

ACTION OF CHLORINE ON SEMIDRY STARCH¹

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ABSTRACT

Wheat starch of 13.2% moisture is oxidized with chlorine gas at starch-to-chlorine molar ratios of 1:1 and 1:3 in the dark and under 500 ft.-candles of light. Aldehyde, carboxyl, and total carbonyl groups increase with the extent of oxidation. Solubility increases and viscosity decreases as a result of depolymerization, which is shown to result from oxidative as well as hydrolytic cleavage of D-glucosidic bonds. The major sugar derivative found is D-gluconic acid, but small amounts of D-glucuronic acid and D-glucosone are also produced. Fragments seem to be extensively oxidized, since the only other oxidation products observed are oxalic acid and carbon dioxide. The oxidation differs from that in an aqueous solution in that no observed cleavage occurs between carbon atoms C2 and C3.

An understanding of the action of chlorine gas on wheat starch would aid in interpreting the chlorine bleaching of flour. In particular, it is desirable to know more about the rate of formation of oxidized groups and about the nature of the products formed. Uchino and Whistler (1) showed that semidry wheat starch is depolymerized by chlorine and that the reaction is very sensitive to photochemical catalysis. They also showed that the principal oxidation product is D-gluconic acid and that little oxidation occurred at carbons C2 and C3, since no cleavage products from this reaction were observed. The oxidation of semidry starch is thus different from that which occurs in aqueous starch slurry (2).

The present work was undertaken to obtain more detailed information on the rate of oxidation and on the nature of the products

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formed in both dark and photoactivated reactions. Oxidations were followed over an extended period of time in order to find and identify products that might be produced in very small amounts.

Materials and Methods

The wheat starch used was kindly supplied by Ogilvie Flour Mills Co., Ltd., Montreal, Canada. It was defatted by extraction with 85% methanol (3), air-dried for 4 days, sieved through a 200-mesh screen, and stored in a glass container. It contained 13.2% moisture. Other analysis calculated on dry weight basis showed 0.01% ash, less than 0.1% water-solubles, and 0.1% protein.

Chlorine and hydrogen chloride were used as received in cylinders from Matheson Co., Inc.

Oxidations were conducted at 18° (\pm 2°) C. in 1-liter Pyrex bottles. The chlorine held by each bottle was determined by analysis. Empty bottles used for calibration and starch-containing bottles were filled in the same manner. A bottle was evacuated and immediately filled with chlorine to atmospheric pressure. It was then re-evacuated and refilled rapidly two more times. At the final filling, the pressure of chlorine gas was slightly greater than atmospheric, and the excess gas was released through a mercury trap. The chlorine present in the bottle for calibration or in a bottle following a starch oxidation was estimated by flushing it with nitrogen into 4*N* sodium hydroxide solution, and the hypochlorite produced was determined iodometrically. Bottles of starch and chlorine were continuously rotated slowly on a horizontal roller during oxidation. For determination of the effect of light, incandescent lamps were positioned above and below the rotating bottle to provide light intensity of 500 ft.-candles on the surface of the bottle. Light intensity was measured by Weston illumination meter model 603 (Weston Electrical Instrument Corp., Newark, N.J.). Carbon dioxide formed in certain oxidations was measured by absorption in 2*N* sodium hydroxide solution, and the excess of alkali was titrated with oxalic acid as described by Whistler and Schweiger (2).

Following oxidation, the residual starch was washed free of hydrochloric acid and oxalic acid with ether and dried over phosphorus pentoxide and sodium hydroxide at 25°C. in a vacuum desiccator.

Solubility of the oxidized starch was determined by suspending a large excess in 2 ml. of distilled water at 25°C. with stirring; the mixture was centrifuged, and the material was dissolved in a 1-ml. aliquot determined by drying to constant weight. Solubility is expressed in g. per 100 ml. of the solution.

Viscosities of 1% aqueous solutions were measured in an Ostwald-Cannon-Fenske viscometer at 25°C. and the solutions were then used for pH determination.

The number of aldehyde groups per 100 D-glucose units of the oxidized starch were determined by a modification of the procedure of Martin *et al.* (4). A 0.2- to 0.4-g. portion of the oxidized starch was added to 10 ml. of 0.05M borax solution at pH 9.7, and 20 ml. of 0.03N iodine solution was added. After it was kept for 2 hr. at 25°C. in the dark, the reaction mixture was acidified with 30 ml. of 0.1N hydrochloric acid solution and titrated with 0.01N sodium thiosulfate. Values were corrected for blank titrations.

The number of carboxyl groups per 100 D-glucose units in the oxidized starch were determined by the calcium acetate method of Mattisson and Legendre (5).

The total number of carboxyl and carbonyl groups per 100 D-glucose units in the oxidized starch were measured by the use of hydroxylamine hydrochloride according to the method described by Gladding and Purves (6) for estimating total carbonyl groups in oxy-starch and oxycellulose. The reagent was prepared by dissolving 5.0 g. of hydroxylamine hydrochloride in 50 ml. of water, adding 12 ml. of 1N sodium hydroxide solution, and diluting to 100 ml. A portion (0.2 to 0.4 g.) of oxidized starch was added to 10 ml. of the reagent and this was allowed to stand for 18 hr. at 25°C. The unreacted hydroxylamine was titrated with 0.1N hydrochloric acid using bromthymol blue as an indicator. A blank titration was also made.

Chromatographic examination of oxidized starch before and after hydrolysis was made on Whatman paper No. 1. Irrigants were:

- (a) 1-butanol:acetic acid:water (4:1:1 v/v)
- (b) 1-butanol:acetic acid:water (4:1:5 v/v)
- (c) t-butanol:methyl ethyl ketone:formic acid:water (40:30:15:15 v/v)

The spray reagents employed were:

- (d) an acetone solution of silver nitrate followed by an ethanolic solution of sodium hydroxide (7)
- (e) a solution of resorcinol in 1-butanol containing hydrochloric acid (8)
- (f) an iodate-iodide mixture (9)
- (g) 0.4% bromcresol green adjusted to pH 7.5 (10).

Electrophoresis was performed according to the procedure described by Foster (11). Samples were applied to Whatman paper No. 3, and electrophoresis was conducted for 2 hr. at a voltage gradient of

20 v. per cm. in sodium acetate buffer at pH 5 for acidic components and in borate buffer at pH 10 for neutral components.

Results and Discussion

Oxidations were conducted at molar ratios of starch to chlorine of 1:1 and 1:3. Parallel oxidations were made in the dark and with the bottle exposed on two sides to 500 ft.-candles of incandescent light. Chlorine consumed during oxidations is shown in Fig. 1. It is evident

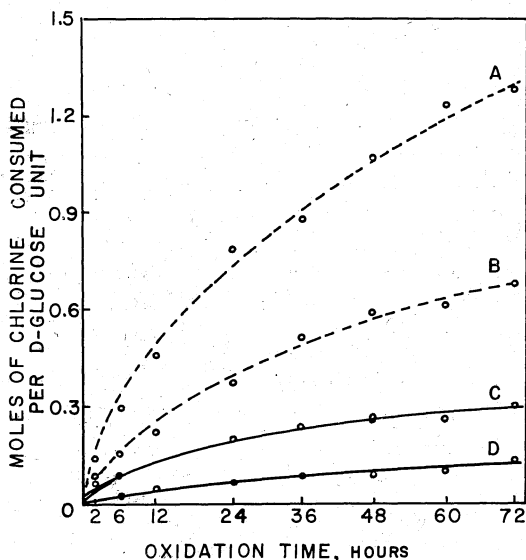


Fig. 1. Chlorine consumption for semidry starch treated with three moles of chlorine for each mole of D-glucose unit in light (A) and in dark (C), and with one mole of chlorine for each mole of D-glucose unit in light (B) and in dark (D).

that the oxidation rate is photosensitive because of the photoactivation of chlorine, which absorbs light over a wide range of the visible as well as the ultraviolet spectrum with high quantum yields. The major difference seems due only to light activation of chlorine molecules and not to free radical effects, since the oxidation products are apparently the same for dark and photoactivated reactions.

Dry starch yields, even after 72 hr. of reaction in the dark, are 98–99%. In photoactivated reactions, the yield may decrease to 95% after a 72-hr. reaction period. Loss is mainly due to oxalic acid formation and carbon dioxide liberation (Fig. 2). Formation of oxalic acid is proved by its isolation from the ether washings of the oxidized starches. The oxalic acid is obtained pure by isolation from paper

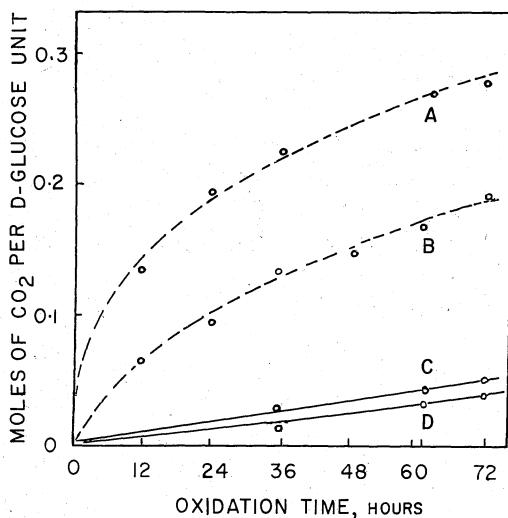


Fig. 2. Carbon dioxide production from semidry starch oxidized with three moles of chlorine for each mole of d-glucose unit in light (A) and in dark (C); and with one mole of chlorine for each mole of d-glucose unit in light (B) and in dark (D).

chromatograms. It crystallizes from water as needles, m.p. 186°. These crystals give a blue color with diphenylamine (12) and a yellow solution with resorcinol-sulfuric acid which turns blue on standing. On neutralization with ammonium hydroxide, the solution becomes pink (13). It also moves at the same rate as authentic oxalic acid on a paper chromatogram in irrigants a and c as indicated by spray reagents f and g. The origin of oxalic acid and carbon dioxide is not indicated, but these substances must result from extended oxidation of sugar

TABLE I
FORMATION OF OXIDIZED GROUPS IN SEMIDRY STARCH OXIDIZED BY CHLORINE
(1:3 RATIO) AT 18° ± 2°C. IN LIGHT

| TIME hr. | GROUPS PER 100 D-GLUCOSE UNITS | | | |
|-------------|--------------------------------|----------|------|----------|
| | Aldehyde | Carboxyl | Sum | Carbonyl |
| 1 | 5.7 | 7.4 | 13.1 | 11.8 |
| 2 | 7.3 | 10.1 | 17.4 | 16.5 |
| 3 | 7.0 | 10.1 | 17.1 | 18.8 |
| 4 | 6.5 | 9.8 | 16.3 | 16.6 |
| 5 | 7.7 | 11.1 | 18.8 | 18.9 |
| 6 | 8.2 | 11.4 | 19.6 | 19.3 |
| 12 | 8.4 | 16.2 | 24.6 | 24.4 |
| 24 | 10.6 | 21.0 | 31.6 | 31.4 |
| 36 | 9.1 | 22.7 | 31.8 | 32.1 |
| 48 | 9.2 | 24.5 | 33.7 | 33.5 |
| 60 | 9.4 | 22.6 | 32.0 | 33.7 |
| 72 | 9.7 | 24.8 | 34.5 | 34.6 |

fragments. Since no evidence of intermediate-size fragments is found, they must be more susceptible to oxidation than the initial derivatives of six carbon sugars.

Rates of formation of aldehyde, carboxyl, and combined carbonyl and carboxyl groups, as determined by different analytical procedures, are indicated in Tables I, II, III, and IV. The sum of the aldehyde numbers and the carboxyl numbers is nearly the same as the separately determined sum of carbonyl and carboxyl groups per 100 D-glucose units. This observation suggests that there are but few ketonic carbonyl groups in the oxidized starch.

When samples of the ether-extracted oxidized starch are dissolved in water and chromatographed, components are observed which corre-

TABLE II
FORMATION OF OXIDIZED GROUPS IN SEMIDRY STARCH OXIDIZED BY CHLORINE
(1:1 RATIO) AT $18^{\circ} \pm 2^{\circ}\text{C}$. IN LIGHT

| TIME | GROUPS PER 100 D-GLUCOSE UNITS | | | |
|------------|--------------------------------|----------|------|----------|
| | Aldehyde | Carboxyl | Sum | Carbonyl |
| <i>hr.</i> | | | | |
| 1 | 6.9 | 9.7 | 16.6 | 15.7 |
| 2 | 7.3 | 10.7 | 18.0 | 19.1 |
| 3 | 8.0 | 12.8 | 20.8 | 22.0 |
| 4 | 7.8 | 11.4 | 19.2 | 21.0 |
| 5 | 7.9 | 11.5 | 19.4 | 21.5 |
| 6 | 8.4 | 12.3 | 20.7 | 20.6 |
| 12 | 13.3 | 14.8 | 28.1 | 26.7 |
| 24 | 12.5 | 17.0 | 29.5 | 28.4 |
| 36 | 13.0 | 16.8 | 29.8 | 31.3 |
| 48 | 11.7 | 18.3 | 30.0 | 31.0 |
| 60 | 10.2 | 18.4 | 28.6 | 31.9 |
| 72 | 11.1 | 19.3 | 30.4 | 32.1 |

TABLE III
FORMATION OF OXIDIZED GROUPS IN SEMIDRY STARCH OXIDIZED BY CHLORINE
(1:3 RATIO) AT $18^{\circ} \pm 2^{\circ}\text{C}$. IN DARK

| TIME | GROUPS PER 100 D-GLUCOSE UNITS | | | |
|------------|--------------------------------|----------|------|----------|
| | Aldehyde | Carboxyl | Sum | Carbonyl |
| <i>hr.</i> | | | | |
| 1 | 6.2 | 4.5 | 10.7 | 12.8 |
| 2 | 6.5 | 4.9 | 11.4 | 11.9 |
| 3 | 6.7 | 5.5 | 12.2 | 12.5 |
| 4 | 7.4 | 5.9 | 13.3 | 13.6 |
| 5 | 6.9 | 6.3 | 13.2 | 13.6 |
| 6 | 8.6 | 7.8 | 16.4 | 15.1 |
| 12 | 6.0 | 11.4 | 17.4 | 18.3 |
| 24 | 7.4 | 11.9 | 19.3 | 19.1 |
| 36 | 6.2 | 14.1 | 20.3 | 21.5 |
| 48 | 7.5 | 13.8 | 21.3 | 22.0 |
| 60 | 6.6 | 15.6 | 22.2 | 22.5 |
| 72 | 6.8 | 15.0 | 21.8 | 22.6 |

TABLE IV
FORMATION OF OXIDIZED GROUPS IN SEMIDRY STARCH OXIDIZED BY CHLORINE
(1:1 RATIO) AT $18^{\circ} \pm 2^{\circ}\text{C}$. IN DARK

| TIME | GROUPS PER 100 D-GLUCOSE UNITS | | | |
|------------|--------------------------------|----------|------|----------|
| | Aldehyde | Carboxyl | Sum | Carbonyl |
| <i>hr.</i> | | | | |
| 1 | 4.1 | 3.7 | 7.8 | 9.4 |
| 2 | 4.2 | 4.6 | 8.8 | 11.0 |
| 3 | 4.7 | 6.6 | 11.3 | 12.5 |
| 4 | 4.9 | 5.6 | 10.5 | 10.2 |
| 5 | 5.5 | 6.2 | 11.7 | 13.6 |
| 6 | 5.1 | 6.2 | 11.3 | 13.0 |
| 12 | 4.2 | 7.0 | 11.2 | 12.2 |
| 24 | 5.1 | 11.2 | 16.3 | 16.5 |
| 36 | 6.1 | 10.8 | 16.9 | 17.6 |
| 48 | 5.0 | 12.4 | 17.4 | 18.3 |
| 60 | 3.9 | 12.6 | 16.5 | 17.2 |
| 72 | 4.2 | 14.7 | 18.9 | 20.5 |

spond to small amounts of D-glucose and D-gluconic acid. Traces of oligosaccharides are also detected.

Hydrolysis of the oxidized starch produces D-glucose, D-gluconic acid, and D-gluconic acid. In some samples, mainly those obtained from oxidation conducted without photoactivation, the presence of D-glucosone was also detected. For its isolation, oxidized starch is hydrolyzed in 10% sulfuric acid solution for 12 hr. at 90° . Sulfuric acid is then removed by extraction with a solution of methyl *n*-dioctylamine in chloroform. The aqueous solution is stirred with barium carbonate on a steam bath for 1 hr. and filtered. Concentration of the filtrate to a syrup and addition of two volumes of ethanol precipitates the barium salts of organic acids. The acidic material is mainly D-gluconic acid, with a small quantity of D-gluconic acid. D-gluconic acid is separated from the mixture as its insoluble calcium salt by treating the solution of barium salts with the requisite quantity of sulfuric acid and neutralizing the acidic solution with calcium carbonate. The insoluble calcium salt of gluconic acid is separated by centrifugation, decationized, and converted into its crystalline brucine salt, m.p. $151\text{--}152^{\circ}\text{C}$., $[\alpha]_D^{24} = -17.5^{\circ}$ (c, 1 in water). D-Gluconic acid is observed

in starches oxidized for more than 24 to 36 hr. The presence of uronic acid is indicated by the carbazole test (14). The presence of D-gluconic acid is confirmed by oxidizing the calcium salt of D-gluconic acid with sodium hypoiodide to obtain a product electrophoretically identical with known D-glucosaccharinic acid. Chromatography of the solution from which the organic acids are separated shows the presence of D-glucose mainly, and a small amount of D-glucosone (*D-arabino-*

hexosulose), which is present in a greater amount in starches oxidized for more than 12 hr. This oxidation product of D-glucose units is present in greater amounts in oxidations conducted in the dark than in photoactivated oxidations, as observed by chromatographic examination. Amounts of D-glucosone formed, though not quantitized, seem to parallel the amounts of ketonic carbonyl by the differences in columns 3 and 4 in Tables I, II, III, and IV. The presence of D-glucosone is proved by separating it on a paper chromatogram in irrigants a and b (Rg, 1.1) and eluting it from the strips with water. The aqueous solution gives a strong test for ketoses (8) and runs parallel to the authentic sample of D-glucosone prepared according to the procedure described by Bayne (15). The presence of D-glucosone is also confirmed by converting it to a known crystalline, 2-(arabino-tetrahydroxybutyl)quinoxaline, m.p. and mixed m.p. 188°–190°C. Isolation of this oxidation product shows that some oxidation of C2 occurs. At present there is no evidence for oxidation of carbon C3. This observation shows again that oxidation of semidry starch with chlorine proceeds in a manner differing from that of chlorine oxidation of aqueous starch slurries, as observed by Whistler and Schweiger (2). In aqueous slurry the oxidation is degradative, with cleavage of glycosidic bonds, but there is also an extensive attack to cleave the bond between carbon atoms C2 and C3 with conversion of these atoms to carboxyl groups. No such oxidation is evident in the semidry oxidation of starch, regardless of whether the oxidation is photoactivated or not.

The finding of large amounts of D-gluconic acid on hydrolysis of the oxidized starch is indicative of extensive cleavage at the glycosidic bonds with simultaneous or secondary oxidation of anomeric carbons. The D-gluconic acid units can form either by direct oxidative cleavage of the D-glucosidic bonds or by the oxidation of aldehydic end units produced by prior hydrolytic cleavage of the glycosidic bonds by hydrochloric acid. Most of the hydrochloric acid will be produced by reaction of chlorine and water present as moisture. A small amount will be generated by actual chlorine oxidation of the starch.

Extensive depolymerization of the starch under chlorine treatment is indicated by the rapid fall in viscosity and the rapid rise in water solubility (Table V and Fig. 3). To obtain some idea of the amount of degradation that might result from the hydrochloric acid, a portion of starch was placed in an atmosphere of hydrogen chloride under conditions normally used for oxidation. The increase in aldehyde content was followed, and observed to rise rapidly during the first 3–4 hr. and nearly level off after 12 hr. at a value corresponding to

TABLE V
DEPOLYMERIZATION OF STARCH UNDER CHLORINE TREATMENT

| CONDITION | RATIO, STARCH : CHLORINE | SHORT-TERM EXPERIMENTS (1 hr. to 6 hr.) | | LONG-TERM EXPERIMENTS (12 hr. to 72 hr.) | |
|-----------|--------------------------------|--|---------|---|---------|
| | | Reduced Viscosity | pH | Reduced Viscosity | pH |
| Light | 1:3 | 0.08-0.06 | 2.9-2.7 | 0.06-0.05 | 3.2-3.0 |
| Light | 1:1 | 0.08-0.07 | 2.7-2.6 | 0.08-0.05 | 3.3-3.1 |
| Dark | 1:3 | 0.1-0.09 | 3.0-2.9 | 0.17-0.15 | 4.5-3.8 |
| Dark | 1:1 | 0.12-0.1 | 3.2-3 | 0.2-0.16 | 4.5-3.6 |

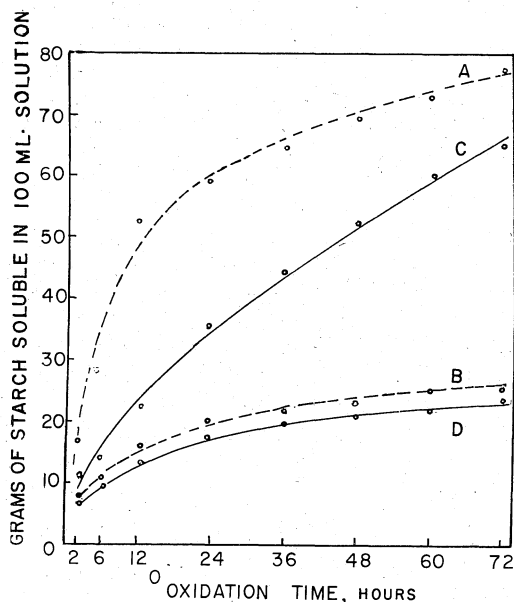


Fig. 3. Solubility in water of the oxidized starch obtained by treatment of semi-dry starch with three moles of chlorine for each mole of D-glucose unit in light (A) and in dark (C); and with one mole of chlorine for each D-glucose unit in light (B) and in dark (D).

12 aldehyde groups per 100 D-glucose units. Under chlorine treatment, nearly 35 chain cleavages per 100 D-glucose units occurred, as indicated by the sum of aldehyde and carboxyl groups, which may represent only about one unit of uronic acid per 100 D-glucose units (Table I, column 3). Thus under these conditions, about 23 cleavages per 100 D-glucose units must result from the oxidative attack.

These results suggest that the principal action of chlorine gas on wheat starch is depolymerization by attack at the glycosidic bond. Though strongly photoactivated, the reaction in the light remains similar to that in the dark. While hydrolysis may account for some

cleavage, the major cleavage must be oxidative. The mechanism of this oxidative cleavage is not established, but work on model substrates should provide an explanation.

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