

The Nitrogen Dioxide Oxidation of Starch*

J. W. MENCH, with ED F. DEGERING, Purdue University

Although the literature contains over two hundred references to the oxidation of starch, little is known concerning the structures of the products because various oxidants may simultaneously attack one or more of the hydroxyl groups to produce a complicated structure containing carboxyl, aldehyde, and ketone groupings. An exception appears to result when periodic acid is used, since this oxidant seemingly exhibits a specificity towards parts of the carbohydrate molecule containing adjacent, secondary hydroxyl groupings.^{1,2} The observations of Unruh, Yec-
kel, and Kenyon,^{3,4} that cellulose can be oxidized with nitrogen dioxide to a polyanhydroglucuronic acid, indicate that this reagent preferentially attacks the primary hydroxyl groups in certain glycosides.

We have attempted to apply to starch both the static and cyclic method of oxidation reported by these men, in an effort to produce the *alpha*-linked polyanhydroglucuronic acids. However, the tendency of the powdered carbohydrate to pack made these procedures unsatisfactory, since the reactions were difficult to control and at least partial hydrolysis of the starch frequently occurred.

We have found, however, that under suitable conditions, starch may be oxidized by nitrogen dioxide to produce a new type of oxidized starch containing, predominately, uronic acid residues. This point was confirmed, qualitatively, by a positive *beta*-naphthol test,⁵ which indicated the presence of glucuronic acid, although attempts to hydrolyze the products to glucuronic acid, and to prepare the cinchonine salt or oxime of this compound were without success.

The procedure and diluent utilized in this work was suggested by the work of Maurer and Drefahl,⁶ who oxidized galactose, methyl glucoside, and methyl galactoside with nitrogen dioxide in carbon tetrachloride or chloroform—obtaining mucic acid and the corresponding substituted uronic acids, respectively. The method of oxidation is readily controlled so that oxidized starches of any desired carboxyl contents can be produced.

In marked contrast to the behavior of untreated starch, these products, even those with low carboxyl contents, do not dissolve in hot water. Since these products, presumably, exist as the free acids or as lactones, this would indicate that the carboxyl group is less water-solubilizing than is the primary hydroxyl grouping or considerable lactone formation has occurred, although nitrogen dioxide-oxidized celluloses show enhanced water-susceptibility with increasing carboxyl contents.⁴ On the other hand, samples containing even small amounts of carboxyl groupings are

* This is an abstract of a portion of a thesis submitted by J. W. Mench to the Faculty of Purdue University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1944. American Maize-Products Co. fellow 1941-44. Present address, Eastman Kodak Company, Rochester, New York.

completely soluble in dilute, aqueous alkalis, and concentrated solutions of the alkali metal salts of these products do not retrograde upon cooling as do dispersions of untreated starch.

Preliminary work has indicated that some of the salts of these polyanhydroglucuronic acids might find application in the replacement of certain other modified starches and polyanhydrouronic acids as thickeners, gel-formers, dispersants, and stabilizers. The free acids have possible use as acidulants, dispersants, and disintegrates in certain food and medicinal products, and as a source of glucuronic acid.

Glucuronic acid, in turn, is a useful intermediate for the preparation of saccharic acid by the further oxidation with halides, electrolytically-generated halogens, or chlorous acids.⁷ The production of *D-arabo*-trihydroxyglutaric acid from this intermediate is also feasible.⁸ A further commercial application of glucuronic acid would be in the synthesis of ascorbic acid by methods analogous to those recently divulged by Regna and Caldwell⁹ and Isbell.¹⁰

Experimental

All of the oxidations were conducted on a commercial grade of air-dried corn starch¹¹ which was given an additional 24 hour drying treatment in a vacuum oven at 55°C. and at a pressure of 10 mm. of Hg before use. The nitrogen dioxide employed was a commercial, Du Pont product, and the term refers to the equilibrium mixture of nitrogen dioxide and nitrogen tetroxide.

The apparatus consisted of a 250 ml. three-neck flask fitted with an efficient, rapid stirrer and a six-inch, spiral type reflux condenser. In all cases, 16.5 g. of starch was oxidized, which is equivalent to 0.1 mole when the residual moisture is taken into account. The diluent selected for the reaction was carbon tetrachloride, and it was usually used in the amount of 150 ml., although smaller amounts were also tested in order to determine the effect of higher initial concentrations of the oxidant. The nitrogen dioxide addition was facilitated by the use of a water-jacketed burette, the weight of the oxidant being calculated from density and temperature data.

All reactions were run at room temperature (25° to 29°C.), and after the desired time of oxidation, the products were filtered off on a Pyrex fritted-glass funnel, washed with carbon tetrachloride, and the excess solvent allowed to evaporate into the atmosphere. The oxidation products were then washed thoroughly with distilled water until the washings were free of acidity, followed by successive washings with acetone and ether. The latter solvent was allowed to evaporate, and the products were then given a more thorough drying over phosphorus pentoxide in a vacuum desiccator. The washing treatment just described completely removes all soluble materials such as excess oxidant or nitric acid, glucose, dextrans, or the organic acids which might be present because of partial hydrolysis and oxidation of the hydrolysis products of the starch.

The weight of oxidized starch obtained was always at least as great as the weight of starch used, and in the more highly oxidized samples, was usually somewhat greater.

Analysis of the Products:

The carboxyl contents of the products were determined by titration with standard alkali, as suggested by Unruh and Kenyon.³ Although this is not as accurate as the method involving carbon dioxide evolution resulting from decarboxylation of the uronic acid residues, it was felt to be sufficiently accurate for comparison purposes. On oxidized celluloses, the method gives somewhat lower values than the carbon dioxide evolution method, especially at low carboxyl values, although it approaches the true value at the theoretical 25.5 per cent carboxyl content.

The method as used consisted in weighing 1 g. samples of the dried, oxidized starches into 250 ml. Erlenmeyer flasks, adding 30 ml. of 1 to 2 pyridine-water mixture, and heating on a steam bath until the samples were substantially in solution. The sides of the flasks were then washed down with 30 ml. of distilled water, and the contents titrated to a phenolphthalein end point with N/2 sodium hydroxide. The values obtained were corrected for by a blank titration on the solvent mixture.

Data:

Pertinent data and the results obtained are summarized in Table 1, and Figures 1, 2, 3, and 4 illustrate the relationships existing between

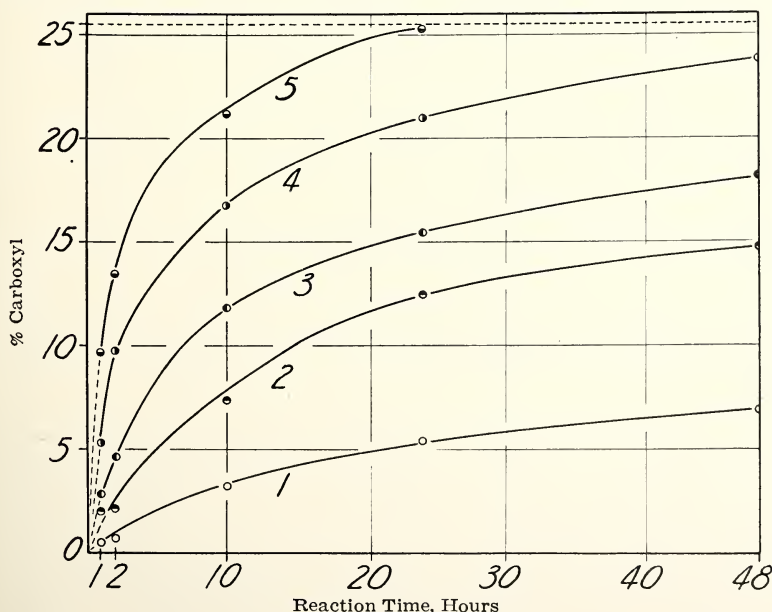


Fig. 1. The Relationship of Carboxyl Contents to the Time of Reaction at Various Ratios of Nitrogen Dioxide to Starch. Legend: Molar Ratio of Nitrogen Dioxide to Starch, 1. 1 to 1; 2. 2 to 1; 3. 3 to 1; 4. 5 to 1; and 6. 10 to 1.

the carboxyl contents of the products and some of the factors influencing the same.

Discussion:

A study of the data given in Table 1 and Figures 1 to 4 indicates that the reaction between nitrogen dioxide and starch is influenced by at least several factors:

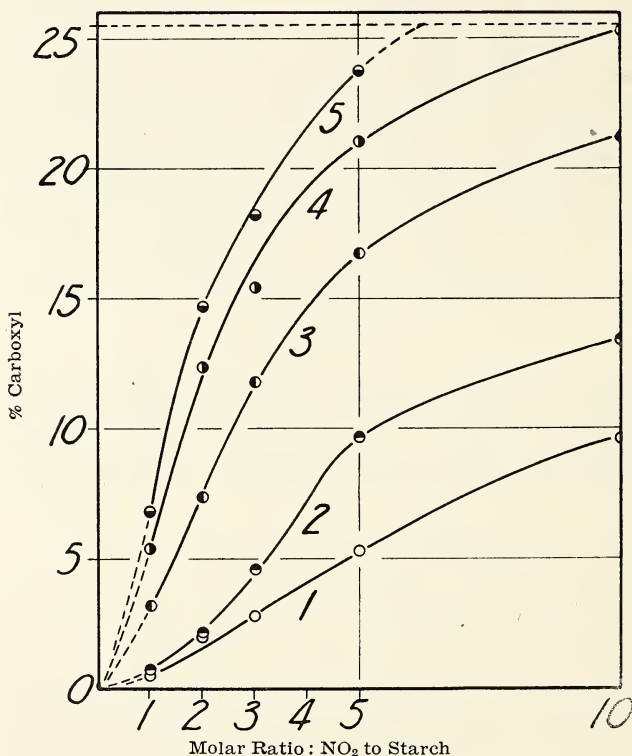


Fig. 2. The Relationship of Carboxyl Contents to the Ratio of Nitrogen Dioxide to Starch at Various Reaction Times. Legend: Reaction Time, 1. 1 hr.; 2. 2 hrs.; 3. 10 hrs.; 4. 24 hrs.; and 5. 48 hrs.

1. At a given molar ratio of oxidant to starch, the carboxyl content of the product is a function of the time of oxidation, increasing with the length of time of reaction. As shown in Fig. 1, the nitrogen dioxide rapidly oxidizes the starch in the early stages of the reaction, and then oxidation slows considerably. This may be due to loss of a portion of the oxidant through the reflux condenser and stirrer bearings over a long period of time, although the same effect was observed by Yackel and Kenyon⁴ in their static cellulose oxidations, which were conducted in sealed containers.

2. At a given time of oxidation, the carboxyl content of the product is a function of the molar ratio of oxidant to starch, increasing as the

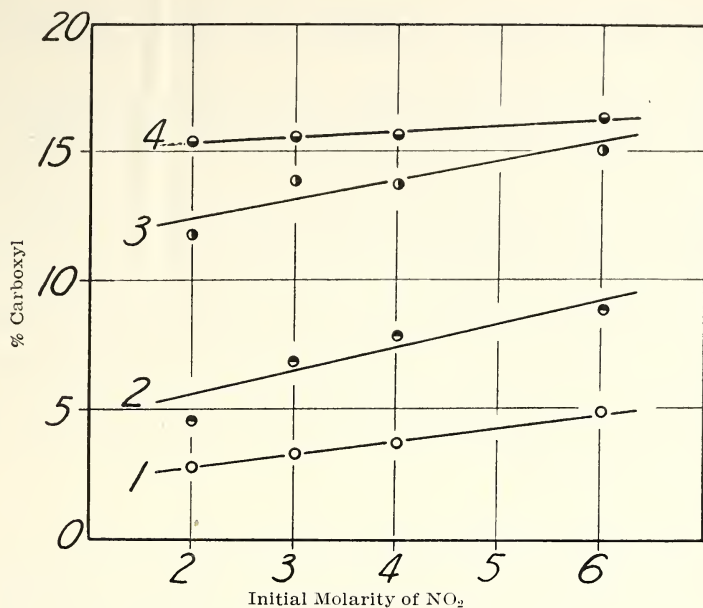


Fig. 3. The Relationship of Carboxyl Content to the Initial Molarity of Nitrogen Dioxide at Various Reaction Times. Legend: Reaction Time, 1. 1 hr.; 2. 2 hrs.; 3. 10 hrs.; and 4. 24 hrs.

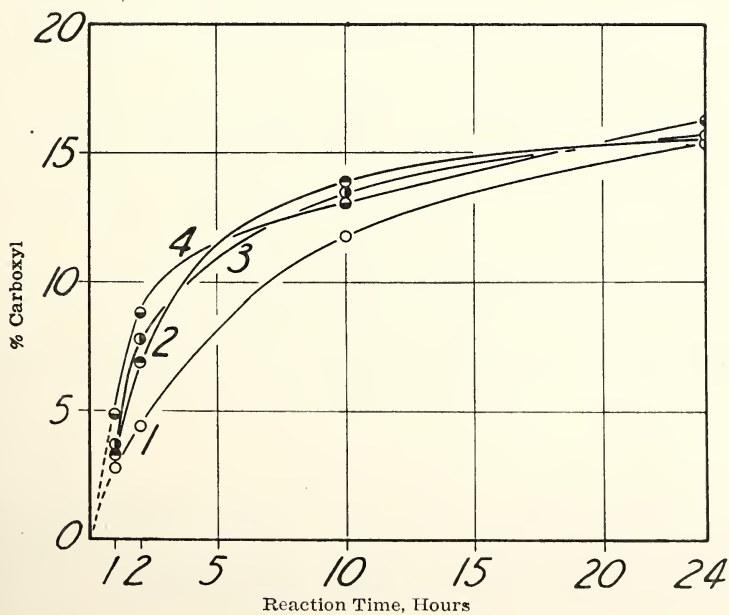


Fig. 4. The Relationship of Carboxyl Contents to the Time of Reaction at Various Initial Molarities of Nitrogen Dioxide. Legend: Initial Molarity of NO_2 , 1. 2 M; 2. 3 M; 3. 4 M, and 4. 6 M.

molar ratio of the oxidant is increased. This effect is illustrated in Fig. 2.

It was thought that an increase in the initial concentration, or molarity, of the nitrogen dioxide might have the effect of increasing the carboxyl content without the use of large excesses of oxidant. This was tested by arbitrarily choosing an oxidant to starch ratio of 3 to 1, which is somewhat over the theoretical ratio of 2 to 1 required for the conversion of a primary hydroxyl group to carboxyl, and decreasing the amounts of carbon tetrachloride used as diluent. The results of these experiments, however, (cf. Fig. 3) indicate that at a given molar ratio (3 to 1) of oxidant to starch, and at a given time of oxidation, the carboxyl content of the product increases only slightly with an increase in the initial concentration of the oxidant. This effect is further illustrated in Fig. 4, in which carboxyl content has been plotted against the time of reaction at constant values of initial oxidant molarity. These curves almost coincide, with the exception of the one run at the lowest concentration (2.0 molar).

Properties of the Products:

One of the most surprising properties of these oxidized starches, even those with comparatively low carboxyl contents, is their insolubility in hot water. This is in marked contrast to the behavior of untreated starch, which is easily dispersed at elevated temperatures. All of the products, however, dissolve readily, when warmed, in dilute bases such as 2 per cent sodium hydroxide, 2 per cent sodium carbonate, dilute ammonium hydroxide, aqueous solutions of quaternary ammonium hydroxides such as Triton B, and aqueous pyridine. They are also soluble in 5 per cent sodium bicarbonate, liberating carbon dioxide from the solution. Unlike untreated starch, these products do not retrograde when fairly concentrated solutions containing their salts are cooled. The free salts may be obtained by precipitation of their aqueous solutions with ethanol or other water-miscible solvents, but the free acids are not easily regenerated by rendering the solutions acidic. This suggests that the uronic acid residues are hydrolyzed with ease.

As is the case with oxidized celluloses,⁴ salts of other metals may be readily prepared by treatment of the oxidized starches with aqueous solutions of the metal acetates, since the polyanhydroglucuronic acid is strong enough to displace acetic acid from its salts. Polyvalent cations, as in the case of oxidized celluloses, yield water-insoluble salts.

Acknowledgment

The authors wish to express their sincere appreciation to the American Maize-Products Company and to its Director of Research, Dr. H. H. Schopmeyer, for the generous provision of funds, in the form of a fellowship, which made this and other research possible.

Summary

1. A method for the oxidation of starch with nitrogen dioxide is described, which results in the production of *alpha*-linked polyanhydroglucuronic acids. The oxidation is readily controlled to yield products of any desired carboxyl content.
2. The carboxyl content of the product is dependent, mainly, on the time of oxidation and the molar ratio of oxidant to starch, whereas the initial concentration of the nitrogen dioxide seems of less importance.
3. Methods of preparation of various salts of the oxidized starches are described.
4. The properties of the oxidized starches, and of their salts, have been described, and uses based on these are suggested.

Table 1. The Oxidation of Starch with Nitrogen Dioxide

Run	Molar ratio: NO ₂ /starch	MI of CCl ₄	Initial Molarity of NO ₂	Time of Oxidation (hours)	Yield of washed product (grams)	% COOH
1	1	150	0.67	1	16.20	0.5
2	1	150	0.67	2	16.60	0.7
3	1	150	0.67	10	16.90	3.2
4	1	150	0.67	24	17.40	5.4
5	1	150	0.67	48	17.72	6.9
6	2	150	1.33	1	16.50	2.0
7	2	150	1.33	2	16.92	2.1
8	2	150	1.33	10	17.36	7.4
9	2	150	1.33	24	17.12	12.4
10	2	150	1.33	48	16.70	14.7
11	3	150	2.00	1	16.30	2.8
12	3	150	2.00	2	16.80	4.6
13	3	150	2.00	10	18.46	11.8
14	3	150	2.00	24	17.28	15.4
15	3	150	2.00	48	16.68	18.2
16	3	100	3.00	1	17.43	3.3
17	3	100	3.00	2	17.79	6.9
18	3	100	3.00	10	18.25	13.9
19	3	100	3.00	24	18.34	15.6

Run	Molar ratio: NO ₂ /starch	Ml of CCl ₄	Initial Molarity of NO ₂	Time of Oxidation (hours)	Yield of washed product (grams)	% COOH
20	3	75	4.00	1	17.90	3.7
21	3	75	4.00	2	17.68	7.8
22	3	75	4.00	10	19.56	13.7
23	3	75	4.00	24	18.34	15.7
24	3	50	6.00	1	18.40	4.9
25	3	50	6.00	2	18.23	8.8
26	3	50	6.00	10	18.38	15.1
27	3	50	6.00	24	18.45	16.3
28	5	150	3.33	1	17.36	5.3
29	5	150	3.33	2	17.87	9.7
30	5	150	3.33	10	19.12	16.7
31	5	150	3.33	24	18.59	21.0
32	5	150	3.33	48	17.72	23.8
33	10	150	6.67	1	18.15	9.6
34	10	150	6.67	2	18.60	13.4
35	10	150	6.67	10	19.13	21.2
36	10	150	6.67	24	18.85	25.3

Bibliography

1. Jackson and Hudson, J. Am. Chem. Soc., **59**:2049 (1937), **60**:989 (1938).
2. Grangaard, Gladding, and Purves, Paper Trade J., **115**:41 (1942).
3. Unruh and Kenyon, J. Am. Chem. Soc., **64**:127 (1942).
4. Yackel and Kenyon, J. Am. Chem. Soc., **64**:121 (1942).
5. Thomas, Bull. soc. chim. biol., **7**:102 (1925).
6. Maurer and Drefahl, Ber., **75B**:1489 (1942).
7. Isbell, U. S. 2,338,115 (Jan. 4, 1944).
8. Isbell, U. S. 2,338,114 (Jan. 4, 1944).
9. Regna and Caldwell, J. Am. Chem. Soc., **66**:243-50 (1944).
10. Isbell, Abstracts of the Cleveland Meeting of the A.C.S., 1944.
11. Supplied by the American Maize-Products Co., of Roby, Indiana.