OXIDATION OF WHEAT STARCH WITH CHLORINE[1]

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ABSTRACT

Hard wheat starch was more rapidly oxidized by chlorine gas in the light than in the dark and at 25°C. than at 15°C. Oxidation rate increased rapidly with moisture content of the starch. Hydrolysis of the oxidized starch yielded D-gluconic acid with very small amounts of D-glucuronic acid. It is suggested that wheat starch at 15.3% moisture reacts with chlorine gas, first undergoing hydrolysis and then oxidation of the freed aldehyde groups to produce starch chains with D-gluconic acid end units.

Quality of flour, particularly that of cake flour, is improved by chlorine bleaching. It is generally recognized that chlorine bleaching increases the hydrogen-ion concentration and alters the characteristics of both the gluten and the starch. Sollars (6) investigated, by means of a reconstitution technique, the effect of chlorine bleaching on flour fractions in cakes and cookies. Interaction between oxidatively modified gluten and starch is possible, and Hlynka and Bass (1) theorize that simple carbohydrates, through their reducing groups, may act as cross-linking agents between protein molecules. Neukom and Deuel (4) find marked improving action on dough and on baking quality when periodate-oxidized polysaccharides are incorporated.

The present work was undertaken to determine the nature of the initial mode of attack of chlorine on dry or semidry wheat starch.

Materials and Methods

Starches. Commercial hard wheat starch was defatted by Soxhlet extractions with 85% methanol for 2 days, and was air-dried and equilibrated to a moisture content of 8.0%. A portion was stored over water in a closed container to reach a moisture content of 15.3%. Another portion was vacuum-dried at 55°C. for 48 hours.

Amylopectin was prepared from commercial corn starch by butanol fractionation (5).

Oxidation of Amylopectin. Ten grams of amylopectin were dissolved in 600 ml. of hot water and the solution was filtered through cloth and divided into two parts, each of which contained 0.0264 mols. of D-glucose residues. To one part 0.0792 mols. of sodium hypochlorite solution were added and to the second part, 0.0792

mols. of fresh chlorine water. Solutions were adjusted to 1.5 liters and to acidity of pH 5 by addition of either hydrochloric acid or sodium hydroxide, and buffered at this pH with phosphates. Solutions were maintained in the dark at 25°C. and the pH adjusted when necessary. Unreacted oxidant was determined iodometrically on aliquots (Fig. 1).

Oxidation of Wheat Starch with Chlorine. Starches (4.43 × 10⁻³ mols.) of 0, 8.0, and 15.3% moisture contents were placed in 300-ml.
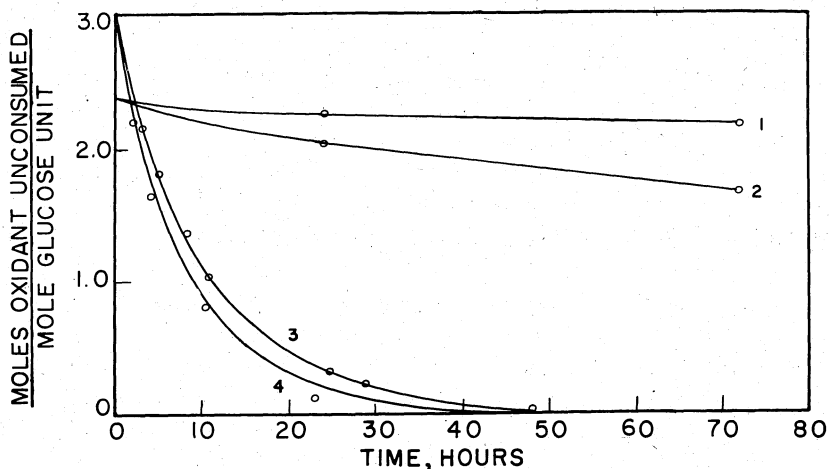

Fig. 1. Chlorine gas on wheat starch: 1, in the dark, and 2, in light. 3, Aqueous hypochlorite, and 4, aqueous chlorine on corn amylopectins in the dark at pH 5.
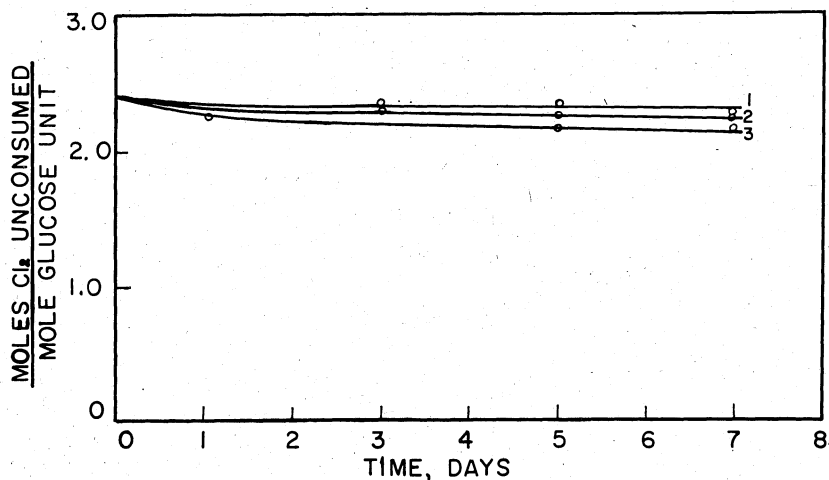

Fig. 2. Oxidation of wheat starch with chlorine gas at 15°C.: 1, dry; 2, 8% moisture; 3, 15.3% moisture.
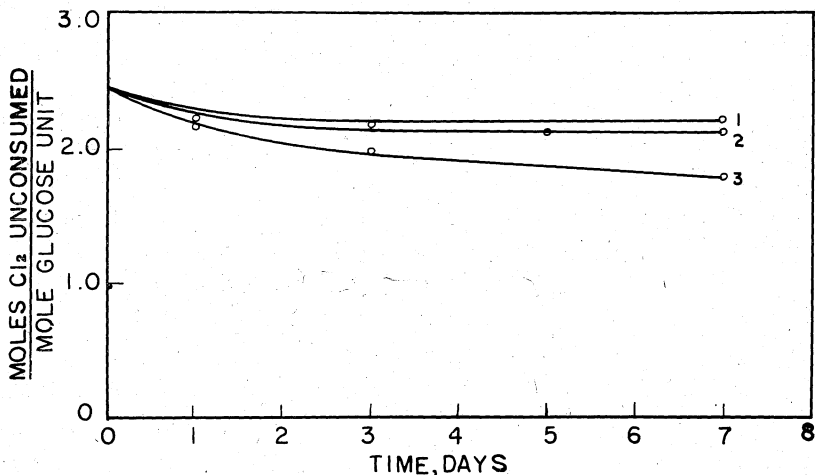
Fig. 3. Oxidation of wheat starch with chlorine gas at 25°C.: 1, dry; 2, 8% moisture; 3, 15.3% moisture.

containers which were then rapidly evacuated and filled with chlorine gas. Some containers were maintained in the dark at 15°C. and others at 25°C. One group was maintained at 25° and exposed to a 15-watt fluorescent lamp at a distance of 15 cm. After definite reaction periods, the unreacted chlorine was removed by passage of air through the reaction containers to carry the chlorine into alkaline solutions where it was determined iodometrically (Figs. 1, 2, and 3).

In two other oxidations 0.0302-mol. portions of wheat starch at 0 and 8.0% moisture contents respectively were shaken at 15°C. for 3 days with 0.0906 mols. of chlorine gas. The starch was then washed with 95% ethanol and dried. Carboxyl contents were determined by titration (2). Results are given in the table below.

Carboxyl Content of Oxidized Wheat Starch

Moisture Content %	Carboxyl Content %	Mols. COOH per mol. D-glucose Residue
0	0.14	1/198
8	0.47	1/58

In another oxidation 54 g. of wheat starch at 15.3% moisture content were oxidized with a threefold molar equivalent of chlorine gas at 25°C. for 7 days. Toward the end of the reaction the starch became slightly sticky. It was hydrolyzed in 1N sulfuric acid solution on a steam bath for 5 hours and neutralized with barium hydroxide. Paper chromatography of the hydrolysate showed the presence of D-glucose, D-gluconic acid, a small amount of D-glucuronic acid, and a trace of D-erythronic acid. The hydrolysate was passed through a column (3.8 ×

25 cm.) of Dowex 3 basic resin and the effluent concentrated and crystallized as D-glucose, m.p. 150°C., $[a]_D^{23}+53.2$ (c, 4 in water). The phenylosazone melted at 209°C. The resin, containing the acid components of the syrup, was placed on top of a 6 × 80 cm. Dowex 3 column in the acetate form, and the combination was irrigated successively with 0.2N acetic acid, 1N acetic acid, 2N acetic acid, 1N formic acid, and 0.5N trifluoroacetic acid. Sequential fractions were concentrated to dryness, weighed, and analyzed by paper chromatography.

Irrigant and Volume	Residue	Product Found
liters	*g*	
0.2N acetic acid (17)	0.4	D-glucose
1N acetic acid (33)	7.4	D-gluconic acid
		D-glucuronic acid (minor)
		D-erythronic acid (trace)
2N acetic acid (58)	1.5	D-gluconic acid
		D-glucuronic acid (minor)
1N formic acid (18)	0.4	D-gluconic acid
0.5N trifluoroacetic (57)	1.7	D-gluconic acid

The syrups on dissolution in warm water and neutralization with calcium carbonate yielded crystals of calcium D-gluconate. On decationization and treatment with brucine, crystalline brucine D-gluconate was obtained, m.p. 151–152°C., $[a]_D^{23}-18.0$ (c, 2 in water).

Another 8.5-g. portion of 15.3%-moisture wheat starch was oxidized with chlorine gas as described above. The product when dissolved in water had a pH of 0.25. It was hydrolyzed in 1N sulfuric acid solution as described. The concentrated barium salts of sugar acids when precipitated by ethanol, filtered, and dried, weighed 2.8 g. Uronic acid determination (3) on 805.6 mg. gave 9:7 mg. of carbon dioxide, indicating that the concentrated barium salts contained 0.2 g. of barium D-glucuronate. The remaining barium salt was chromatographed on paper with ethyl acetate:acetic acid:formic acid:water (18:3:1:4 v/v); the portion of the paper containing D-gluconic acid was cut out and eluted with water and the extract concentrated to dryness at 105°C. After 8 hours' heating at this temperature the product was dissolved in warm 0.1N sodium hydroxide solution and back-titrated with standard acid. Titration value indicated that the 2.8 g. of the concentrated barium salts of sugar acids contained 2.3 g. of barium D-gluconate.

Discussion

Oxidation of aqueous starch amylopectin at pH 5 proceeds at the same rate regardless of whether chlorine or hypochlorite is the starting reagent (Fig. 1, curves 3 and 4). This is expected because of the rapid

interconversion of chlorine and hypochlorite to establish an equilibrium ratio at pH 5. The oxidation of wheat starch is very sensitive to photochemical catalysis. Thus the mere presence of radiation from a 15-watt fluorescent tube at 15 cm. distance causes a marked increase in the rate of chlorine consumption (Fig. 1, curves 1 and 2).

Under aqueous conditions of oxidation Whistler and Schweiger (7) show that the predominant oxidative attack of hypochlorite on starch is at carbons C2 and C3 of D-glucose residues. This attack presumably creates a carbonyl group which, in the neutral pH range, enolizes and allows further rapid oxidation to occur at the double bond with consequent cleavage between carbon atoms C2 and C3. Proof to this site of attack is the isolation from among the hydrolysis products of large quantities of D-erythronic and glyoxylic acids.

In contrast to its action in aqueous solution, chlorine gas oxidizes semidry wheat starch rather slowly. The oxidation is temperature-dependent, as seen in Figs. 2 and 3. In addition, the oxidation rate is markedly affected by the moisture content of the starch. Starch of 15.3% moisture is much more rapidly and extensively oxidized at 25°C. than starch of lower moisture content. Information on the nature of the oxidation at the 15.3% moisture level is obtained by examination of the hydrolysis products from the oxidized starch. Observation of large amounts of D-gluconic acid occurring with little D-glucuronic acid and no significant amounts of D-erythronic acids suggests that the principal attack is on carbon atom C1. Very little oxidation of carbon atom C6 occurs even during a 7-day period, and practically no oxidative attack takes place on carbon atoms C2 and C3 as evidenced by the lack of the four-carbon-fragment, D-erythronic acid, which would result. Extensive production of D-gluconic acid at high moistures might suggest that the low pH levels induced hydrolysis of the glycosidic linkages freeing reducing groups which would rapidly oxidize to gluconic end units. Approximately 42% of the chlorine consumed in the oxidation is required for production of the isolated D-gluconic acid.

Since oxidation is extensive at low moisture and even under dry conditions, it would seem that a second nonhydrolytic depolymerization occurs, possibly induced by a free radical mechanism. If this is so, then it might be expected that the two types of depolymerization would occur simultaneously at intermediate moisture levels. Further work is necessary to bring to light the details of the depolymerization, and particularly that at low moisture contents.

Nonetheless, it is apparent that during chlorine-gas bleaching of

wheat flour some depolymerization of the starch probably occurs with the formation of D-gluconic acid end units, which may in turn also influence the properties of the resulting dough or cakes produced therefrom.

Acknowledgment

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