Reactions of Aqueous Chlorine and Chlorine Dioxide with Model Food Compounds

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Chlorine and chlorine dioxide (ClO_2), common disinfecting and bleaching chemicals used in the food industry, are potent oxidizing and chlorinating agents. Unfortunately, little is known about the nature of the reactions of chlorine with organic food constituents. This presentation reviews published information concerning the reactions of chlorine gas ($Cl_2[g]$), aqueous chlorine, and ClO_2 with model food compounds, the fate of chlorine during the chlorination of specific food products, and the potential toxicity of the reaction products. Fatty acids and their methyl esters react with chlorine with the degree of incorporation corresponding to their degree of unsaturation. Aqueous chlorine oxidizes and chlorinates lipids and amino acids much more readily than ClO_2. Several amino acids are highly susceptible to oxidation and chlorination by chlorine compounds. Reactions of chlorine and ClO_2 with several food products, including flour and shrimp, have also been characterized. In one model system, 99% of $Cl_2(g)$ either reacted with components of flour or was consumed by oxidation/chlorination reactions. The lipids extracted from the chlorinated flour contained significant amounts of chlorine. Exposure of shrimp to hypochlorous acid (HOCl) solution resulted in significant incorporation of chlorine into the edible portion. Although significant quantities of chlorine can be incorporated into specific model compounds and food products, the health risks associated with exposure to chlorinated organic products are unknown. Preliminary studies using the Ames Salmonella/microsome mutagenicity assay indicate that the reaction products from mixtures of aqueous chlorine and various lipids or tryptophan are nonmutagenic. Nevertheless, additional studies are warranted, so that the toxicological significance of these reaction products can be understood more fully.

Introduction

Chlorine has been used for many years by the food industry as the principal sanitizing and disinfecting agent (1) (Table 1). The history of the discovery and use of chlorine in the food industry has been reviewed by Mercer and Somers (2) and Dychdala (3). Aqueous chlorine solutions are used to sanitize food processing equipment, to cool food containers, and to rinse various food products. Chlorine gas is also used in the flour industry as an oxidizing and bleaching agent and to improve the quality of flour. In the United States, chlorine and hypochlorites are considered "generally recognized as safe" (GRAS) for specific uses in food processing and for bottled water. In some cases, chlorine is considered a food additive, and therefore use levels have been established by the Food and Drug Administration.

This report is a review of the reactions of aqueous chlorine and chlorine dioxide with organic compounds

Table 1. Use of chlorine in the food industry.[a]

Use	Concentration, ppm
Bleaching flour (chlorine gas)	1200.0–2500.0
Container cooling water	0.5–10.0
Water for transporting products	0.5–7.0
Recycled product	0.5–10.0
Belt sprays	3.0–15.0
Sanitizing equipment	100.0–200.0
Cleaning equipment	2.0–125.0
Rinse water	1.0–5.0
Hydrocooling raw products	5.0–200.0
Fish thawing	5.0–10.0
Thawing of frog legs	200.0–500.0

[a] Adapted from Kirk and Mitchell (1).

found in foods and with specific food products. To understand these reactions more fully, the chemistry of aqueous chlorine and chlorine dioxide is first briefly reviewed. Unfortunately, most of the literature on reactions of chlorine and food chemicals has concentrated on net changes in gross chemical fractions of foods such as lipids, glutens, and fat or lean portions rather than on the formation of potentially hazardous reaction prod-

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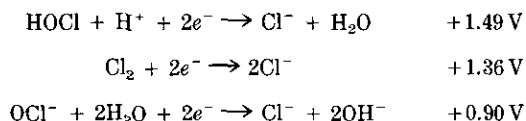
ucts. It is hoped that the information presented in this review will help in evaluating the possible health risks associated with chlorination in food processing. Certainly, more information is needed concerning the level and reactivity of chlorine used in various processes, the idenity and toxicity of the reaction products, and the levels of these compounds to which consumers are exposed before we can fully understand the risks of using chlorine in the food industry.

Chemistry of Aqueous Chlorine and Chlorine Dioxide

Chlorine gas ($Cl_2[g]$) hydrolyzes rapidly and almost completely in water to form hypochlorous acid (HOCl) and hypochlorite (OCl^-) as follows:

$$Cl_2(g) + H_2O \rightleftharpoons HOCl + HCl$$
$$HOCl \rightarrow OCl^- + H^+$$
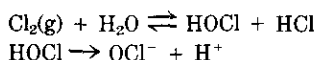
All three species, Cl_2, HOCl, and OCl^-, are present in an aqueous chlorine solution and their relative concentrations are pH-dependent. At pH 6, HOCl is the predominant species, representing greater than 90% of the chlorine in solution at 25°C. However, at pH 8, OCl^- is the major species (4). Chlorine gas is present only in minute amounts. All three species will readily oxidize organic compounds; however, HOCl is the most reactive as reflected by its higher redox potential (5):

$$HOCl + H^+ + 2e^- \rightarrow Cl^- + H_2O \qquad +1.49\,V$$
$$Cl_2 + 2e^- \rightarrow 2Cl^- \qquad +1.36\,V$$
$$OCl^- + 2H_2O + 2e^- \rightarrow Cl^- + 2OH^- \qquad +0.90\,V$$

In addition, HOCl is the primary bactericidal agent in aqueous chlorine, possessing approximately 80 times the germicidal potency of OCl^- (6).

The reactivity of HOCl is based on the electrophilic nature of the molecule at either the oxygen or the chlorine atom (4). Reactions occurring at the oxygen atom produce a chloride ion by displacement. In reactions with organic compounds, the chlorine atom may become electrophilic and may combine with an electron pair in the substrate. This behavior accounts for the reactions of HOCl with ammonia and amines, with phenols and other aromatic substances, and in the formation of chloroform from organic substrates.

Chlorine dioxide (ClO_2) is a polar gas that readily dissolves in, but does not react with, water (7). This compound has a larger oxidation capacity than that of HOCl because it can accept 2.5 times more electrons than HOCl, but its oxidizing potential is less, as indicated by its redox potential (7):

$$ClO_2 + e^- \rightarrow ClO_2^- \qquad +0.95\,V$$
$$ClO_2^- + 2H_2O + 4e^- \rightarrow Cl^- + 4OH^- \qquad +0.78\,V$$
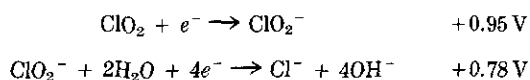
In addition, there are no appreciable differences in the bactericidal activity of ClO_2 as a function of pH (8).

Chlorine dioxide will oxidize and chlorinate many organic compounds, but at a much slower rate than that associated with HOCl reactions. In most cases, oxidation reactions predominate. Among organic compounds studied thus far, ClO_2 is most reactive in aqueous solutions with aliphatic tertiary amines, aromatic amines, and phenolic compounds (7). In addition, ClO_2 will oxidize aldehydes and ketones to form carboxylic acids. Chlorine dioxide does not react with saturated aliphatic hydrocarbons and aliphatic side chains, although the latter may be split from aromatic rings or other functional groups (7). The chlorination of aromatic and unsaturated aliphatic hydrocarbons by ClO_2 has been reported (5,7); however, no trihalomethanes have been detected as reaction products of ClO_2 with organic materials. Each of these reactions will be discussed in greater detail as it pertains to reactions with lipids, carbohydrates, and proteins.

The mechanisms of chlorination and oxidation of organic compounds by ClO_2 are not known. Chlorination in aqueous solutions may occur indirectly through a progressive reduction of ClO_2, which passes through the HOCl stage (7):

$$ClO_2 + e^- \rightarrow ClO_2^-$$
$$ClO_2^- + 2e^- + 3H^+ \rightarrow HOCl + H_2O$$
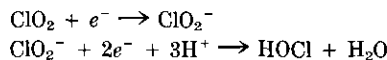
However, the mechanisms of chlorination by ClO_2 and the above transformations are far from being resolved.

Reactions with Carbohydrates

Reactions of $Cl_2(g)$, aqueous chlorine, or ClO_2 with carbohydrates generally result in oxidation products. In reactions with $Cl_2(g)$ or aqueous chlorine, glucose is oxidized at carbons C-2 and C-3 to form ketones. Further oxidation results in cleavage of the C-2 to C-3 bond and formation of carboxylic acids (9–11). Aqueous ClO_2 will also react with glucose; the principal reactions involve oxidation of the hydroxyl groups to form carbonyl derivatives, which are subsequently oxidized to carboxylic acids (12). Reaction products of ClO_2 and glucose have not been identified. β-D-Glucose reacts more rapidly with ClO_2 than α-D-glucose (13).

The reactions of $Cl_2(g)$, aqueous chlorine, or ClO_2 with polysaccharides are primarily oxidative. The actions of $Cl_2(g)$ and aqueous chlorine on starch were extensively studied by Whistler and co-workers (9,14–19). These investigators found that the major effect of $Cl_2(g)$ on starch is depolymerization induced by cleavage at the glycosidic bond between C-1 and the glycosidic oxygen atom. Depolymerization also occurs in starch slurries treated with aqueous chlorine. The mechanism of the opening of the glycosidic bond may involve formation of a carbonium ion at carbon C-1 or a chloro-oxonium complex at the glycosidic oxygen (19). The chloro-oxonium cation can be displaced with either hydroxyl anions from water to form normal sugar end units, or with the hydroxyl group at C-6 to form 1,6 anhydro rings, or levoglucosan units. The extent of levoglucose for-

mation depends on the moisture content of the flour; a higher moisture content results in reduced formation. Oxidation of glucose units at the C-2 and C-3 carbons also occurs, but to a much lesser extent than depolymerization. Huang et al. (11) reported that depolymerization occurs mainly in the amylopectin fraction of starch. The improved baking properties of treated starch are attributed to depolymerization of the glycosidic bond and cleavage between the C-2 and C-3 carbons of glucose (10,11,20,21). The oxidation and depolymerization of starch by $Cl_2(g)$ or aqueous chlorine also occurs with the polysaccharide cellullose (19).

Oligosaccharides have been used as model compounds of cellulose to study ClO_2 bleaching (22). As with reactions of $Cl_2(g)$ and aqueous chlorine, two principal reactions of ClO_2 with the oligosaccharides occur, namely, oxidation of the glycosidic bond and oxidative cleavage of the C-2 and C-3 carbons to form carboxylic acids. The latter reaction proceeds through 2-ketoglucose and 2,3-diketoglucose intermediates. Oxidation may also occur in the cellulose molecules to produce gluconic acid units in the cellulose chains. Hemicellulose, which coexists with cellulose in natural products, reacts much more with ClO_2 than does cellulose (7). However, the reaction products have not been identified.

Although oxidation is the predominant reaction of carbohydrates, Cranet et al. (23) demonstrated that low levels of volatile halocarbons may be produced when D-mannitol is mixed with aqueous chlorine. The mechanism of this reaction is believed to proceed via formation of a ketolactone, which can undergo ring opening by chloride, followed by the classical haloform process. Before this study, carbohydrates were not considered to be significant precursors of haloform formation (24).

The toxicological significance of chlorine-modified carbohydrates is not known. The potential formation of halocarbons from carbohydrates raises some concern about their production in treated flour and other food products. There is no evidence to determine whether other oxidation products are toxic after chronic exposure.

Reactions with Lipids

Lipids constitute a class of organic compounds in foods that readily reacts with chlorine. Leopold and Mutton (25) found that aqueous chlorine completely saturates triolein, the major reactions being addition of chlorine and hydroxyl groups to the double bonds. Chlorine dioxide will also react with triolein, modifying 25% to 50% of the double bonds present. Gilles et al. (26) found that $Cl_2(g)$ decreases total unsaturation in flour lipids. Although significantly modified, the lipid fraction of flour does not play a significant role in the cake-baking potential of flour (27,28).

Using $HO^{36}Cl$ and $^{36}ClO_2$, Ghanbari et al. (5) measured the extent of incorporation of chlorine into free fatty acids and their corresponding methyl esters. The lipids were suspended in a buffered solution, and the

Table 2. Incorporation of [36]chlorine into lipids mixed with aqueous chlorine ($HO^{36}Cl$) or chlorine dioxide ($^{36}ClO_2$) solutions.[a]

Lipid	Number of double bonds	Chlorine incorporated, moles per mole lipid[b]	
		$HO^{36}Cl$	$^{36}ClO_2$
Oleic acid	1	0.155	0.006
Linoleic acid	2	0.247	0.013
Linolenic acid	3	0.387	0.021
Arachidonic acid	4	0.434	0.023
Methyl oleate	1	0.025	0.0039
Methyl linoleate	2	0.054	0.0075
Methyl linolenate	3	0.091	0.0094
Methyl arachidonate	4	0.137	0.0080

[a] Adapted from Ghanbari et al. (5).
[b] Mixtures contained 3.4 mM lipids and 5.07 mM available chlorine equivalent to $HO^{36}Cl$ or $^{36}ClO_2$ in 0.1 phosphate buffer, pH 6.0. The mixtures were incubated for 60 min.

radiolabeled aqueous chlorine or ClO_2 solutions were then added. After 60 min, the modified lipids were extracted with chloroform:methanol (3:1), and the amount of radiolabel in the organic solvent fraction was determined. The amount of label present in the organic extract represented the amount of chlorine incorporated into the lipid. In reactions with either $HO^{36}Cl$ or $^{36}ClO_2$, the percentage of chlorine incorporation correlated with the number of double bonds in the lipid (Table 2). Obviously, the increased number of reaction sites in the more unsaturated fatty acids allowed for greater interaction with chlorine. The free fatty acids incorporated significantly more ^{36}Cl than the methyl esters with the decrease in reactivity of the methyl esters caused in part by their increased hydrophobic nature. After suspending the lipids in the buffered solution, the methyl esters separated out of solution and formed lipid droplets more rapidly than their corresponding fatty acids. Chlorine incorporation was more prevalent with aqueous chlorine than with ClO_2. As previously mentioned, the major reaction of ClO_2 is oxidation rather than chlorination. These results confirm the results of other investigators (25, 29) that HOCl and ClO_2 react with unsaturated fatty acids at their double bonds.

In another study (30), chloroform extracts from aqueous chlorine-oleic acid reaction mixtures were methylated with diazomethane and analyzed by gas chromatography-mass spectrometry (GC-MS). The chromatogram suggested that six compounds were present in the solvent extractable fraction. The only chlorinated compound identified was 9-chloro-10-hydroxymethyl stearate. The other products were identified as hydroxylated products of oleic acid. The chlorinated derivative was the major product formed, representing greater than 95% of the modified lipids. The mechanism of reaction most likely involved addition of chlorine across the double bond to form a carbonium ion, which then combined with a hydroxyl group to form the chlorohydrin (30).

Reactions of ClO_2 with methyl oleate, like aqueous chlorine, will result in oxidation and chlorination across

the double bonds (25, 29). However, the levels of chlorine incorporation into methyl oleate were much less than that with aqueous chlorine. In addition, unsaturated ketones and chloroketones are the result of a different reaction mechanism than that of aqueous chlorine. These products result from the formation of allyl radicals rather than from direct addition to the double bonds (29). Autoxidation also proceeds through this type of radical formation. The carbon–carbon double bond in the unsaturated hydroperoxide formed by autoxidation of methyl oleate has a *trans* configuration. The *trans* configuration of the unsaturated ketones obtained by ClO_2 oxidation agrees with formation through allyl radicals.

Cunningham and Lawrence (31–36) conducted a series of experiments to determine the biological fate and toxicity of chlorinated fatty acids in rats. Chlorinated lipids are moderately toxic; the acute oral LD_{50} for chlorinated oleic acid was approximately 20 g/kg body weight (32). In addition, chlorination reduced the absorption and deposition of all lipids in tissues, the reduction being less severe with chlorinated oleic acid than with chlorinated linolenic or linoleic acid (31,33). Radiolabeled chlorinated fatty acids administered orally to pregnant and lactating rats may be transferred to the fetus and to suckling rats (34). Exposure to chlorinated flour lipids in the diet for 2 weeks reduced the growth rate and increased relative liver weights in rats (36). A decrease in body weight and a corresponding increase in relative organ weights are considered deleterious effects (37). Based on these results, the risks associated with exposure to chlorinated lipids are still not clear. The doses of chlorinated lipids used in these studies were much higher than would be expected from our food supply. However, the biological and possibly toxic effects of chlorinated lipids in laboratory animals should raise some concern about the chronic effects of chlorinated food commodities in consumers.

We conducted some preliminary experiments to determine the mutagenic potential of chloroform:methanol (3:1) extracts of reaction mixtures of aqueous chlorine and fatty acids using the Ames Salmonella/microsome mutagenicity assay (38). Approximately 5.0 mM aqueous chlorine plus 3.4 mM fatty acid in 0.1 M phosphate buffer, pH 6.0, were mixed for a period of time necessary for the levels of available chlorine, measured by iodometric titration, to drop to 25% of the original levels. The reactions were stopped by addition of an equal volume of 0.1 M sodium thiosulfate. The modified lipids in the mixture were extracted three times with equal volumes of chloroform:methanol (3:1). The organic solvent fractions were combined, the solvents were removed using a rotary evaporator, and the lipid residues were weighed and dissolved in dimethyl sulfoxide. None of the solvent-extractable lipids (up to 1.0 mg/plate) possessed mutagenic activity using strains TA 98 or TA 100 with and without metabolic activation. At the higher levels tested, cytotoxicity was observed. These results do not preclude the formation of mutagenic products. Highly volatile and aqueous reaction products and the

products from ClO_2 and lipid reactions have not been tested for their mutagenic potential. Also, the bacterial strains used in the mutagenicity assay may not be sensitive to the mutagenic effects of the reaction products. The formation of carbonyl compounds from reactions of ClO_2 and lipids suggests a potential hazard since carbonyl compounds constitute a potentially important source of human mutagens (39). A new Salmonella tester strain (TA 104) has recently been developed for detecting these carbonyl mutagens (39). Studies are currently underway to determine the mutagenic potential of the chlorinated and oxidized lipid products using this new strain.

Reactions with Amino Acids, Peptides and Proteins

Numerous amino acids, peptides and proteins in biological systems can readily undergo oxidation or chlorination by chlorine compounds. Reactions of various amino acids with aqueous chlorine and ClO_2 have been observed by many other investigators.

Margerum et al. (40) and Isaac and Morris (41) have studied the reactions of HOCl with several amino acids in an aqueous solution at neutral pH. All the amino acids tested reacted rapidly with HOCl. The formation of N-chloro compounds from the reaction mixtures was also characterized. Kirk and Mitchell (1) observed rapid changes and formation of an insoluble precipitate when they mixed aqueous chlorine with tryptophan. Using structurally similar indole compounds as tryptophan models, they concluded that the heterocyclic ring is involved in the reactions of the amino acid with aqueous chlorine. Although the reaction products were not conclusively identified, a monochlorinated product was isolated and partially characterized.

Although most amino acids do not readily react with ClO_2, several amino acids including tyrosine, tryptophan, and cysteine will react rapidly (42–46). Tyrosine forms dopaquinone and dopachrome when oxidized by ClO_2 (43). This reaction has been used as the basis for a colorimetric method to determine the concentration of ClO_2 in water (42). Phenolic compounds such as tyrosine are especially susceptible to oxidation and chlorination by ClO_2 (7). Chlorine dioxide oxidizes tryptophan to form insatin, indoxyl, indigo red, and unidentified yellow-orange substances (43). Oxidation of cystine by ClO_2 results in the formation of cysteic acid through the disulfoxide intermediate (7).

Other investigators have studied the reactions of ClO_2 with amino acids under varying conditions. Schmidt and Braunsdorf (47) found that the least reactive amino acids were glycine, leucine, serine, alanine, phenylalanine, valine, hydroxyproline, and aspartic and glutamic acids. When a mixture of tryptophan, tyrosine, proline, threonine, methionine, valine, and lysine was reacted with diaphanol (a 50% solution of glacial acetic acid saturated with ClO_2), the aromatic amino acids disappeared after 18 hr (48). These results indicate that aromatic amino

acids tend to be more susceptible than aliphatic amino acids to oxidation by ClO_2.

When exposed to aqueous chlorine solutions, peptides and proteins are subject to oxidation, substitution, and addition reactions (49). Chlorine incorporation into proteins has been demonstrated in shrimp (50), poultry and meat (51), egg albumin (52), flour proteins (43,53), gelatin (54), casein, and bovine serum albumin (55,56).

When flour was treated with ten times the commercial level of $Cl_2(g)$, the levels of methionine, cysteine, tyrosine, and histidine decreased by 68.3, 40.8, 31.6, and 16.8%, respectively (44). Tsen and Kulp (53) observed that the amount of water-extractable proteins in treated flour increased as chlorine concentration increased. The authors suggested that proteins were progressively cleaved with increasing chlorine concentration. In addition, dipeptides will form N,N-dichlorodipeptides in a concentrated aqueous chlorine solution (57). However, the amino group involved in the peptide bond is not attacked by aqueous chlorine at room temperature.

Meredith et al. (58) treated wheat flour with high levels of ClO_2 and did not find any protein or amino acid chloroderivatives. However, the levels of tryptophan and cystine were reduced to about 92 and 75%, respectively, of their original levels. In addition, the oxidation of cysteine to cysteic acid and of methionine to various oxides has been observed in flour treated with ClO_2 (59). Other changes observed when wheat flour is exposed to high levels of ClO_2 include an increase in water solubility of the flour proteins (44). When solutions of wheat proteins are exposed to ClO_2, a pink-yellow color develops as a result of oxidation of tyrosine and tryptophan (44).

Very little is known about the toxicity of the oxidized or chlorinated derivatives of amino acids and proteins. Certain amino acids, such as aspartic acid, tryptophan, and tyrosine, as well as proteinaceous material may undergo halogenation to form dihaloacetonitriles and trihalomethanes (60), some of which have been shown to be genotoxic (61,62). In addition to tryptophan, Morris and Baum have identified proline and hydroxyproline as potential precursors of chloroform formation during water chlorination (24). The toxicities of the nonvolatile, halogenated, or oxidized products, however, are not known.

Süssmuth (63) tested the genotoxicity of the products formed from the chlorination of amino acids. Chlorination of methionine, tyrosine, phenylalanine, cysteine, and glycine resulted in the formation of mutagenic products in the Ames assay; however, the products were not identified. Horth et al. (64) also tested the mutagenic activity of extracts of chlorinated amino acids using the Ames assay. With the exception of glycine, mutagenic activity was observed in all of the extracts tested with the most potent precursors being methionine, tyrosine, phenylalanine, and a mixture of heterocyclic amino acids. They identified several compounds in the extracts by gas GC-MS. Only one of the identified products, dichloracetonitrile, is a known mutagen and was considered unlikely to account for a significant proportion of

Table 3. Incorporation of [36]chlorine during chlorination of flour with chlorine gas [[36]Cl_2(g)].[a,b]

Flour component	Cl incorporated, %
Total flour	99.3
Chloroform extract (lipids)	33.3
Chloroform extract:	
Nonpolar lipids	31.2
Polar lipids	68.8
Water-insoluble fraction	21.0
Water-soluble organics	4.7
Chloride ion ([36]Cl⁻)	40.3

[a] Adapted from Wei et al. (65).

[b] 20 g of unbleached flour, pH 5.8, was chlorinated to pH 5.25 using radiolabeled chlorine gas with a specific activity of 1.26×10^6 dpm/mg.

the observed mutagenic activity. Nonvolatile products of chlorination were not tested for their mutagenic activity.

We are currently investigating the potential genotoxicity of reaction products of amino acids and chlorine compounds. In a preliminary study, using the Ames mutagenicity assay, we tested the mutagenic activity of the nonvolatile precipitate that forms immediately when tryptophan and aqueous chlorine in 0.1 M phosphate buffer, pH 6.0, are mixed. The precipitate was not mutagenic to TA 98 (up to 0.5 mg/plate) with and without metabolic activation. However, these preliminary results do not prove conclusively that the reaction products of tryptophan are not toxic. For example, antagonistic effects of two or more reaction products present in the precipitate may cancel or mask any mutagenic response. In addition, reaction conditions may not accurately reflect those found in the food industry.

Reactions with Food Products

Flour is commonly treated with 1,200 to 2,500 ppm $Cl_2(g)$ as a bleaching agent and to enhance its baking properties. The ability of chlorine to improve the baking properties of flour prompted many investigations into the reactions of chlorine with flour components.

Early studies demonstrated that the gluten and lipid fractions of flour were susceptible to chlorination (20, 21). After fractionating bleached flours, approximately half the chlorine can be recovered in the water soluble fraction, presumably in the form of the chloride ion. Gilles et al. (26) analyzed chlorine content in bleached flour using X-ray spectroscopy. The lipid and water contents, which represent approximately 5% of the total flour, were found to contain greater than 90% of the chlorine introduced during the bleaching process. Using $^{36}Cl_2$ (g), Wei et al. (65) demonstrated almost complete incorporation of the Cl_2 (g), into the flour (Table 3). The highest levels of the ^{36}Cl in the flour were present as chloride ion or were incorporated into the lipids.

The presence of the chloride ion indicates a reaction occuring in which chlorine is reduced to the ion. A likely target of chlorine oxidation is the starch fraction, which can be readily oxidized and depolymerized. Uchino and Whistler (14) determined that, under normal chlorina-

Table 4. Incorporation of [36]chlorine in shrimp following
immersion in a 150 ppm solution of [36]Cl-labeled hypochlorous
acid.[a,b]

Biological component	[36]Cl incorporated, %
Total from original solution	2
Of total:	
Nonedible portion	25
Edible portion	75
Of edible portion:	
$CHCl_3$:MeOH fraction (lipids)	3
Trichloroacetic acid precipitate (proteins)	22
[36]Chloride ions ([36]Cl⁻)	73
Water-soluble organics	2

[a]Adapted from Johnston et al. (50).
[b]Shrimp were incubated for 30 min, pH 6-7, at room temperature.

tion treatment of flour, 42% of the chlorine that is consumed is required for the resulting starch depolymerization. Interestingly, Wei et al. (65) found 47% of chlorine in their model system to be in the form of the chloride ion. Other studies have indicated that the actions of chlorine on the starch fraction are responsible for the improved baking qualities of bleached flour (10, 11, 20, 21).

Considerable research has been conducted to determine the toxicity of chlorinated flour. Cunningham et al. (66) reported that weanling rats fed flour chlorinated at 2,000 and 10,000 ppm showed reduced growth rates and increased liver and kidney weights after 2 weeks of exposure. As mentioned previously, similar effects were noted in rats fed chlorinated proteins or lipids derived from bleached flour (36). In a 4-week feeding study, Fisher et al. (67) exposed rats to diets containing chlorinated (1257 or 2506 ppm chlorine) flour. Although a slight dose-related increase in relative kidney and liver weights was found in the treated animals, no pathological tissue changes were observed. In a chronic feeding study, rats fed flour chlorinated at levels used commercially (1200 to 2500 ppm), did not develop any pathological lesions, and their body and organ weights were the same as those of controls (68). In a similar study with mice, a dose-related increase in heart and kidney weights, and a decrease in ovary weights were observed (69). However, no evidence of carcinogenicity was apparent following treatment with chlorinated flour. The evidence indicates that chlorination of flour at levels used commercially does not pose a significant human health risk.

Chlorine incorporation into shrimp immersed in a 150 ppm solution of $HO^{36}Cl$ was recently determined (Table 4) (50). Approximately 2% of the chlorine was incorporated into shrimp, with 75% of this amount found in the edible portion. Most of the chlorine associated with the edible portion was present as chloride ion. Lipid analysis indicated that the more highly saturated fatty acids (i.e., cholesterol esters) incorporated less chlorine. In addition, phospholipids incorporated more chlorine than neutral lipids. The hydrophilic moiety of the phospholipid increased its solubility in the aqueous chlorine

solution, allowing for the greater incorporation of chlorine.

Cunningham and Lawrence measured the incorporation of [36]Cl into pieces of beef, pork, and poultry immersed in a 200 ppm aqueous [36]Cl solution (51). Within 2 hr, over 50% of the chlorine reacted with both the lean and fat fractions of the meats. Most of the chlorine was reduced to chloride ion, which leached into the water. Nevertheless, significant amounts of chlorine reacted with tissue lipids and remained in the meat.

Robinson et al. (70) investigated the formation of chloroform in freshly eviscerated poultry carcasses exposed to water containing aqueous chlorine or chlorine dioxide. Treatment conditions simulated commercial broiler chicken processing. Low levels of chloroform were detected in carcasses that were treated with aqueous chlorine or ClO_2 solutions. The highest concentration of chloroform (447 ppb) was found in carcasses immersed in water containing 50 mg/L of aqueous chlorine. The chloroform concentration was higher in depot fat than in the other tissues examined. The source of the chloroform is unknown, although the chloroform may have been present in the aqueous chlorine or ClO_2 solutions before the chickens were immersed. Whether the immersion water was analyzed for chloroform content before the carcasses were treated was not mentioned. Nevertheless, the possibility that chloroform was formed from the reaction of chlorine or ClO_2 with organic chemicals found naturally in the food product cannot be ruled out.

Summary

This report has focused on the reactions of chlorine compounds with carbohydrates, lipids, and proteins. Not mentioned are many other organic chemicals present in food that may react with chlorine, including the nucleic acids, vitamins and vitamin precursors, complex lipids, steroids, and other chemicals and essential trace elements. In most cases, the reactions of chlorine compounds with these food components have not been studied.

To determine adequately the risks of using chlorine compounds in the food industry, the amounts of chlorine used and the reactivities of the chlorine compounds with food chemicals must be examined. Also, the reaction products must be identified and their toxicities assessed. Finally, the amounts of these reaction products to which consumers are exposed should be determined. At this time, it is too early to assess the risks of using chlorine compounds in foods. It is hoped that we may have an answer in the near future.

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