

Effect of Immersion Chilling of Broiler Chicken Carcasses in Monochloramine on Lipid Oxidation and Halogenated Residual Compound Formation

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ABSTRACT

This study was conducted to evaluate the effect of immersion chilling of broiler chicken carcasses in tap water (TAP) or TAP containing 50 ppm of monochloramine (MON) with respect to chloroform formation, total chlorine content, 2-thiobarbituric acid (TBA) values, and fatty acid profiles. Ten broiler chicken carcasses were chilled in TAP or MON for 6 h. After exposure, the carcasses were removed and cut in half along the median plane into right and left halves. After roasting the left halves, samples of the breast, thigh, and skin (with fat) were collected, subjected to fatty acid profiling, and assayed for chloroform, total chlorine, and TBA. The uncooked right halves of each carcass were stored at 4°C for 10 days and then roasted. After roasting these right halves, samples of breast, thigh, and skin (with fat) were collected from each carcass half, subjected to fatty acid profiling, and assayed for chloroform, total chlorine, and TBA. There were no statistical differences between TAP- and MON-treated fresh or stored products with regard to chloroform levels, total chlorine content, TBA values, or fatty acid profiles.

Free available chlorine (FAC), composed of hypochlorous acid (HOCl) and the hypochlorite ion (OCl⁻), is highly reactive and rapidly oxidizes, bleaches, or otherwise reacts with any number of substances (22). The widespread use of FAC as a disinfectant in food processing has raised food safety concerns regarding the potential for trihalomethane (THM) formation and chlorine incorporation into the food. Several studies have reported on the incorporation of chlorine into beef, pork, chicken, and shrimp (2, 5–7). The immersion of shrimp in 150 ppm of chlorine for 30 min resulted in 2% of the FAC being incorporated into the shrimp, and 75% of this amount was detectable in the edible portion (6, 7). These authors found that chlorine bound more readily to unsaturated fatty acids. High levels of unsaturated fatty acids in poultry products, coupled with research that determined HOCl to have the ability to destroy antioxidants, such as that conducted by Kanner and Kinsella (8), further raised the level of concern with the widespread use of FAC in the poultry processing industry.

The U.S. Department of Agriculture Food Safety and Inspection Service allows the addition of chlorine to poultry processing water at levels of up to 50 ppm in carcass wash applications and chiller make-up water (18). If an immersion chiller is treated directly, the concentration of FAC in the chiller must be <50 ppm before the first carcass exits the chiller, and once the carcasses have begun exiting the chiller, the FAC in the water returning from the red water chilling system must be <5 ppm before that water reenters the chiller (19).

Research has shown that a FAC residual in a loaded immersion chiller cannot be established under practical conditions. One study demonstrated that the addition of 400 ppm of FAC to equilibrated chiller water was insufficient to establish a FAC residual, because the chlorine demand in the chiller exceeded the 400-ppm dose (15). The entire amount of added FAC was consumed in the chiller, some of which reacted with chiller contents, such as ammonia and organic amines from the chicken carcasses, to randomly form various chlorinous compounds, such as the beneficial biocide monochloramine (MON) and other undesired nonbiocidal organic chloramines. Because none of the added FAC remained as free chlorine, the total chlorine content of the chiller was composed entirely of combined chlorine. The use of FAC as a chiller chlorination control metric does not capture the combined chlorine component of the water.

When the U.S. Environmental Protection Agency (USEPA) published the disinfection by-products rules establishing the maximum allowable concentrations of THMs in potable water systems (20), the managers of many potable water treatment plants that used FAC to disinfect water in the water treatment plant and broader distribution systems found themselves out of compliance. The problem of excess THM formation caused by FAC reacting with components of the natural organic material (NOM) in potable water systems was solved in numerous potable water treatment plants by switching from FAC to MON treatments in their distribution systems.

The National Primary Drinking Water Regulations of the USEPA establish maximum residual disinfection levels in potable water for both chlorine (FAC) and chloramine at

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4.0 mg/liter (calculated as Cl_2) (16). The U.S. Food and Drug Administration adopted the USEPA's maximum residual disinfection levels as the allowable levels of chlorine and chloramine in the quality standard regulation for bottled water (17).

A large body of research has established that chloramination of potable water with MON is safe. Switching from FAC to MON in the distribution systems of publicly owned treatment works not only achieved the primary goal of reducing the quantities of THMs produced, but also often improved the water quality by eliminating chlorinous tastes and odors and reducing biofilm formation (22). The characteristics of MON that made its use successful in the potable water treatment industry gave rise to consideration for its use in the food processing industry (12).

MON is tasteless, odorless, stable, highly soluble, and persistent in water, as well as biocidal (21). MON does not tend to react with organic material and become deactivated upon contact, as does FAC. To the contrary, it remains available to inactivate microbial populations and is therefore a more effective disinfectant than FAC in systems in which high organic loads exist (12). Although MON has historically been assumed to be ineffective as a process water treatment because of its relatively low specific lethality to microorganisms in fresh water, research has shown that, given contact times of about 1 h, its antimicrobial efficacy is equivalent to FAC (22). Contact times of about 1 h are typical in poultry immersion chillers, with some processes lasting as long as 6 h.

When FAC reacts, it generally loses its biocidal properties and can no longer act as a disinfectant (22). One notable exception is when it combines with ammonia to produce MON. Previous studies have indicated that MON at concentrations not exceeding 50 ppm of total chlorine is a superior antimicrobial treatment in immersion chillers for reducing bacterial populations on broiler chickens (12). This study was undertaken to assess the effect of exposure to MON on lipids and the formation of halogenated compounds in broiler carcasses in a poultry chiller application.

MATERIALS AND METHODS

The experimental design for this study was $2 \times 5 \times 2 \times 4$ (treatment, chicken, storage condition, and assay, respectively). Means were separated by the analysis of variance procedure from SAS (13), with significance evaluated at the $\alpha = 0.05$ level. MON was manufactured at the time of use by the controlled mixing of 6% sodium hypochlorite and a solution of Food Chemical Codex-grade ammonium chloride at a 5:1 ratio of chlorine (calculated as Cl_2) to ammonia-nitrogen ($\text{NH}_3\text{-N}$) in tap water (TAP). Treatment concentrations were measured and verified by Hach model CN-21P high-range chlorine test kits (Loveland, Colo.) with sulfite and sulfamic acid powder pillows and sodium thiosulfate reagents.

Ten broiler chicken carcasses were collected before entering the immersion chiller from a commercial poultry-processing facility. Two plastic containers were prepared containing (i) 28.4 liters of TAP with an initial nominal concentration of 1 ppm of FAC as the untreated control or (ii) 28.4 liters of TAP with 50 ppm of MON. Then, five carcasses each were placed into the two solutions, which were then aerated and chilled for 6 h. After 3 h, the MON solution was tested and adjusted to maintain the 50-

ppm treatment level. After 6 h, the carcasses were removed and cut in half along the median plane into right and left halves. The left halves were roasted in an oven at 350°F for 45 min. The uncooked right halves were stored at 4°C for 10 days, after which they were roasted in an oven at 350°F for 45 min. Immediately after roasting, samples of breast, thigh, and skin (with fat) were collected from each carcass half and individually bagged, labeled, and shipped on ice overnight to the ABC Research Corporation (Gainesville, Fla.), where they were frozen at -12°C pending fatty acid profiling and analysis for the concentration of chloroform, total chlorine, and 2-thiobarbituric acid (TBA).

Chloroform was used as the indicator for comparing the residual THM levels on the products treated with TAP and MON. Samples were assayed for chloroform by AOAC Official Method 977.18, Gas Chromatographic Method, which was modified as follows. Chloroform was extracted by blending 100 ml of isopropyl alcohol (CAS 67-63-0, pesticide grade, Fisher, Fair Lawn, N.J.) with each chicken sample. The resulting solution was filtered with a syringe and a Whatman 0.45- μm nylon filter (cat. no. 6870-2504, Florham Park, N.J.) prior to injection on a Varian CP-3800 gas chromatograph (Palo Alto, Calif.). Instrument parameters were as follows. The injector temperature was 150°C. The oven was programmed at 50°C with a 5-min hold time and then a 10°C/min increase to 120°C with no hold and a 45°C/min increase to 250°C with a 1-min hold, resulting in a total run time of 15.89 min. The temperature of the electron capture detector was 180°C. Varian wall-coated open tubular fused silica gas chromatography columns (cat. no. CP 8510) were used in place of the packed columns identified in the standard method. A Star Chromatography Workstation version 6.2 with Service Pack 1 software (Varian) was used in place of chart recorders.

Total chlorine levels were measured to compare differences in the amount of chlorine that was incorporated into the samples between the TAP and MON treatments. Total chlorine levels were assayed by Hach method 10014 with a DR/4000 spectrophotometer (Hach), which is a USEPA-accepted DPD (*N,N*-diethyl-*p*-phenylene diamine) method for determining trace levels of total chlorine in treated domestic and industrial wastewater, and was adapted from *Standard Methods for the Examination of Water and Wastewater* (1). Assayable slurries were prepared by working 2 to 4 g of each product sample into 20 ml of deionized water. To avoid chlorine loss, turbidity that could interfere with sample analysis was removed with a 3- μm membrane filter after reacting the DPD with the chlorine in the sample. This method incorporated several modifications to normal DPD chlorine methods, including the use of a Hach Pour-Thru Cell to give more stable readings than are possible with movable sample cells and liquid reagents packaged in ampules and sealed under argon gas to ensure stability (ULR Chlorine Reagent Set, Hach). Test results were measured at 515 nm.

TBA assays were used to compare differences in the amount of lipid oxidation that occurred in the samples between the TAP and MON treatments. TBA values were determined by AOAC Official Method 935.47 (Turner), in which partially frozen samples were treated with TBA (Sigma-Aldrich, Milwaukee, Wis.), trichloroacetic acid (Sigma, St. Louis, Mo.), and pyridine (Fisher) solutions and digested from their prior fat oxidation forms. The solutions were then recovered and quantified on a spectrophotometer at 538 nm.

Fatty acid profiles were used to compare differences in the amount of oxidation that occurred in individual fatty acids between the TAP and MON treatments. Fat extraction for fatty acid profile assays was accomplished by a Bligh Dyer technique that was modified as follows. A 300-ml solution of two parts chloro-

TABLE 1. Comparison of chloroform concentrations in roasted chicken breast meat, thigh meat, and skin with fat treated by 6 h of immersion chilling in tap water or tap water containing monochloramine (50 ppm)^a

	Tap water control, fresh meat	Monochloramine, fresh meat	Monochloramine, stored meat
Breast meat	0.06 ± 0.07	0.03 ± 0.05	0.10 ± 0.08
Thigh meat	0.27 ± 0.04	0.19 ± 0.06	0.13 ± 0.10
Skin with fat	0.22 ± 0.04	0.18 ± 0.03	0.23 ± 0.15

^a Values are parts per million. $n = 5$ for each data point. For samples that were below the level of detection, the minimum detectable level of 0.01 ppm was applied.

form (CAS 67-66-3, pesticide grade; Fisher) to one part 99.8% methanol (CAS 67-56-1, Caledon, Georgetown, Ontario, Canada) was blended with about 50 g of sample for 2 min. The mixture was filtered through a Büchner funnel into an Erlenmeyer flask under vacuum. Extraction was repeated two times for a total volume of ca. 900 ml of filtrate, which was then transferred to a separation funnel. One hundred milliliters of saturated NaCl (CAS 7647-14-5, certified ACS; Fisher) solution was added, and the layers were allowed to separate for 2 h. The lower layer was filtered through sodium sulfate (CAS 7757-82-6, anhydrous, certified ACS; Fisher), and the chloroform was evaporated on a rotovap. The final extract of fat was purged under nitrogen (compressed; Airgas, Radnor, Pa.) to remove any residual chloroform.

Fatty acids were derived as follows. Eight milliliters of 0.5 M NaOH (CAS 1310-73-2, certified; Fisher) was added to 0.5 g of fat and refluxed for 15 min to dissolve all fat. Nine milliliters of boron trifluoride (CAS 7637-07-2, 14% in methanol; Sigma-Aldrich) was added to the solution, which was then refluxed for another 2 min. Five milliliters of hexane (CAS 110-54-3, 99.5%; Caledon) was added and refluxed for another minute. Twenty-five milliliters of saturated NaCl (CAS 7647-14-5, certified ACS; Fisher) was added to the solution, which was then shaken vigorously. The hexane layer was transferred to an amber vial containing a few grams of sodium sulfate to remove any water in the sample. One milliliter of this sample was transferred to an amber vial containing 7 ml of hexane (CAS 110-54-3, 99.5%; Caledon) and 0.5 ml of C23:0 internal standard (methyl tricosanoate) (CAS 2433-96-7, >99%; Nu-Chek Prep, Elysian, Minn.). About 1 ml of this solution was transferred to a gas chromatography vial for injection on a Varian CP-3800 gas chromatograph. Instrument parameters were as follows. The temperature of the injector was 250°C. The oven was programmed for a 1-min hold time at 180°C and then a 1°C/min increase to 200°C with no hold and a 5°C/min increase to 230°C with an 18-min hold, resulting in a total run time of 45 min. The temperature of the pulsed flame photometric detector was 50°C. The SGE (Scientific Glass Engineering) capillary gas chromatography columns were coated with BPX70 (70% cyanopropyl polysilphenylene-siloxane) as the stationary phase and were 60 m long with an inner diameter of 0.25 mm (cat. no. GCO-5513, Phenomenex, Torrance, Calif.). The gas chromatograph was controlled with a Star Chromatography Workstation version 6.2 with Service Pack 1 software (Varian).

RESULTS AND DISCUSSION

There were no significant differences in chloroform levels detected in fresh or stored breast meat, thigh meat, or skin and fat between the chickens treated with the TAP control and MON (Table 1). These chloroform assays are

TABLE 2. Comparison of total chlorine concentrations in roasted chicken fat and skin treated by 6 h of immersion chilling in tap water or tap water containing monochloramine (50 ppm)^a

	Tap water control	Monochloramine
Fresh skin and fat	0.7 ± 0.5	0.3 ± 0.2
Stored skin and fat	0.4 ± 0.2	0.3 ± 0.2

^a Values are parts per million. $n = 3$ to 5 ($n < 5$ when individual sample sizes were insufficient to conduct assay). For samples that were below the level of detection, the minimum detectable level of 0.2 ppm was applied.

consistent with the literature that characterizes MON as slow to react with organic material and therefore likely to minimize trihalomethane formation.

For the TAP control treatments, total chlorine was below the detection limit of 0.2 ppm in all fresh and stored breast meat samples and was detected in 33% of both fresh and stored thigh meat samples. For the MON treatments, total chlorine was below the detection limit in all fresh and stored breast meat and fresh thigh meat samples and was detected in 25% of the stored thigh meat samples. However, analysis for total chlorine showed that it was detected in 100 and 50% of the TAP-treated fresh and stored skin or fat samples, respectively, and in 67 and 60% of the MON-treated fresh and stored skin or fat samples, respectively. These results are in consonance with the literature that indicates the probability of chlorine incorporation to be substantially greater in skin and fat than in muscle tissue (5, 7). Also, as anticipated by the low reactivity of MON with respect to organic material, as found in the literature (22), no differences were found in incorporated chlorine levels between the skin and fat samples of chickens treated with TAP and those treated with MON (Table 2).

Because of the many variables that can affect TBA values, such as analytical methods used, lipid composition of individual carcasses, cooking parameters, and storage conditions, data obtained from the various studies reported in the literature (11, 14) were not compared directly to the results from this study. Within the confines of this experiment, the values for fresh and stored products treated with MON were not statistically different from products treated with the TAP controls (Table 3). These results are consistent with the literature on the topic that characterizes MON

TABLE 3. Comparison of TBA values in roasted chicken breast meat, thigh meat, and skin with fat treated by 6 h of immersion chilling in tap water or tap water containing monochloramine (50 ppm)^a

	Tap water control, fresh meat	Monochloramine, fresh meat	Monochloramine, stored meat
Breast meat	3.86 ± 1.12	3.16 ± 0.39	2.73 ± 0.41
Thigh meat	3.59 ± 0.46	3.62 ± 0.11	3.39 ± 0.03
Skin with fat	2.58 ± 0.34	2.96 ± 0.43	2.03 ± 0.29

^a Values are milligrams of malonaldehyde per kilogram of chicken. $n = 5$ for each data point. Detection limit, 0.05 mg/kg.

TABLE 4. Comparison of the fatty acid content in roasted chicken treated by 6 h of immersion chilling in tap water or tap water containing monochloramine (50 ppm)^a

	Oleic		Linoleic		Linolenic		Arachidonic	
	TAP	MON	TAP	MON	TAP	MON	TAP	MON
Fresh breast	33.9 ± 1.9	35.6 ± 2.0	16.9 ± 1.5	16.0 ± 1.1	0.18 ± 0.06	0.16 ± 0.09	1.00 ± 0.90	0.10 ± 0.00
Stored breast	42.4 ± 1.9	40.4 ± 1.0	18.9 ± 0.9	18.8 ± 0.7	0.28 ± 0.02	0.26 ± 0.05	0.10 ± 0.00	0.24 ± 0.19
Fresh thigh	35.6 ± 3.3	35.8 ± 1.1	16.1 ± 1.3	16.5 ± 1.8	0.21 ± 0.13	0.15 ± 0.11	0.14 ± 0.09	0.10 ± 0.00
Stored thigh	38.9 ± 11.2	40.1 ± 3.3	16.7 ± 2.1	17.7 ± 1.6	0.30 ± 0.05	0.33 ± 0.03	0.10 ± 0.00	0.17 ± 0.15
Fresh skin and fat	38.1 ± 1.8	39.5 ± 1.4	16.3 ± 1.5	17.7 ± 1.0	0.17 ± 0.08	0.38 ± 0.13	0.10 ± 0.00	0.10 ± 0.00
Stored skin and fat	39.7 ± 11.2	45.2 ± 1.4	16.2 ± 1.3	17.0 ± 0.8	0.34 ± 0.03	0.39 ± 0.02	0.10 ± 0.00	0.10 ± 0.00

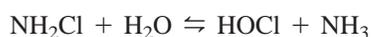
^a Values are percentages of total fat. *n* = 5 for each data point.

as an exceptionally weak oxidizer that cannot bleach and is not prone to react with organic material (22).

The fatty acid profiles were conducted with a focus on the effect that MON has on oleic and linoleic acids, the major unsaturated fatty acids in poultry fat, as well as on arachidonic, linolenic, and linoleic acids, which are generally recognized as the primary targets for exogenous oxidants such as FAC (10). Because the fatty acid profiles of chicken tissues are influenced by a number of variables, such as the age of the bird and the fatty acid composition of the dietary fat (4), no attempt was made to compare the results obtained in this study with those reported in other research. With respect to oleic acid, linoleic acid, linolenic acid, and arachidonic acid, the experimental data obtained in these experiments indicate that no significant differences exist between fresh and stored products that have been treated with either TAP or MON (Table 4). These results are supported by other experimental data reported in the literature that show, for example, that no chlorine incorporation occurred in laboratory studies when oleic acid was reacted with chloramine (5).

By eliminating high doses of FAC and substituting controlled doses of MON, the concern with chlorine incorporation in products from chiller treatments can be alleviated. Because naturally occurring ammonia from chicken carcasses is present in all immersion chillers, adding FAC to these chillers will produce varying levels of MON; however, far more organic nitrogen in the form of proteins and amino acids is present, which, when combined with FAC, produces nonbiocidal organic chloramines. Because there is no residual free chlorine in an equilibrated immersion chiller (15), the combined amounts of the inorganic chloramines (i.e., MON) and organic chloramines make up the total chlorine content of the chiller water. When FAC is used to treat the chiller water, the quantity of MON produced is a small percentage of the total chlorine. Conversely, when MON is used, 100% of the total chlorine dose is composed of MON, the biocidal species of the various combined chlorine compounds.

Some molecules of MON, however, can hydrolyze over time, yielding the chemical species according to the following equilibrium (22):



In a poultry chiller, the resultant FAC would either quickly

recombine with the ammonia or react with another constituent of the chiller. These experiments produced no differences between TAP- and MON-treated products, which indicates that insufficient FAC was produced from the hydrolysis of MON to affect chlorine incorporation.

Previous research indicates that the ratio of FAC to total organic carbon must be taken into account when attempts are made to predict how much chloroform is produced. The propensity of THMs to form in potable water distribution systems may be relatively high because the chlorine concentration is high compared to the NOM in the water supply (3). Chlorination of NOM results in the production of *N*-chloro compounds, some of which are chloroform (9), but three chlorine atoms must combine with a single carbon for this to occur. When the organic load in the water becomes exceptionally high compared to the concentration of FAC, as in poultry immersion chillers, the probability of three chlorine atoms combining with the same carbon atom will become much more unlikely. For example, in a potable water system, the chlorine concentration may be 2.0 ppm when the NOM is 0.1 ppm, yielding a Cl₂-to-NOM ratio of 20:1. In a poultry chiller, if the chlorine concentration were 50 ppm and the organic load were 1,000 ppm, the Cl₂-to-NOM ratio would be 1:20. The lack of discernible differences between the incorporation of chloroform into TAP- and MON-treated poultry samples in any of the metrics analyzed in these studies is understandable given the low reactivity of MON, the low production of transient FAC from hydrolysis, and the high relative concentration of organic material.

If total chlorine were used as the control metric in an immersion chiller, the sum of the various chlorine species present could never exceed the amount based on this metric, because total chlorine is the sum of FAC plus all the species of combined chlorine. Additionally, dosing with MON would maximize the combined biocidal chlorine residual and preclude excessive dosing with FAC in an unproductive effort to establish a FAC residual.

These studies indicate that 50-ppm concentrations of MON in a poultry chilling operation for up to 6 h do not contribute to lipid oxidation or halogenated residual compound formation beyond what occurs from chilling in TAP. The same characteristics of MON that led to its widespread use in drinking water distribution systems make it an at-

tractive treatment in food processing waters such as those found in poultry immersion chillers.

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