Action of Chlorine on Wheat Flour Polysaccharides[1]

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ABSTRACT

As a result of the present and earlier work from this laboratory, it is
now possible to explain the reactions which occur between polysaccharides
and chlorine during flour bleaching. Chlorinolysis of wheat-straw hemi-
cellulose proceeds in the same manner as earlier established for starch and
cellulose. The principal difference is the lack of 1,6-anhydro ring forma-
tion in hemicelluloses. Hence under dry and semidry conditions they develop
higher numbers of aldehydic end units and possibly more intermolecuar
grafting than observed with hexose polymers. Work with isolated poly-
saccharides such as cellulose, starch, and hemicellulose leads to the belief
that direct oxidation of these occurs, at most, to only a minor degree when
flour is bleached with chlorine. Instead, a chlorinolysis proceeds with
depolymerization through breakage of glycosidic bonds. In dry or semidry
reactions, cellulose and starch cleave with development of levoglucosan units
at the potentially reducing chain end. Xylans, whose units cannot form
stable anhydro rings involving carbon Cl, yield either reducing chain ends
through acceptance of hydroxyl groups from water or, to a minor degree,
produce grafts through union of the active chain ends with hydroxyls of
neighboring chains.

This laboratory has been concerned with the chemical reactions occurring
in the polysaccharides of wheat flour when it is treated with chlorine gas.
To be sure, chlorine reacts with most of the components of flour during
bleaching; it attacks the lipids and proteins as well as the carbohydrates.
Furthermore, the attack is kinetically directed, most of the chlorine being
consumed in the fastest reactions. Rate of reaction depends not only on the
free energy change, which may be altered by the degree of stabilization of a
flour component through combination with other ingredients, but also upon
its physical accessibility to diffusing chlorine molecules.

The proportion of chlorine reacting with individual flour components
during bleaching is not known. However, even though one component may
react with only a small proportion of the chlorine, its contribution to flour
characteristics may be significantly altered. Polysaccharides strongly influence
the rheology of flour doughs. Hence, minor changes in them, either through
direct modification by chlorine or through interaction with other chlorine-
modified flour components, may greatly alter flour characteristics. We have
sought to obtain a fuller understanding of the way in which flour polysac-
charides react with chlorine by examining their reactions in isolated systems.
As a consequence, we find it possible not only to explain the mechanism of
the polysaccharide-chlorine reaction but also to see how, in a minor way, the
polysaccharides may react with each other and with other flour components.

Previous work (1,2,3) has defined the action of chlorine gas on starch
and on cellulose. Results reported here show the way in which chlorine
reacts with a typical hemicellulose. The interaction of chlorine with each
polysaccharide can now be said to follow a similar, predictable, mechanistic

pattern. Furthermore, there is ample evidence to indicate the possibility of polysaccharide interaction during bleaching to produce grafts.

MATERIALS AND METHODS

Hemicellulose A (called hemicellulose hereafter) was isolated from delignified wheat straw by alkaline extraction (4). It was dissolved in $1N$ sodium hydroxide and filtered to remove insoluble material. The clear solution was adjusted to pH 5 with acetic acid and dialyzed for 10 days against deionized water. The dialyzed solution was concentrated to a small volume and 3 volumes of ethanol was added. The purified product was isolated by centrifugation, washed several times with 99.5% ethanol, and dried with ether. Hydrolysis and quantitative paper chromatography showed an approximate composition of 6% L-arabinose, 9% D-glucuronic acid, and 85% D-xylose.

Methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside was prepared from 2,3,4-tri-O-acetyl-β-D-xylopyranosyl bromide according to the procedure of Helferich and Ost (5), m.p. 114°–115°C. Methyl β-D-xylopyranoside was obtained by deacetylating the above product with sodium in absolute methanol. After neutralization with IR-120 (H⁺) and removal of the resin by filtration, the clear solution was concentrated by dryness. The residue, methyl β-D-xylopyranoside, was recrystallized from ethanol, m.p. 156°C.

Chlorine, obtained from Mateson Company, Inc., was dried by passage through sulfuric acid. Acetic acid and carbon tetrachloride were Baker's analyzed reagents. The latter was dried over sodium sulfate.

Sugars were chromatographed on Whatman No. 1 paper; irrigants employed were: A, ethyl acetate:pyridine:water (25:7:8 v./v.), and B, 1-propanol:ethyl acetate:water (7:3:1 v./v.); components were located by spraying dried chromatograms with irrigant C, an acetone solution containing 0.5% silver nitrate followed by an ethanolic solution of sodium hydroxide, and irrigant D, a solution of 2.66 g. aniline acid phthalate in 100 ml. water-saturated butanol, and heating at 110°C. for 10 min.

Thin-layer chromatography was performed on plates 5 × 12 cm., coated with Silica Gel G obtained from Brinkmann Instruments Inc., Westbury, N. Y., and Silica Gel G impregnated with borate (6). Irrigants employed were: E, chloroform:methanol (4:1 v./v.), and F, butanol:acetic acid:water (5:4:1 v./v.). Components were located by spraying with 5% sulfuric acid in ethanol and heat-charring, or by spraying with aniline acid phthalate and heating at 110°C. until spots were developed.

Aldehyde groups were determined on the oxidized material by the procedure of Martin and co-workers (7). Total carbonyl groups were determined by the hydroxylamine method (8). Intrinsic viscosity was measured in $0.5M$ cupriethylene diamine at 25°C. (9). Degree of polymerization was estimated from viscosity data by use of the equation $[\eta] = 2.2 \times 10^{-2} \times \text{D.P.}^{0.72}$ (10).

Hemicellulose was treated with chlorine in 1-liter Pyrex bottles fitted with ground-glass joints. At appropriate time intervals, residual chlorine was removed by passage of dry air through the flask and finally by placing the flask under reduced pressure.

Samples of hemicellulose were also treated with chlorine in glacial acetic acid and in carbon tetrachloride at 25°C. At appropriate intervals, a flask was opened, and solvent and residual chlorine were removed under reduced pressure. To ensure complete removal of chlorine, fresh solvent was added several times and removed under reduced pressure after each addition.

In all chlorinolysis reactions the ratio of chlorine to hemicellulose was 3:1.

Samples of hemicellulose (500 mg.) were treated with a 3:1 molar ratio of chlorine in acetic acid and in carbon tetrachloride at 25°C. for 4 hr. After removal of residual chlorine and solvent under reduced pressure, the sample treated in acetic acid was deacetylated with sodium in absolute methanol. The samples were then suspended in distilled water and the insoluble fraction removed by filtration. The water-soluble fractions were concentrated to dryness under reduced pressure at 50°C. Quantitative paper chromatography in irrigant A revealed D-xylose, L-arabinose, xylobiose, and xylotriose in both samples, although only traces of each appeared in the sample treated in acetic acid. Hydrolysis of the water-insoluble fractions in $2N$ sulfuric acid solution and chromatography in irrigant A revealed D-xylose and L-arabinose in each.

Methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (1 g.) was suspended in carbon tetrachloride containing 735 mg. chlorine in a sealed tube. The tube was heated at 100°C. for 12 hr., then cooled in dry ice-acetone, and opened. The residual chlorine and solvent were removed under reduced pressure. The residue was dissolved in 20 ml. of a 5% solution of silver nitrate-pyridine complex in absolute ethanol and heated at 80°C. for 90 min. After filtration to remove silver chloride, the solution was evaporated to dryness and the residue extracted with chloroform. After removal of chloroform under reduced pressure, the residue was taken up in absolute methanol saturated with ammonia to remove any chlorinated acetyl groups. Methanol and ammonia were removed under reduced pressure and the residue partitioned between water and ether. The water phase was concentrated to dryness. The residue was acetylated in 7 ml. of pyridine and 5 ml. of acetic anhydride at 25°C. for 18 hr. Isolation of the acetylated product and repeated recrystallization from 95% ethanol yielded ethyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside, m.p. 105°–106°C. The compound did not depress the melting point of an authentic sample.

Methyl β-D-xylopyranoside (205 mg.) was placed in glacial acetic acid containing 325 mg. of chlorine at 50°–55°C. for 18 hr. After deacetylation with sodium in absolute methanol, quantitative paper chromatography in irrigant A revealed less than 1% D-xylose and 15% methyl β-D-xylopyranoside. The major component was a nonmobile oligosaccharide which was isolated by elution from Whatman 3MM paper. Chromatography of this fraction in irrigant B revealed two major and two minor components. The oligosaccharide mixture on dissolution in $0.01N$ sulfuric acid and heating on a steam bath for 8 hr. followed by chromatography revealed no D-xylose. This suggests the presence of only D-xylopyranosyl units.

A similar reaction at 25°C. for 24 hr. revealed methyl β-D-xylopyrano-

side and free D-xylose as major products. Traces of two unknowns with R_f values corresponding to xylobiose and xylotriose were also detected.

Methyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside (2 g.) was treated with a 3:1 molar ratio of chlorine in acetic acid as described above. After 20 hr. the reaction mixture was concentrated to 10 ml. under reduced pressure and added slowly to a solution of 1 g. of commercial cellulose diacetate in 100 ml. pyridine. After heating at 50°C. for 20 hr., the polysaccharide was precipitated in ethanol, isolated by centrifugation, washed several times in ethanol, and finally dried with ether. Thin-layer chromatography, with iso-propyl ether as irrigant, revealed no D-xylose derivatives in the isolated product. Hydrolysis in $2N$ sulfuric acid for 4 hr. on a steam bath and chromatography in irrigant A revealed small amounts of D-xylose in addition to D-glucose.

RESULTS

Dry hemicellulose is rapidly depolymerized by chlorine, as indicated in Fig. 1. Depolymerization is most rapid with gaseous chlorine and with a
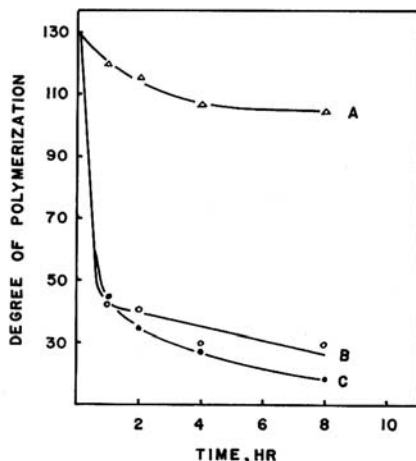

Fig. 1. Decrease in degree of polymerization of dry hemicellulose upon treatment with chlorine in: acetic acid (A), chlorine in carbon tetrachloride solution (B), and chlorine gas (C).

carbon tetrachloride solution of chlorine, which is expectedly similar. A lower rate of depolymerization, similar to that observed with amylose, takes place in acetic acid.

The lower apparent rate of observed depolymerization of xylan and amylose in acetic acid-chlorine as compared to that in carbon tetrachloride-chlorine or in chlorine alone may be due to grafting by the reactive chain ends formed during chlorinolysis. The consumption of chlorine is the same in either acetic acid or the carbon tetrachloride system. Although the number of ketonic carbonyl groups produced in both acetic acid and carbon tetrachloride is approximately the same, the number of aldehyde groups in products from the acetic acid-chlorine reactions is much lower (Table I).

The lower number of aldehyde groups and the higher viscosity of the products suggest that fragments produced by chlorinolysis are more often

TABLE I
GROUPS PER 100 D-XYLOSE UNITS

	CHLORINE GAS			CHLORINE IN CCl₁			CHLORINE IN AcOH		
OXI-DATION	Alde-hyde	Ketonic	Intrinsic Viscosity	Alde-hyde	Ketonic	Intrinsic Viscosity	Alde-hyde	Ketonic	Intrinsic Viscosity
hr.									
1	2.6	5.7	0.33	5.6	1.9	0.34	0.5	6.4	0.70
2	3.6	1.9	0.32	7.4		0.28	0.7		0.66
4	3.5	6.5	0.25	7.6	6.5	0.24	0.7	10.1	0.64
8	4.1	9.0	0.25	6.7	14.8	0.18	0.7	13.3	0.61

grafted to polysaccharide chains in an acetic acid system than those in a carbon tetrachloride system. This view is supported by the presence of relatively large amounts of small oligosaccharide fragments in the water-soluble fraction of hemicellulose after treatment with chlorine in carbon tetrachloride as compared to the acetic acid system. Likewise, in the chlorinolysis of methyl β-D-xylopyranoside in acetic acid, polymerization is more extensive, with production of higher-molecular-weight polymers, than in comparable reactions in carbon tetrachloride. Polymerization and grafting reactions are not due to hydrochloric acid formed, since no polymerization or grafting is observed when the chlorine is replaced by hydrochloric acid in these reactions.

Glycosides of pentose sugars are cleaved with chlorine in much the same way as glycosides of hexose sugars. Thus, methyl β-D-xylopyranoside triacetate, on treatment with chlorine in carbon tetrachloride, is cleaved to the D-xylopyranosyl chloride as indicated by recovery of ethyl β-D-xylopyranoside triacetate after treatment of the chloride with ethanol and silver nitrate. A further significant observation is that methyl β-D-xylopyranoside, when treated with chlorine in acetic acid, undergoes extensive polymerization to oligosaccharides. This condensation occurs through such reactive intermediates as the D-xylopyranosyl chloride and also, but probably to a lesser extent, through the C1 carbonium ion or the 1,2-anhydro-D-xylopyranose. High yield of oligosaccharides differentiates the pentoside reaction from the somewhat similar hexoside reaction.

In view of these results, it was not surprising to find that chlorination of methyl β-D-xylopyranoside triacetate, when mixed with a commercial cellulose acetate having 0.65 free hydroxyl groups per unit, produced grafting. This was proved by isolation of the reacted polysaccharide and hydrolysis to a mixture of D-xylose and D-glucose.

The hemicellulose used in this work was hemicellulose A isolated from wheat-straw holocellulose and may be regarded as a typical hemicellulose. Endosperm hemicelluloses found in wheat flour (11) are highly branched and contain larger amounts of L-arabinose. Save for the possible difference due to L-arabinose content, the two hemicelluloses will be expected to react similarly under conditions of chlorinolysis.

DISCUSSION

It is clear that the major action of chlorine on dry or semidry polysaccharides is a co-ordination with glycosidic oxygen atoms which leads to cleavage of the glycosidic bonds. Under neutral to rather strongly acidic condi-

tions, only a minor action of chlorine is oxidation at carbon C2, C3, or C6 (3,12).

Chlorine accountably reacts with the glycosidic oxygens in a polysaccharide to form a chloro-oxonium cation. This complex can undergo cleavage at the C1-oxygen bond in a variety of ways, depending upon whether or not attacking anions are present and upon their nature. In the absence of anions, cleavage of the C1-oxygen bond can proceed, leading to development of a carbonium ion at C1 and, as in all cases of cleavage, to a hypochlorite ester group at C4 of that portion of the chain serving as the alcohol function (see Fig. 2 where cleavage of a starch chain is illustrated). The
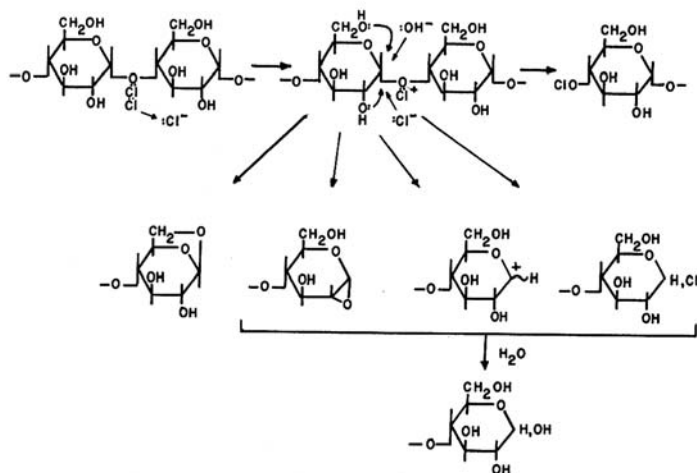

Fig. 2. Cleavage of a starch chain.

resulting carbonium ion can react with anions if present or with hydroxyl groups. Carbonium ion formation will not be extensive in chlorinolysis reactions because of the abundance of anions.

It is reasonable that in the great majority of cleavages the chloro-oxonium cation is displaced by an attacking anion. The anion may be chloride which would lead to the formation of a glycosyl halide, which in turn could undergo reaction with water, if present, to form a normal sugar end unit or with an alcohol group of the carbohydrate. If the alcohol group, or displacing alkoxide anion, is derived from a sugar unit in a neighboring chain, a grafting reaction ensues wherein the reactive end unit becomes glycosidically bound to the neighboring polysaccharide chain. Reactions resulting in grafting of the reactive chain ends to a hydroxyl position of a neighboring chain is indicated in model systems. Thus methyl β-D-xylopyranoside triacetate, in the presence of chlorine and partially acetylated cellulose, is observed to produce a product wherein D-xylose units become glycosidically bound to the cellulose molecule. The resultant grafted polymer, on purification, can be hydrolyzed to D-xylose and D-glucose. Therefore, in chlorine treatment of wheat flour it may be expected that some transglyco-

sylation can occur, with resultant grafting of fragments from various polysaccharides to other polysaccharide molecules or even to other adjacent flour components. The extent of such grafts will depend on the amount of water present and on the proximity of fragmented chain ends to hydroxyl groups of neighboring chains. Kinetic considerations would suggest that such grafts, though entirely possible, are likely to occur only in small numbers, mainly because of the lower mobility of the severed chain ends.

Earlier work by us (1) has indicated that breakage of glycosidic bonds during chlorinolysis could take place through attack of the hydroxyl oxygen at carbon C2 on the carbon C1 position with displacement of the chlorooxonium cation. The occurrence of this reaction in the chlorinolysis of cellobiose was shown in the finding of D-mannose among the products. The intermediate in this reaction has to be 1,2-anhydro-D-glucopyranose. The active oxirane ring can, of course, be opened by anions other than hydroxyl. If opened by attack at carbon C1 by the oxygen of a hydroxyl from a neighboring polysaccharide chain, the result would be grafting to the neighboring chain with the same resultant structure as indicated in the preceding paragraph, from the reaction of a chain having an active glycosyl chloride end unit.

Chlorinolysis reactions producing glycosyl chloride or 1,2-anhydro-D-glycose end units will, in the presence of water, yield glycose end units. In the presence of excess chlorine these reducing-sugar end units could be oxidized to glyconic acid units.

If the chloro-oxonium cation is displaced by the hydroxyl oxygen at carbon C6 (Fig. 2), the end unit assumes a levoglucosan structure becoming nonreducing. Such reactions occur (2) when water is limiting, and account for the observation that starch, when chlorinolyzed under water-limiting conditions, undergoes the usual rapid depolymerization but without the development of significant amounts of either aldehyde or glyconic acid end units. Since the hydroxyl at carbon C6 is positioned close to carbon C1, internal displacement with formation of stable 1,6-anhydro rings is a preferred reaction under dry or semidry chlorinolysis conditions. It is interesting to observe that when pentosans are chlorinolyzed, aldehyde functions continue to increase with extent of reaction (Table I). Since pentosans cannot form 1,6-anhydro rings, the principal reactions must be with water to form reducing-sugar end units. In water-deficient systems, the principal reaction must be the formation of glycosyl chlorides or 1,2-anhydro rings, both of which could undergo secondary reaction, either with water to form reducing end units or with hydroxyls of neighboring molecules to produce grafts. Grafting is a major reaction in model systems. Thus methyl β-D-xylopyranoside, when treated with chlorine in acetic acid, undergoes extensive polymerization to oligosaccharides containing D-xylopyranose units. Methyl β-D-glucopyranoside under similar conditions does not polymerize as extensively, but instead, produces levoglucosan in about 25% yield. For these reasons it is expected that any grafting which occurs in wheat flour is likely to contain hemicellulose fragments. These may graft to starch or to active groups in other flour components.

In all of the above chlorinolysis reactions, polysaccharide chains are fragmented by opening of the glycosidic bond between carbon C1 and the glycosidic oxygen atom. The cleavage may be due to formation of a semi-stable carbonium anion on carbon C1 or to several types of displacement of the chloro-oxonium cation. Thus, in every cleavage a hypochlorite ester is formed at the alcoholic side of the glycosidic bond. With starch, cellulose, or a xylan this will be at position C4 of the sugar unit serving as the alcohol function. In the presence of water the hypochlorite ester could quickly hydrolyze to yield a normal sugar unit at the newly formed nonreducing end of the chain. Even in the presence of water, but more prominently in its absence, the hypochlorite ester can undergo dehydrochlorination to produce a carbonyl function at carbon C4. The occurrence of this reaction has been shown in an earlier part of our work (1,2).

At this point it is possible to understand and correlate the reactions which ensue among flour polysaccharides in the presence of chlorine during the bleaching process.

It may be concluded that during the bleaching of wheat flour, some portion of the chlorine is consumed in reaction with the polysaccharides. Very little oxidation occurs to produce uronic carboxyls, carbonyl groups, or modifications of the secondary alcohol groups of the sugar units. Under normal moisture conditions the major interaction of chlorine with polysaccharides results in depolymerization induced by cleavage of glycosidic bonds. The most probable cleavage would result from a complex formed between chlorine and the glycosidic oxygen atom. The cleavage mechanism we propose would occur through nucleophilic displacement of the chloro-oxonium complex with either hydroxyl anions from water to give normal sugar end units, or, in the case of starch or cellulose, with the hydroxyl group at position C6. The latter reaction would give rise to 1,6-anhydro rings, or levoglucosan units. Since the hydroxyl at C6 competes with hydroxyl ions from water for the chloro-oxonium complex, the extent of levoglucosan formation will depend upon the moisture content of the flour.

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