 12.8, 13.4%.

A second sample methylated five times, extracted from the residue with methyl alcohol but not chloroform, had a methoxyl content of 22.3, 22.3, 22.2%. Some sodium sulfate was still present. The methylated material could not be extracted from aqueous solution by chloroform on the alkaline or acid side. It was thought that methylation was not taking place very readily. Similarly Hirst and Jones (158) have found that in the methylation of apple pectin by dimethyl sulfate and alkali the pectic acid was destroyed.

Another sample of 4Br oxidized starch methylated according to the procedure which Levene and Tipson (196) used on the aldobionic acid from gum arabic gave no better results until the dry methylated solid was ground with concentrated sulfuric acid and extracted with cold chloroform. The methoxyl content of the extracted material was 30.7, 30.1%.

Although this last product suffered some decomposition in the sulfuric acid treatment and could not be used further, its methoxyl content indicated that the difficulty was not in the methylation procedure but in the extreme solubility of the methylated products in water. Samples methylated later by a dry procedure to a methoxyl content of 42% were still soluble in water.

From these methylations there was obtained along with the sodium sulfate a considerable quantity of a colorless crystalline material, M.P. 180-185°C (with decomposition), which was undoubtedly sodium methyl sulfate.

Calculated for $NaCH_3SO_4$, MeO: 23.1; SO_4: 71.6%.
Found MeO: 23.6; SO_4: 69.6%.

b. *Sodium and methyl iodide in liquid ammonia.* Five grams of 4Br oxidized starch were methylated in liquid ammonia by means of sodium and methyl iodide according to the directions given by Freudenberg and Boppel (86). None of the product was extractable by chloroform. On solution in water and precipitation with ethyl alcohol to remove the sodium iodide 27% of the original material was recovered.

Found, MeO: 3.95%.

On evaporation of the filtrate to dryness and subsequent extraction with 95% alcohol a large crop of colorless crystals (1.7 grams) separated. On purification and analysis these were found to be trimethyl ammonium iodide.

Calculated for $(CH_3)_3NHI$, N_2: 7.48%; I_2: 67.8%.
Found, N_2: 7.38, 7.47%; I_2: 62.9, 64.3%.

The residue from the alcoholic extraction was likewise not methylated to any extent as its solubility indicated.

Three grams of the oxime of 4Br oxidized starch were methylated by the same procedure with no better results. No chloroform soluble material was obtained. Trimethyl ammonium iodide was again isolated. It was noticed, however, that during the methylation more than the required amount of sodium was used. This suggested some of the side reactions mentioned in the review by Fernelius and Watt (74), such as esters forming sodium derivatives of amides, aldehydes and ketones forming ammonia addition products which could react with sodium, etc.

In fact, the production of trimethyl ammonium iodide from some such side reactions is analogous to the experience of Engel (64). He attempted to methylate fructose methylphenylosazone in liquid ammonia by means of sodium and methyl iodide. Cleavage of the N-N linkages took place instead of methylation of the hydroxyl groups. The only crystalline product obtained was trimethylphenyl ammonium iodide, $(CH_3)_3C_6H_5NI$.

c. Methyl iodide on the sodium derivative. Three grams of the 4Br oxidized starch were allowed to stand for seven days in 80% ethyl alcohol which contained 21% sodium hydroxide. The sodium derivative thus formed had an orange-brown color and contained 37% sodium. On treating the dry sodium derivative with methyl iodide for several days, a product was obtained whose methoxyl content was only 1.85%.

Simultaneous experiments on raw starch showed that refluxing was necessary to form a true sodium derivative instead of the easily formed sodium hydroxide addition compound. Because of the coloration of the 4Br oxidized starch by sodium hydroxide even at room temperature it was deemed inadvisable to reflux the oxidized starch in sodium hydroxide solution.

d. Methyl iodide and silver oxide. Purdie's (246) reagents, viz. methyl iodide and silver oxide, were used on the silver salt of the 4Br oxidized starch in order to esterify the carboxyl groups and simultaneously methylate the hydroxyl groups.

(i) Preparation of the silver salt of the oxidized starch. 33.0 grams of the calcium salt of 4Br oxidized starch (5.02% Ca) were dissolved in the minimum amount of water (about 150 ml.) and stirred while a solution of 14.5 grams (slight excess) of silver nitrate in 8 ml. of water was added. The heavy precipitate was filtered with suction. Two volumes of alcohol were added to the filtrate to precipitate more of the silver salt. These two precipitates were combined, washed well with 67% alcohol, then with 95% alcohol and dried in vacuo. Yield 37.8 grams. Per cent of carbohydrate recovered, 96.4. Silver found: 20.4%; calculated: 22.2%.

(ii) Methylation procedure and simultaneous esterification. For methylation and esterification the dry silver salt was placed in a three-neck round bottom flask fitted with a reflux condenser and mercury seal stirrer. The top of the condenser was fitted with a calcium chloride tube. All cork stoppers were sealed with collodion. A large excess of methyl iodide was added to the flask, being five times the weight of silver salt used. An equal volume of anhydrous methyl alcohol was also added. If, at this point, the suspension was not fluid enough to be stirred easily more methyl alcohol was added.

Five mols of silver oxide (approximately five times the weight of the silver salt) were added in four portions at intervals of four to six hours as stirring was continued. A total of one day was generally allowed for each methylation. The excess methyl iodide and methyl alcohol were removed by distillation from a hot water bath. Hot methyl alcohol was then used to wash the methylated material from the silver residues, followed by filtration. On evaporation of the methyl alcoholic filtrate the methylated product was obtained.

The same methylation procedure was applied four successive times to the silver residues from the first methylation. The final silver residue was extracted overnight with anhydrous methyl alcohol in a Soxhlet extractor. The products from this extract and from the five methylations were combined for further methylation. The composite at this stage contained about 31% methoxyl and constituted a recovery of 73-74%. Two more methylations of the composite in the absence of methyl alcohol brought the methoxyl content to 35 or 38%. Continued methylation with methyl iodide and silver oxide increased the methoxyl content a small amount each time but did not appear to approach any limiting value. This is shown in Figure 11. This continued increase beyond the estimated value of 35% methoxyl for 4Br oxidized starch was not due to mechanical loss of material during methylation because, as Table IX shows, the increase in methoxyl content is not related to the percentage loss. Some other explanation must be sought for the steady increase of methoxyl content such as a chemical change during methylation which would liberate more hydroxyl groups for the next methylation.

For the hydrolyses described below samples of the methylated composite which had reached a methoxyl content of 33 to 35% were used because these were beyond the point where the slow increase in methoxyl content was noted. See Figure 11.

(iii) Hydrolysis of the methylated product. 9.7 grams of a methylated composite sample of 32.7% methoxyl was dissolved in 300 ml. of anhydrous methyl alcohol containing 2% dry hydrogen chloride. The solution was refluxed slowly in a soil flask fitted with a ground glass condenser closed by a calcium chloride tube. After 32 hours the optical rotatory

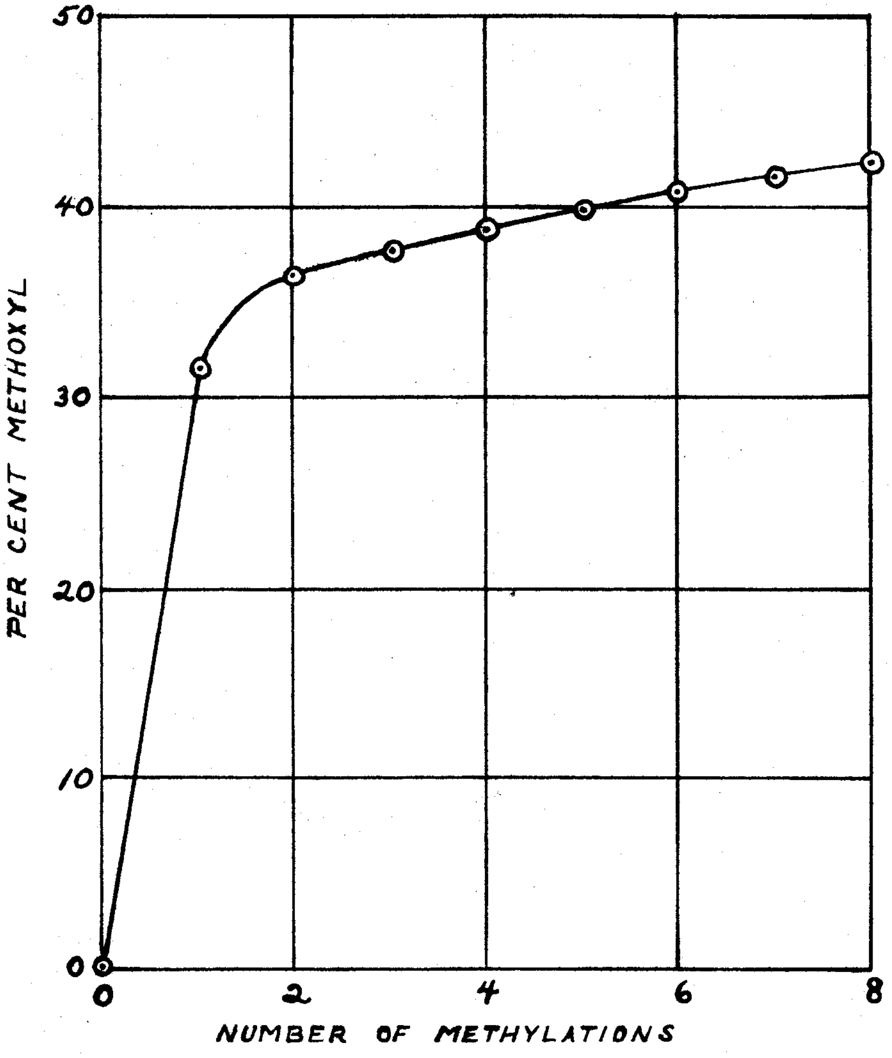


FIG. 11. METHYLATION OF 4Br OXIDIZED STARCH

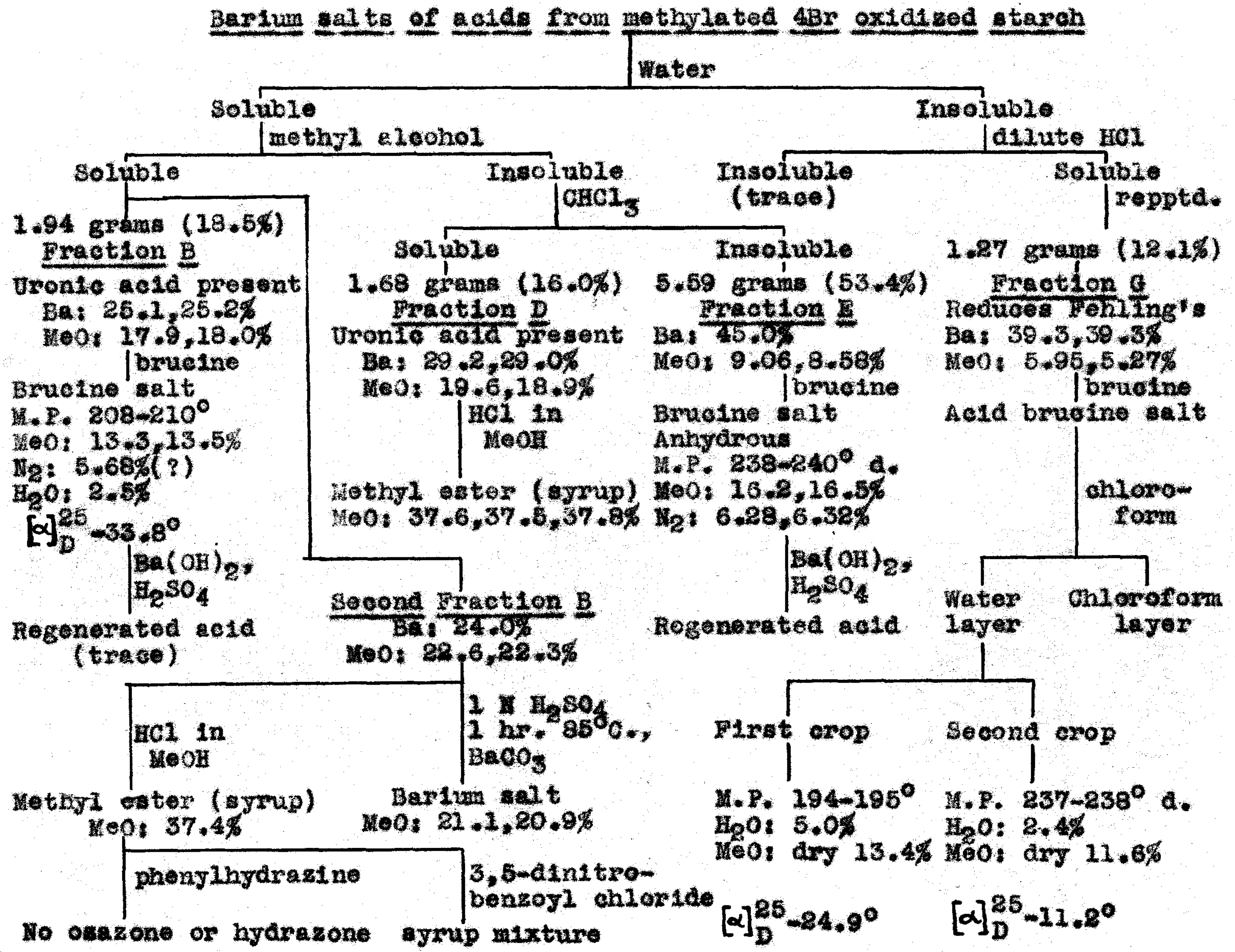
Table IX. Methoxyl Content and Loss During Methylation of 4Br Oxidized Starch

Number of methylations	Methoxyl content %	Increase of methoxyl %	Loss %
1	31.5	-	-
2	36.3	4.8	7.5
3	37.8	1.5	7.6
4	38.8	1.0	1.7
5	39.8	1.0	7.0
6	40.7	0.9	5.3
7	41.5	0.8	3.3
8	42.1	0.6	1.5

power was constant. The solution was neutralized with silver carbonate and evaporated to dryness in vacuo at 40°C. The residue was converted to barium salts of carbohydrate acids by heating in 0.1N barium hydroxide solution at 60°C until the titer of the solution was constant. The water insoluble barium salts were filtered off, carbon dioxide was passed into the filtrate until neutral, and the resulting barium carbonate removed by filtration. The aqueous filtrate containing water-soluble barium salts was evaporated to dryness at 60°C in vacuo and fractionated in the manner described below. Total recovery of water-soluble and water-insoluble barium salts was 11.4 grams.

(iv) Fractionation of the hydrolysate. A diagram of the fractionation is shown on the following page. The water-insoluble barium salts were dissolved in dilute

Diagram of Fractionation



hydrochloric acid, filtered, and reprecipitated by adding sodium hydroxide solution just to neutrality. The barium salts were filtered off, washed until free from chlorides and dried. Yield 1.27 grams of Fraction G(12.1%).

The water-soluble barium salts were exhaustively extracted in a Soxhlet extractor with anhydrous methyl alcohol. 1.94 grams of Fraction B(18.5%) were recovered from the methyl alcohol extract. Exhaustive chloroform extraction of the residue insoluble in methyl alcohol removed 1.68 grams of Fraction D (16.0%). The remainder, insoluble in methyl alcohol or chloroform, constituted Fraction E of 5.59 grams (53.4%). All fractions gave negative tests for keto-hexoses and gave no osazones.

<u>Fraction B</u>, soluble in methyl alcohol, gave a fairly good test for uronic acids. From two fractionations the data were:

Found, Ba: 25.1, 25.2%; MeO: 17.9, 18.0%.
Ba: 24.0%; MeO: 22.6, 22.3%.

Calcd. for Barium dimethylglucuronate,
Ba: 25.0%; MeO: 22.6%.

Calcd. for Barium dimethyl ketoglucuronate,
Ba: 23.9%; MeO: 21.5%.

The analyses indicate a methylated monobasic acid of a hexose.

This fraction was converted to a brucine derivative by quantitative removal of the barium with sulfuric acid and treatment of the aqueous filtrate with a slight excess of brucine according to the usual procedure. The brucine derivative was purified by recrystallization from 95% alcohol until colorless, then once from water. M.P. 208-210°. $[\alpha]_D^{25}$ -33.8°.

Found, MeO: 13.3, 13.5, 13.4%; N_2: 5.68%(?); H_2O: 2.5%.

The nitrogen and methoxyl values are not equivalent and give no clue about the identity of the derivative.

The barium salt was regenerated by heating an aqueous solution of the brucine derivative with barium hydroxide for three hours. After extracting the brucine with chloroform, removing the barium with sulfuric acid and evaporating the solution to dryness only a tract of organic acid was recovered.

The portion of the original barium salt with a methoxyl content of 22.6, 22.3% was converted to the methyl ester by refluxing for 8 hours in methyl alcohol containing 2.5% hydrogen chloride (285). A syrup was isolated by chloroform extraction.

Found, MeO: 36.8, 37.4%.
Calcd. for Dimethyl methylglucuronate, MeO: 39.4%.

When the ester was reacted with phenylhydrazine, according to Smith (285), no phenylhydrazide or osazone crystallized out.

Smith's directions for the preparation of the p-nitrobenzoate were applied to the preparation of the 3,5-dinitrobenzoate of the above ester. A mixture resulted which gave no crystalline fractions.

When the barium salt of methoxyl content 22.6, 22.3% was heated for one hour at 85°C in 1N sulfuric acid, reconverted to the barium salt by means of barium carbonate and extracted by means of methyl alcohol, it was expected that one methoxyl group (glucosidic) would be lost. This was not the case.

Found, MeO: 20.9, 21.1%.

Therefore none of the methoxyl is present in glucosidic form. The analysis of the methyl ester and the failure to get a derivative with phenylhydrazine all point to the absence of an aldehydic group.

Fraction D, soluble in chloroform but insoluble in methyl alcohol, gave a good test for uronic acid and gave reduction of Fehling's solution only after heating in acid. This last indicated a glucoside. The analyses indicated a methylated monobasic acid of a hexose.

Found, Ba: 29.2, 29.0%; MeO: 19.6, 18.9%.
Calcd. for Barium dimethyl glucuronate,
Ba: 25.0%; MeO: 22.6%.

Conversion of the barium salt to the methyl ester by means of hydrogen chloride in methyl alcohol gave a syrup.

Found, MeO: 37.6, 37.5%.
Calcd. MeO: 39.4% for dimethyl methyl glucuronate.

Fraction E, insoluble in methyl alcohol or chloroform, gave no test for uronic acid or reducing groups. The analyses point toward dibasic acids of fewer than six carbon atoms.

Found, Ba: 45.0%; MeO: 9.06, 8.58%.
Calcd. for Monomethyl barium glutarate,
Ba: 41.6%; MeO: 9.4%.
Calcd. for Monomethyl barium tartrate,
Ba: 45.8%; MeO: 10.36%.

Attempts to form an amide according to the Hirst and Jones (159) prodecure gave non-crystallizing syrups.

A brucine salt was formed, recrystallized from alcohol, and found to melt with decomposition at 238-240°C.

Found, MeO: 16.2, 16.5%; N_2: 6.28, 6.32%.
Calcd. for Di-brucine monomethyl tartrate,
MeO: 16.3%; N_2: 5.89%.

On regenerating the acid from the brucine derivative a low yield of acid was obtained which could not be crystallized. Treatment with various solvents showed that the acid was originally a mixture or formed a mixture of acids during regeneration from the brucine salt.

Fraction G, insoluble in water but soluble in acids, reduced Fehling's solution strongly. The analyses point toward a mixture of dibasic acids of fewer than six carbon atoms, at least one of which is methylated.

Found, Ba: 39.3, 39.3%; MeO: 5.95, 5.27%.

An acid brucine salt (monobrucine salt) was prepared and separated by extraction of the aqueous solution with chloroform into two fractions. From the aqueous layer were crystallized two brucine derivatives, which were purified by several recrystallizations from water and analyzed.

First crop, M.P. 194-195°C; $[\alpha]_D^{25}$ -24.9°.
Found, H_2O: 5.0%; MeO: 12.7% (dry 13.4).

Second crop, M.P. 237-238° (with decomposition);
$[\alpha]_D^{25}$ -11.2°.

Found, H_2O: 2.4%; MeO: 11.3% (dry 11.6).

Because the latter of these agreed in methoxyl content with that of a monobrucine tartrate (11.4%) an acid brucine salt of d-tartaric acid was prepared. This derivetive is not reported in the literature.

Found, M.P. 247-248° (with decomp.); $[\alpha]_D^{25}$ -24°.

Therefore neither of the above salts is monobrucine d-tartrate.

IV. DISCUSSION

The electrolytic oxidation process developed in this work was found to be very useful for studying in the laboratory the various physical and chemical changes taking place during the oxidation of raw starch. There are certain advantages in the use of the electrolytic process over the chlorine and caustic soda process as used in the starch industry. A more exact control of the amount of active oxidizing agent produced can be made in the electrolytic process because of the ease with which the current may be controlled and measured. Purer products may be obtained and with better recovery because of the smaller amount of salt to be washed free from the electrolytically oxidized starch.

It is believed that loss of starch upon oxidation is due in part to removal of soluble oxidized fragments during the washing operation. Although swelling experiments showed that the oxidizing agent penetrates deeply into the starch granule it seems that oxidation is localized to some extent in the regions between starch crystallites instead of occurring over all of the starch substance simultaneously. Localized attack of this nature produces visible radial fissures on the granules as was shown by the photomicrographs. It appears from the chemical evidence that once oxidation has begun on a certain portion of the starch molecule oxidation continues on that same portion to produce highly degraded acidic fragments. These acidic fragments become soluble in the alkaline medium and are, for the most part, washed out of the oxidized starch product. The evidence for this view other than the percentage recovery comes from quantitative determinations of birefringence and of chemical change during the oxidation of raw starch. The quantitative birefringence of starch granules was unchanged by oxidation, a fact which indicates that the gross crystalline structure was not altered. No increase in reducing power was noted until the oxidized starch had become quite soluble in hot water. There was likewise no increase in uronic acid content over the 1.5 to 1.65% found in raw starch. Whistler, Martin and Harris (325) similarly removed pectic substances from cellulosic materials by boiling in 1% sodium hydroxide and found no uronic acid in the resulting celluloses.

When an attempt is made to apply the electrolytic process to oxidation on a commercial scale several problems are presented. It seems probable that for commercialization a substitute electrode would have to be found because of the cost of platinum. This substitute electrode must withstand the oxidizing effect of the chlorine and hypochlorite and operate at a high current density in order to decrease the total electrode surface. The cost of electricity would be another deciding factor in the commercial feasibility of the process because by far the largest item is the cost of the current.

A series of electrolytically oxidized starches produced by the use of sodium hypochlorite in amounts varying from 0.025 to 0.5 equivalent of chlorine per glucose anhydride unit (1/2 to 11% chlorine) was examined by various physical and chemical measurements. It was at once noted that methods were lacking for characterizing slightly modified starches and for following the modifications of starch. This led to the development of several improved methods for following the modification of starch, viz., the use of turbidity, the susceptibility to digestion by soybean beta-amylase, the loss of birefringence and a rapid method for determining reducing power. Several other methods of characterization developed recently in this laboratory by Brimhall and Hixon (25,161) were also employed, viz., those for determining hot viscosity, rigidity, gel strength and volume of centrifuged sediment.

Because the existing methods for determining the reducing power of slightly solubilized starch products were either of questionable accuracy or time consuming, there was developed a rapid method for determining the reducing power of starch products. This method consists in heating the starch sample in ferricyanide solution, acidifying and titrating the reduced iron with ceric sulfate directly. Many of the difficulties of other methods have been eliminated and the time for a determination has been shortened to about twenty minutes from the three-hour period used by Richardson, Higginbotham and Farrow (259) whose values for starches the present method checks remarkably well. This simplified determination of reducing power has been used not only to follow the acid and alkaline conversion of starch, e.g., on the thin-boiling starches, on the Gore (105) starches, and on the dextrins formed by acid and alkaline catalyst, but also to follow some of the chemical changes taking place during oxidation.

During the first stages of electrolytic oxidation of raw starch no increase in reducing power is experienced; often a slight decrease results. Presumably both of these effects are due to the oxidation of reducing groups to acid groups. Any terminal aldehydic groups in the presence of alkaline hypochlorite would be expected to oxidize to carboxyl groups. Only when extensive degradation sets in to produce oxidized starches soluble in hot water to a clear solution does the reducing power increase. Since it seems improbable that any aldehydic groups could remain unoxidized in the presence of alkaline hypochlorite this reducing power is attributed to ketone groups produced by oxidation.

The susceptibility of the oxidized starches to digestion by soybean beta-amylase was measured by the method of Newton, Farley and Naylor (226). The total maltose equivalent producible from starch was gradually reduced as oxidation proceeded. For starch oxidized with 11% chlorine (0.5 equivalent) the yield of maltose was decreased by 15%. Oxidation obviously changes some of the glucose units of starch to structures which are not attacked by beta-amylase. In confirmation of this fact is the digestion of starch which was gelatinized previous to extensive oxidation by bromine. Bromine in the amount of 0.5 equivalent reduced the maltose yield by 25%; this maltose yield was gradually decreased as more oxidation took place until, with any quantity beyond two equivalents of bromine, no maltose could be produced from the product. Myrbäck (218) reported that oxidation of the reducing end-groups had no effect on amylolytic hydrolysis of starch but the quantity of iodine used for the oxidation (0.054 equivalent per glucose unit) was very small and just sufficient to destroy the reducing action of the starch. He concluded that the reducing groups were of no significance in the hydrolysis by amylases. The dextrin of Brown and Millar (29) was partially susceptible to diastatic hydrolysis even though the terminal glucose unit had been oxidized to a five-carbon acid. A larger amount of oxidation, e.g. such as is reported in this thesis, is apparently necessary to decrease the extent of enzymic digestion of starch. Oxidation to the extent which markedly decreases the digestibility of starch has been shown to cause deep-seated changes in many of the glucose units of the starch chain.

The change in susceptibility to beta-amylase digestion changes upon oxidation parallel to the change of the gelatinizing properties of the starch. These two effects were shown by

Newton, Farley and Naylor (226) to be correlated. The gelatinizing characteristics were measured microscopically by counting the per cent of granules showing the polarization cross. Oxidation lowered the gelatinization temperature and changed the rate at which the granules would gelatinize as the temperature was raised. The correlation of these two effects, viz., loss of birefringence and susceptibility to digestion by beta-amylase, may possibly be explained on the basis of the hydration capacity of the starch granules. Oxidation has caused the granules to become hydrated (and consequently susceptible to digestion by beta-amylase) at a lower temperature.

The sudden drop in hot viscosity resulting from a very small amount of oxidation is probably a large physical change due to a very small chemical change of the starch substance. The increase in hot viscosity beyond the minimum point is probably a hydration effect which reached a maximum at 0.2 equivalent (4.5%) of chlorine. Centrifugation tests on the starch pastes showed that the viscosity minimum and maximum was correlated with the minimal and maximal volume of the swollen granules. This was confirmed by microscopic observations. When more than 0.2 equivalent (4.5%) of chlorine was used the usual decrease in hot viscosity attendant upon most degradations of the starch molecule followed proportional to the amount of oxidation. This drop in viscosity was paralleled by a decrease in the volume of the swollen granules and by irregular swelling or disintegration of the granules followed by solution.

Oxidation produced a uniform decrease in the gel strength and rigidity of raw starch, the latter falling off first at a rapid rate, then at a much slower rate. The change in slope of the rigidity curve coincided with the hot viscosity maximum (at 4.5% chlorine). This is interpreted to mean that, while the hot viscosity (resistance to flow) increased presumably through hydration which allowed greater swelling, the rigidity (resistance to being deformed) of the starch was rapidly being destroyed by internal disintegration of each granule. By the time that degradation began to decrease the hot viscosity of the starch, most of the rigidity had already been destroyed. The internal disintegration beyond this point was so complete that the resulting particles in each granule did not swell noticeably but merely crumbled and separated prior to going into solution. Such complete internal disintegration is believed to be opposed to the formation of a strong and rigid starch gel.

For certain industrial applications starch pastes are desired which form clear pastes and which are also free from retrogradation or aging, i.e., their pastes are permanently clear (249). All of the electrolytically oxidized starches were low in turbidity, an important fact in connection with the above requirements. Only a very small amount of oxidation was necessary to decrease the turbidity of the starch paste by three-fourths of its original value. Also important is the fact that oxidation progressively decreases the extent of retrogradation as is known in practice and as was shown by the low yield of insoluble residue from amylolytic digestion of the electrolytically oxidized starches.

When the effect of heat, alkalinity, anodic current density, salt concentration and starch concentration upon the products of oxidation was studied the most significant results were obtained by varying the temperature and the alkalinity. Large changes in current density and salt concentration had no effect on the oxidation products. Change in starch concentration had an effect only because the total volume of solution was smaller, thus resulting in a higher temperature rise during oxidation. The added rise in temperature produced an oxidized starch which was much more soluble in hot water. Especially when the temperature attained during oxidation is near the gelatinization temperature of the starch is the effect on the oxidized product the most noticeable. The effect of heat on the oxidation product was further evidenced by a series of constant temperature oxidations at 45, 50 and 55°C. As the temperature was raised the oxidation products gave clearer pastes and greater solubility in hot water. This may be the result of a different type of oxidation at the higher temperature or merely of an increase in hydration attending oxidation. Some gelatinization resulted at 55°C, showing that the upper temperature limit for the oxidation of raw cornstarch was between 50 and 55°C.

Even more striking in bringing about extensive conversion of starch to clear and thin pastes with a given amount of oxidant was the addition of sodium hydroxide beyond 0.4%. Each increment of sodium hydroxide to 0.5 and 0.6% had a large effect on the viscosity and rigidity of the oxidized product. The starch oxidized in the presence of 0.6% sodium hydroxide had less than half as much viscosity and rigidity as that oxidized in the presence of 0.4% sodium hydroxide. 0.72% sodium hydroxide gelatinized the starch and indicated that the upper limit of alkalinity for the oxidation was between 0.6 and 0.72% sodium hydroxide. This large effect of sodium hydroxide on the course of the oxidation agrees with the well-known swelling action of sodium hydroxide on starch. Presumably the hydration capacity of the starch granules has been increased by each increment of sodium hydroxide. The result is that more of the starch substance is accessible to the oxidizing solution. The hydration capacity increase has evidently been so great that, during the swelling process, the granular structure is weakened and produces lower viscosity and lower rigidity.

As the second phase of this thesis the action of bromine in neutral solution upon gelatinized starch has been used to study the mechanism of the oxidation of starch. Previously Felton, Farley and Hixon (72) presented analytical evidence that some of the primary alcoholic groups of starch were oxidized to form uronic acid units, that some of the secondary alcoholic groups were oxidized to ketone groups and that some of the ketone and aldehydic groups were oxidized to non-uronic acids.

The presence of uronic acids has now been confirmed by hydrolysis of the oxidized starch and isolation of glucuronic acid and the large production of uronic acids whether glucuronic or keturonic (72) from oxidized starch parallels the similar isolation from oxidized cellulose by Kalb and Falkenhausen (174) and substantiates the work of the following who presented indicative evidence but not proof for the presence of uronic acid units in oxidized starch. Pauli and St. Szper (238) found that the dissociation constants of their soluble starches and dextrins were remarkably close to that of glucuronic acid. Mann, Krüger and Tollens (206) and Syniewski (306,307) obtained furfural from oxidized starch and oxidized dextrin, respectively, and reported a positive color test for uronic acids. The furfural yield of Syniewski was equivalent to 34% glucuronic anhydride. Everett and Sheppard (65) also reported color tests indicative of the presence of uronic acids.

The presence of ketone groups in the oxidized starch has been confirmed by the formation of an oxime of oxidized starch. The nitrogen content of the oxime of 4Br oxidized starch was equivalent to the presence of monoketone groups in 65 to 75% of the glucose units or of diketone groups in half that many units. It is assumed to be highly improbable that any aldehydic groups could remain unoxidized in the presence of bromine. According to this view all of the oxime nitrogen represents ketone groups.

The production of non-uronic acids has been confirmed by the separation from the hydrolysate of mixtures of barium salts of dibasic acids of less than six carbon atoms. Attempts to crystallize or derivatize the dibasic acids have as yet been unsuccessful. The presence of dibasic acids in the hydrolysate is in agreement with the instability of the keto-acids present in the oxidized starch, which are presumed by oxidative rupture to produce dibasic acids of less than six carbon atoms.

There is a great difference between the products of the two types of oxidation, that from raw starch and that from gelatinized starch. This is best shown by the chemical analyses. The two series overlap at one point, viz., where 0.5 equivalent of halogen was used (chlorine and bromine respectively). The reducing values at that point were 65 and 245 milligrams of copper per gram of oxidized starch from the raw and gelatinized starches, respectively. The uronic acid equivalents of the respective types were 1.5 and 10.8% respectively. The reducing power and uronic acid equivalent alone show the great dissimilarity between the two types of oxidation and bear out the theory that in the oxidation of raw starch the oxidized fragments are solubilized by alkali and removed by washing. The above product from gelatinized starch was completely soluble in cold water and had to be precipitated from solution by alcohol. It possessed, of course, no rigidity whereas the product from raw starch still exhibited a measureable rigidity and was insoluble in cold water.

The later work of this thesis was directed toward the discovery of methods of stabilization of the labile 4Br oxidized starch followed by hydrolysis and fractionation to confirm by actual isolation and identification the presence of keturonic acids and other degradational acids which have been concluded in the preceding discussion to be present.

Hydrolysis of the 4Br oxidized starch by enzymes was found to be impossible. The starch had been changed by oxidation to structures which were non-digestible by any of the enzymes tried. Hydrolysis in methyl alcoholic hydrogen chloride, mercaptalation and hydrolysis by ethyl mercaptan in fuming hydrochloric acid, and acetylation of the oxidized starch followed by hydrolysis in acetic acid containing hydrobromic acid all attested to the extreme instability of the oxidized starch in acid solutions and were in agreement with the well-known decomposition of uronic and keturonic acids in acid media (53, 195,200).

Stabilization of the oxidized starch molecule prior to hydrolysis was sought by several means, viz., reduction of the ketone groups, preparation of the methyl ester of the carboxyl groups and formation of an oxime on the ketone groups. Reduction by hydrogen over a platinum oxide catalyst was impractical in that it gave only partial hydrogenation. The yield of methyl ester was too low to encourage the use of this derivative. An oxime was readily formed and offered a certain amount of stability during hydrolysis. A successful methylation procedure was developed meanwhile and was to be preferred because it would label the free hydroxyl groups of the oxidized starch, thereby giving much more evidence about its structure.

Methylation of the oxidized starch was carried out by dimethyl sulfate and alkali, by sodium and methyl iodide in liquid ammonia, by methyl iodide on the sodium derivative of the oxidized starch, but was most successfully effected by an anhydrous procedure which used Purdie's (246) reagents (silver oxide and methyl iodide) on the silver salt of the oxidized starch. This procedure esterified the carboxyl groups and simultaneously methylated the hydroxyl groups.

Upon hydrolysis and fractionation there were obtained barium salts of methylated monobasic acids of hexoses which gave positive tests for uronic acids along with barium salts of methylated and unmethylated dibasic acids of less than six carbon atoms. It has further been shown that crystalline brucine derivatives of these fractions can be prepared.

On the one hand there have been surmounted the difficulties due to instability of the oxidized starch and its hydrolytic products in acid solutions. Uronic and keto-acids contributed chiefly to this instability. On the other hand the difficulties encountered in aqueous alkali have been avoided by anhydrous procedures. On theoretical grounds it is not at first expected that alkali would have such a degrading effect on starch products. Experiments in this laboratory showed very little increase in the reducing power of starch in cold alkali whereas hot alkali produced a large progressive increase in reducing power of the starch, indicating hydrolysis by the alkali. Furthermore it is common experience in methylations by sodium hydroxide and dimethyl sulfate that disaggregation of starch to particles of smaller molecular weight takes place in the later stages of methylation. Partial methylation, as employed by Staudinger and Husemann (292), does not show this decrease in molecular weight. Staudinger and Husemann's conclusions should, therefore, not be applied to fully methylated starches. It has already been mentioned that methylation in dimethyl sulfate and alkali destroyed the pectic acid in apple pectin (158). The 4Br oxidized starch discussed in this thesis resembles a pectic acid very closely and possesses almost identical solubilities before and after methylation. The success of methylation in anhydrous media and the lack of it in alkaline media are not surprising in view of the above statements.

V. SUMMARY AND CONCLUSIONS

1. An electrolytic oxidation process has been developed for oxidizing one-pound samples of raw starch by sodium hypochlorite. The method is convenient for studying the factors which influence the properties of starch oxidized by chlorine in alkaline solution.

2. The feasibility of industrial application of the electrolytic process is discussed and found to depend upon securing low cost electric power and a suitable anode to replace the platinum used in the laboratory. Graphite anodes were found to be unsatisfactory.

3. An improved and rapid ferricyanide method for determining the reducing power of starches and dextrins has been developed. In this method the reduced iron is measured directly by a ceric sulfate titration. For starches hydrolyzed by hot or cold acid or oxidized by alkaline hypochlorite, for raw starches and dextrins, the values for reducing power by this method parallel those determined by the longer procedure of Richardson, Higginbotham and Farrow (259). The time for a determination has been shortened from three hours to twenty minutes. Many other modified starches such as the chlorinated and thin-boiling types have been measured.

4. Tables and graphs have been presented to show the

various changes which raw cornstarch undergoes during oxidation. These changes were followed by means of rigidity, gel strength, viscosity, turbidity, volume of centrifuged sediment, reducing power, enzymic digestion and loss of birefringence. Improvements in several of these techniques, especially in the determination of reducing power, enzymic digestion and loss of birefringence, constitute a portion of the thesis.

5. On the basis of swelling phenomena microscopically observed and recorded on photomicrographs, from the quantitative determination of birefringence and from the correlation between viscosity and the volume of the swollen granules a mechanical theory of the action of the oxidizing agent on starch granules has been presented. This theory agrees with the observed changes in viscosity, rigidity and gel strength of the oxidized starches.

6. The influence of various factors such as heat, alkalinity, anodic current density and salt concentration on the properties of electrolytically oxidized cornstarch and on the course of the oxidation was studied. Salt concentration and anodic current density had no effect whereas heat and alkalinity had a marked effect. The upper temperature limit for electrolytic oxidation of cornstarch was between 50 and 55°C. The upper limit of alkalinity was between 0.6 and 0.72% sodium hydroxide.

7. The oxidation of primary alcoholic groups of gelatinized starch to uronic acid groups by bromine in neutral solution was indicated by earlier analytical studies (72) and has now been confirmed by the isolation of glucuronic acid as the cinchonine salt. This was the first isolation of glucuronic acid from a starch product.

8. The oxidation of secondary alcoholic groups of gelatinized starch to ketone groups (72) has been confirmed by the preparation of an oxime of the oxidized starch. The nitrogen content of the oxime was equivalent to the presence of one ketone group in each of 65 to 75% of the glucose anhydride units.

9. The acid regenerated from the oxidized starch was shown by discoloration and evolution of gas due to autodecomposition to be quite unstable. Mono- or di- ketone units would explain such instability and could be further oxidized to non-uronic acids. The separation of mixtures of barium salts of dibasic acids of less than six carbon atoms confirmed the expected degradation (72) of keto-acid units to non-uronic acids during oxidation.

10. In order to stabilize the starch extensively oxidized by bromine, methylation was carried out by an anhydrous procedure using methyl iodide and silver oxide on the silver salt of the oxidized starch. There resulted simultaneous methylation of the hydroxyl groups and esterification of the carboxyl groups.

11. The methylated product was hydrolyzed and fractionated. Analyses of the fractions indicated the presence of methylated monobasic acids of hexoses which gave positive tests for uronic acids and of methylated and unmethylated dibasic acids of less than six carbon atoms.

12. Crystalline brucine derivatives of these acids were prepared from the various fractions. Analyses are reported which indicate the general character of the acids although identification was limited to the glucuronic acid.

13. Monobrucine d-tartrate has been prepared.

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