Determination of Chlorate and Chlorite and Mutagenicity of Seafood Treated with Aqueous Chlorine Dioxide

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The use of chlorine dioxide (ClO_2) as a potential substitute for aqueous chlorine to improve the quality of seafood products has not been approved by regulatory agencies due to health concerns related to the production of chlorite (ClO_2^-) and chlorate (ClO_3^-) as well as possible mutagenic/carcinogenic reaction products. Cubes of Atlantic salmon (*Salmo salar*) and red grouper (*Epinephelus morio*) were treated with 20 or 200 ppm aqueous chlorine or ClO_2 solutions for 5 min, and extracts of the treated fish cubes and test solutions were checked for mutagenicity using the Ames *Salmonella*/microsome assay. No mutagenic activity was detected in the treated fish samples or test solutions with ClO_2. Only the sample treated with 200 ppm chlorine showed weak mutagenic activity toward *S. typhimurium* TA 100. No chlorite residue was detected in sea scallops, mahi-mahi, or shrimp treated with ClO_2 at 3.9−34.9 ppm. However, low levels of chlorate residues were detected in some of the treated samples. In most cases, the increase in chlorate in treated seafood was time- and dose-related.

Keywords: *Chlorine dioxide (ClO_2); chlorite; chlorate; mutagenicity; seafood*

INTRODUCTION

Chlorine dioxide (ClO_2) as a disinfectant for water treatment and the food industry has drawn great attention in recent years. Problems associated with chlorine (as hypochlorous acid [HOCl] and hypochlorite ion [OCl^-]) for water disinfection have occurred because of the formation of trihalomethanes (THMs) and other potentially mutagenic/carcinogenic reaction byproducts. Chemical analyses of chlorinated water samples have detected hundreds of nonvolatile chlorinated hydrocarbons of high molecular weight, including chlorinated ketones, aldehydes, carboxylic acids, and alcohols (Stevens et al., 1990). Kool et al. (1985) showed that chlorine treatment of drinking water increased the mutagenic activity in *Salmonella typhimurium* strains TA 98 and TA 100.

ClO_2 is considered to be a more effective bactericide than aqueous chlorine, and it reduces the formation of chlorinated organics. Tanner (1989) demonstrated that ClO_2 had the highest biocidal activity against several bacteria and yeast in comparison with sodium hypochlorite, iodine, and quaternary ammonium compounds. Several studies have shown the efficacy of ClO_2 in controlling *Salmonella* and other bacterial contaminants on poultry carcasses (Lillard, 1980; Thiessen et al., 1984; Villarreal et al., 1990). In contrast to aqueous chlorine, the efficiency of ClO_2 disinfection does not vary with pH or presence of ammonia. Kim et al. (1997, 1998) showed that ClO_2 treatment of fish fillets did not greatly affect their fatty acid composition or the components protein, fat, vitamins, minerals, and moisture. ClO_2 is becoming more widely used in the food industry and is garnering regulatory approval for direct food contact. The Food and Drug Administration (FDA) amended on March 3, 1995, the food additive regulations (21 CFR §173.69) to allow a 3 ppm residual ClO_2 for controlling microbial populations in poultry processing water (*Fed. Regist.*, 1995). The increasing use of ClO_2 has warranted the need to monitor the levels of possible hazardous byproducts including chlorite (ClO_2^-) and chlorate (ClO_3^-). Tsai et al. (1995) reported that ClO_2 was formed to chlorite and chlorate, and the formation of chlorite in poultry chiller water was dependent on ClO_2 dose and treatment time. According to the U.S. Environmental Protection Agency (USEPA, 1993), the maximum contaminant level for chlorite in drinking water is 1.0 ppm. Chlorate ion has been listed as a potential candidate for regulation because it is a disinfection byproduct. However, the health effects associated with exposure to ClO_3^- are still incomplete (Bolyard et al., 1993).

ClO_2 is a strong oxidant that reacts with organic material to produce a variety of oxidized byproducts. Neither ClO_2 nor its byproducts, ClO_2^- and ClO_3^-, react with humic or fuvic acids to form THMs, as does chlorine in drinking water treatment (Miltner, 1977). Although ClO_2 is known not to form THMs when used in drinking water treatment, this may not be the case during seafood treatment. Seafoods contain compounds such as proteins, lipids, vitamins, minerals, color, etc., and mutagenic reaction products may be produced following ClO_2 treatment. Therefore, this study was conducted to (1) examine if any potential mutagenic reaction products are generated following treatment of seafood with ClO_2 using the Ames *Salmonella*/microsome assay and (2) determine if chlorite and chlorate ions are produced in seafood following treatment with ClO_2. Such information is useful for the FDA in its evaluation to approve the use of ClO_2 for seafood treatment.

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MATERIALS AND METHODS

Preparation of Chlorine Demand-Free Water and Aqueous Chlorine. Chlorine-demand-free water (CDF water) was prepared following the method of Ghanbari et al. (1982) by passing distilled water through two successive Barnstead deionizing units and then a glass column containing Porapak Q (Supelco, Bellefonte, PA).

The modified method of Ghanbari et al. (1983) was used to generate aqueous chlorine. Chlorine gas was generated by the dropwise addition of 8 mL of 3 N HCl solution to 4 g of potassium permanganate ($KMNO_4$, Fisher Scientific, Fair Lawn, NJ) in a closed gas generation apparatus. Chlorine gas was trapped as HOCl in 200 mL of ice-cold CDF water.

Preparation of Activated ClO_2 Stock Solution. The ClO_2 stock solution was prepared from Oxine concentrate (Bio-Cide International, Inc., Norman, OK) (Kim et al., 1998). This stock solution was used to prepare working solutions in 3.5% brine (for mutagenicity testing) or in tap water (for analysis of ClO_2^- and ClO_3^- by ion chromatography). The concentrations of total available chlorine (TAC), total available ClO_2 (TACD expressed as μg/mL ClO_2), and various chlorine species in the stock and working solutions were determined by iodometric and *N*,*N*-diethyl-*p*-phenylenediamine (DPD) ferrous titration methods (APHA, 1989a,b). These solutions were freshly prepared on the day of the experiment to treat seafood products.

Seafood Treatment for Mutagenicity Testing. Fresh Atlantic salmon (*Salmo salar*) and red grouper (*Epinephelus morio*) were purchased from a local seafood store. The fish were filleted, skinned, and cut into cubes (2.5 × 2.5 × 2.5 cm). Fish cubes were mixed well and randomly sampled (60 g) for treatment with five volumes (300 mL) of aqueous Cl_2, ClO_2, or brine solution (1:5, w/v) for 5 min. The treated aqueous solutions and fish cubes were separately collected and processed to prepare samples for mutagenicity testing.

The outer surfaces of the treated fish cubes were removed using a knife, and 30 g of this tissue were then chopped, minced, and homogenized in a Waring blender with 60 mL of distilled water. The homogenate was extracted with 2 volumes (180 mL) of a methylene chloride (CH_2Cl_2)/methanol (CH_3OH) mixture (1:1, v/v), and the mixture was filtered through a Whatman #1 filter paper. The retained solid samples were homogenized in the blender two more times with 100 mL each of CH_2Cl_2/CH_3OH mixture and filtered again. The filtrates were pooled in a separatory funnel, and the CH_2Cl_2 layer was removed and retained. After the methanol in the aqueous phase was removed using a rotary evaporator, the aqueous phase was extracted two times with equal volumes of CH_2Cl_2. All CH_2Cl_2 extracts were pooled and passed through a column of anhydrous sodium sulfate. After removal of CH_2Cl_2 using a rotary evaporator in vacuo, the residue was dissolved in a small volume of CH_2Cl_2 and the solution was transferred to a preweighed amber vial (1.8 mL) capped with a Teflon-lined closure. Any remaining solvent in the vials was flushed under a stream of nitrogen gas. Various test doses were prepared by adding spectrophotometric-grade dimethyl sulfoxide (DMSO, Schwarz/Mann Biotech, Cleveland, OH) to appropriate amounts of the residue. The reaction products in the treated aqueous solutions were processed similarly using CH_2Cl_2/CH_3OH mixture.

Ames *Salmonella*/Microsome Assay. The standard plate-incorporation assay applying *Salmonella typhimurium* strains TA98 and TA100 was used (Ames et al., 1975; Maron and Ames, 1983). The assay was performed in the presence or absence of rat S9 mix (Molecular Toxicology, Inc., Boone, NC) using four plates for each sample dose. The experiments were repeated at least twice.

The assay was performed by adding 0.1 mL of the overnight cultures with a predetermined dose of the test sample (25 μL) and 0.5 mL of S9 mix or 0.25 M potassium phosphate buffer (pH 7.2) into 3.5 mL of top agar containing small quantities of histidine and biotin. Concurrent positive and negative controls were included in all assays; 2-aminofluorene (2-AF, Aldrich, Milwaukee, WI) was used for both strains in the presence of the S9 mix. Methyl methanesulfonate (MMS, Aldrich) was used for strain TA100 and 2-nitrofluorene (2-NF, Aldrich) for TA98 when the S9 mix was absent. The plates were incubated at 37 °C for 2 days before the colonies were counted.

Preparation of the Treated Seafood for Determination of Inorganic Reaction Byproducts. Three different types of seafood [sea scallops (*Placopecten magellanicus*), white shrimp (*Penaeus setiferus*), and mahi-mahi (*Coryphaena hippurus*)] were used in this study. Sea scallops, representing a common, popular molluscan shellfish, were harvested and initially packaged in Boston prior to frozen shipment to Gainesville, FL. The edible meats or adductor muscle (40−50 count/pound) had been previously hand-shucked, washed, and packed in customary 5-lb wax boxes with polybag liners. White penaeid shrimp, representing a common, popular crustacean shellfish, was shell-on tails (count: 30−35 tails/pound). The shrimp had been harvested by trawler in the Atlantic ocean adjacent to St. Augustine, FL, and then frozen in 5-lb boxes prior to shipment and storage in Gainesville, FL. Mahi-mahi, representing a common, popular marine fish, was harvested in the Pacific Ocean off Quepas, Costa Rica. The gutted fish were shipped fresh to Gainesville, FL, and then skinned and cut to fillet portions (approximately 400−450 g/fillet).

These various seafood samples were exposed in a container for 1, 10, 60, and 180 min to tap water (24 °C) containing 0, 10, 20, or 30 ppm of ClO_2. The treatments involved exposure of approximately 5 kg of the seafood products immersed directly into 10 L of the respective ClO_2 solutions. At each time interval, subsamples of the treated seafood were removed for determination of bacterial load and quantities of chlorite and chlorate. Untreated seafood samples were used as controls. Aliquots of all solutions (100 mL) before treatment were sampled for ClO_2 content using iodometric and DPD titration methods.

Quantify Chlorite and Chlorate in Treated Seafood Samples. The seafood homogenates in Butterfield's buffer were transferred to 50 mL centrifuge tubes and centrifuged at 12 000 rpm for 40 min using an IEC B-20A Centrifuge (Damon/IEC Division, Needham Heights, MA) at 4 °C. The supernatants were removed and filtered through 0.2 μm Acrodisc filters (Gelman Sciences, Ann Arbor, MI). These filtrates were then stored at 4 °C until analysis for chlorite and chlorate using a Dionex 2010i ion chromatograph (Sunnyvale, CA). The U.S. EPA method 300 "Determination of Inorganic Anions in Water by Ion Chromatography" was followed. The columns used were an IonPac AS9-SC analytical column (4 mm, Dionex) and IonPac AG9-SC guard column (4 mm). The eluent was 1.4 mM Na_2CO_3/0.2 mM $NaHCO_3$; the flow rate was 1.5 mL/min; and the injection volume was 100 μL. An anion self-regenerating suppressor was connected to the system, and the detection was a suppressed conductivity in an autosuppression recycle mode.

Mixtures of sodium chlorite (Eastman Kodak Co., Rochester, NY) and sodium chlorate (Fisher Scientific) standard solutions were prepared in distilled and purified water (Photronix Water System [reagent grade], Photronix Corp., Medway, MA). The reagents were certified ACS grade. Concentrations of each ion at 0.125, 0.25, 0.5, 0.75, 1.0, and 1.5 ppm were run daily. Curves showed linear responses ($R^2 = 0.999$) for the dose−peak height relationship. Each test sample was injected twice with the average values reported. All glassware used in this experiment was washed with purified water.

Odor and Visual Assessment of Seafood Samples. Assessment of the overall quality of seafood samples prior to and following ClO_2 treatment were performed by a three-member panel of experts. Their expertise was based on over 35 years of combined experience in commercial and analytical settings assessing seafood quality. The panel was presented samples under normal light in plastic Ziploc bags (Dow Brands, Indianapolis, IN). Their descriptive ratings (visual, smell, touch/texture) were based on direct product observation and comparison among treatments prior to exposure and after exposure, immediately and over 6 days of storage at 2 °C. The

Table 1. Mutagenicity Testing with *S. typhimurium* TA 98 and TA 100 of the Organic Solvent Extracts from Treated Aqueous Chlorine and Chlorine Dioxide Solutions Following Interaction with Red Grouper or Salmon Cubes

sample	test dose[a] (per plate)	no. of revertants/plate[b] TA 98 −S9	TA 98 +S9	TA 100 −S9	TA 100 +S9
bacteria		21 ± 3	22 ± 3	85 ± 5	103 ± 9
bacteria + buffer	25 μL	19 ± 5	22 ± 2	84 ± 6	102 ± 13
bacteria + DMSO[c]	25 μL	21 ± 4	23 ± 7	80 ± 7	90 ± 13
bacteria + mutagen					
MMS (5 μL/25 μL)	30 μL			2130 ± 61	
2-NF (5 μg/25 μL)	30 μL	2544 ± 154			
2-AF (10 μg/25 μL)	30 μL		9968 ± 408		4731 ± 321
red grouper					
brine	250 μg	20 ± 4	19 ± 4	80 ± 10	97 ± 13
20 ppm Cl_2	500 μg	21 ± 5	20 ± 7	84 ± 16	92 ± 10
200 ppm Cl_2	500 μg	27 ± 8	26 ± 4	342 ± 79	238 ± 59
20 ppm ClO_2	500 μg	21 ± 2	25 ± 4	83 ± 11	97 ± 8
	250 μg	20 ± 7	24 ± 6	80 ± 9	106 ± 20
200 ppm ClO_2	500 μg	20 ± 6	24 ± 4	82 ± 7	101 ± 10
salmon					
brine	500 μg	19 ± 4	21 ± 3	79 ± 5	107 ± 11
20 ppm Cl_2	250 μg	19 ± 3	23 ± 4	78 ± 9	105 ± 22
200 ppm Cl_2	2 mg	10 ± 3	24 ± 6	238 ± 21	263 ± 24
	1 mg	27 ± 7	29 ± 6	161 ± 35	179 ± 23
	500 μg	26 ± 4	20 ± 6	146 ± 40	137 ± 14
20 ppm ClO_2	500 μg	20 ± 5	23 ± 5	89 ± 8	95 ± 11
	250 μg	20 ± 4	21 ± 3	84 ± 3	96 ± 14
200 ppm ClO_2	500 μg	20 ± 4	22 ± 7	171 ± 24	114 ± 20
	250 μg	20 ± 10	21 ± 5	112 ± 19	94 ± 12

[a] The test dose per plate is the amount of organic solvent extract of the treated solutions. [b] Mean ± standard deviation from eight plates of duplicate trials. [c] DMSO = dimethyl sulfoxide; MMS = methylmethanesulfonate; 2-AF = 2-aminofluorene; and 2-NF = 2-nitrofluorene.

descriptions were based on direct comparisons with the untreated (0 min exposure) or control products.

RESULTS AND DISCUSSION

Mutagenicity Assay. Only the organic solvent extracts from the treated 200 ppm aqueous chlorine with red grouper and salmon showed a weak mutagenic activity toward *S. typhimurium* TA 100 (Table 1). None of the other treated solutions (brine, aqueous chlorine at 20 ppm, or aqueous ClO_2) showed mutagenic activity toward TA 98 or TA100. The lower mutagenic activity of the treated 200 ppm aqueous chlorine from salmon than red grouper could be related to the difference in their lipid contents. Since TA 100 detects primarily mutagens that cause base-pair substitutions, the reaction products generated in the treated 200 ppm aqueous chlorine from the two fish species are capable of causing base-pair substitution mutations. The mutagenic activity of the reaction products from red grouper to TA 100 was decreased in the presence of the S-9 mix. The binding of the test sample with the S-9 enzyme system may have decreased the availability of the test sample for interaction with bacterial DNA to cause mutations. Also, the production of less reactive metabolites by the mixed function oxidase system in the S-9 mix may have contributed to the reduced number of revertants.

Unlike aqueous chlorine, the reaction products obtained from the interaction of brine or ClO_2 at 20 or 200 ppm with red grouper and salmon were not mutagenic to either tester strain in the presence or absence of the S-9 mix (Table 1). None of the extractants from chlorine and ClO_2 treated salmon or red grouper cubes showed mutagenic activity toward *S. typhimurium* TA 98 or TA 100 in the presence or absence of rat S-9 mix (Figure 1).

Many organic compounds present in water and food can be oxidized following treatment with ClO_2. Wei et al. (1987) showed that none of the fractions from aqueous chlorine and ClO_2 with fatty acids showed mutagenic activity to *S. typhimurium* TA 98 or TA 100. However, the reaction products of some peptides, hydroxyproline, and tyrosine exerted mutagenic activity toward both strains in the presence and absence of rat liver S-9 mix (Tan et al., 1987). Schade et al. (1990) found some mutagens from chlorine-treated waters of high organic content. The treated poultry chiller water with chlorine had a 5-fold more mutagenic activity than that treated with ClO_2 (Tsai et al., 1997). Our results to demonstrate the less reactive nature of ClO_2 in producing mutagenic reaction products as compared to aqueous chlorine were in agreement with the findings of Tsai et al. (1997).

Contents of Various Chlorine Species in Stock and Test Solutions. The 10, 20, and 30 ppm ClO_2 solutions were prepared on each test date from stock solutions on the basis of the results of iodometric titration. However, such test solutions, when titrated by DPD method, were found to have respective values of 3.8, 11, and 15.9 ppm ClO_2 for treatment of sea scallops; 7.6, 16.5, and 25.7 ppm ClO_2 for treatment of mahi-mahi; and 12.5, 23.3, and 34.9 ppm ClO_2 for shrimp treatment. The freshly prepared stock solutions of acidified sodium chlorite had 80.4% ClO_2, 1.4% FAC, 18.2% CAC, and no chlorite (Table 2).

Contents of Chlorite and Chlorate in Treated Seafood. Ion chromatography using a 1.4 mM Na_2CO_3/0.2 mM Na_3HCO_2 mixture as the eluent system well separated chlorite and chlorate . The minimal detection limit for chlorate in sea scallops, mahi-mahi, and shrimp was 1.0, 2.0, and 1.0 ppm (μg/g), respectively. Chlorite

Table 2. Various Chlorine Species Present in Stock and Test Solutions Used for Treatment of Shrimp As Determined by Iodometric and DPD Titration Methods

	test ClO_2 solutions[a]			stock solution
	10 ppm	20 ppm	30 ppm	
iodometric method				
TAC (ppm as Cl_2)	47.3	70.9	85.1	3332
TACD (ppm as ClO_2)	18	27	32.3	1267
DPD method				
TACD (ppm as ClO_2)	12.5	23.3	34.9	875
TACD (ppm as Cl_2)	33 (81.3%)	61.3 (83.9%)	91.7 (81.4%)	2300 (80.4%)
FAC[b] (ppm)	0.2 (0.49%)	2 (2.74%)	4.7 (4.17%)	40 (1.40%)
CAC (ppm)	7.4 (18.2%)	9.8 (13.4%)	16.3 (14.5%)	520 (18.2%)
chlorite (ppm)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
TAC (ppm)	40.6 (100%)	73.1 (100%)	112.7 (100%)	2860 (100%)

[a] The test solutions (10, 20, and 30 ppm ClO_2) were prepared by diluting the stock solution with tap water based on its TACD (1267 ppm ClO_2) as determined by the iodometric method. Each of these test solutions was then titrated by iodometric and DPD methods for TACD and individual chlorine species. The stock solution was also titrated by the DPD method to quantify each chlorine species. The number in parentheses is the percent content of each chlorine species in the ClO_2 solution. [b] FAC, free available chlorine; CAC, combined available chlorine; TAC, total available chlorine; ppm, parts per million (μg/mL).

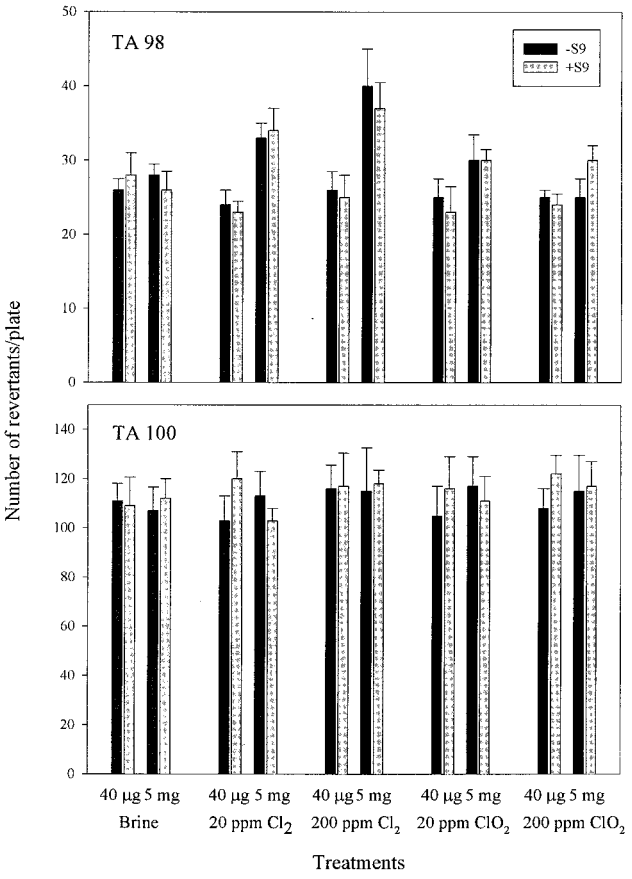

Figure 1. Mutagenicity testing with *S. typhimurium* TA 98 and TA 100 of the organic solvent extracts from treated red grouper with aqueous chlorine and chlorine dioxide.

Table 3. Time-Related Changes of Chlorite (ClO_2^-) and Chlorate (ClO_3^-) Contents (ppm) in Sea Scallops, Mahi-Mahi, and Shrimp Following Treatment with Various ClO_2 Solutions[a]

solution	ClO_2^- and ClO_3^- (μg/g)	1 min	10 min	60 min	180 min
sea scallops					
water	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	0
3.8 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	0
11.0 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	3.26	2.60
15.9 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	0	2.98	6.35	9.30
mahi-mahi					
water	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	0
7.6 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	1.80
16.5 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	1.00	3.30	3.20	6.14
25.7 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	1.50	3.55	6.10	9.24
shrimp					
water	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	0
12.5 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	0	0	0	0
23.3 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	1.14	1.71	2.54	2.59
34.9 ppm	ClO_2^-	0	0	0	0
	ClO_3^-	1.32	1.97	3.55	4.71

[a] No chlorite and chlorate was detected in the homogenates of nontreated sea scallops, mahi-mahi, and shrimp.

was not detected in seafoods even at levels over the 20 ppm spiked level. The minimal detection limits for chlorite and chlorate in deionized water was 0.05 ppm.

No chlorite residue was detected in any of the ClO_2-treated seafood samples (Table 3). However, low levels of chlorate residues were detected in some of the treated samples. The chlorate content increased in a time- and ClO_2 dose-related manner. Miltner (1977) indicated chlorites and chlorates were the primary reaction products following ClO_2 disinfection of surface waters at 50% and 30% of ClO_2 demand, respectively. Pfaff and Brockhoff (1990) demonstrated that ClO_3^- was stable in drinking water, but losses of ClO_2^- were observed within 1 day. The ClO_2^- may react with many other organic compounds in the seafood, thereby leading to its disappearance.

The low level of ClO_3^- found in the treated seafood products can also be changed to other chlorine species. FDA evaluated the petition data and determined that the very low levels of chlorite and chlorate on fresh poultry carcasses following exposure to processing water containing ClO_2, would be converted to low levels of chloride during cooking (*Food Chemical News*, 1995). Therefore, the chlorate residue detected in the treated seafood poses no health hazard to consumers.

Odor and Visual Observation of Treated Seafood and Test Solutions. The treated solutions showed noticeable changes in clarity and color during exposure

to seafood. The treated control solution (0 ppm) for sea scallops showed no change in color. However, the treated ClO_2 solutions at 3.8, 11.0, and 15.9 ppm changed to pink, red, and dark red, respectively, within a 10 min exposure to scallops. The control solution for shrimp was slightly cloudy after 60 min of exposure. The 12.5 ppm ClO_2 solution appeared pinkish-brown after treatment with shrimp for 2 min. It then progressively changed to light brown after 180 min. This progressive change in color also occurred with the 23.3 and 34.9 ppm ClO_2 solutions, but the initial (1 min) and final (180 min) colors were more intense and the solutions were more opaque. The control and test solutions for mahi-mahi became cloudy in a manner similar to shrimp and became more opaque with increasing exposure time and concentrations (7.6−25.7 ppm ClO_2). The color change of the treated solutions is due to the formation of chlorinated reaction products.

Overall quality assessments of sea scallops indicated no discernible differences between products exposed to water or activated ClO_2 until an exposure to ClO_2 at 3.8−15.9 ppm exceeded 10 min. The noted differences in these longer exposures were slight and not objectionable. The loss of surface sheen or slime on the meats was only noticeable in side-by-side comparisons with nonexposed meats. The loss of sheen gave a drier product appearance. Likewise, in these same exposures, fluid or seepage was evident about the product in the containers. The amount of seepage was minimal and expected, and it was noted in side-by-side comparisons with the controls. The slight differences noted for the longer exposure times in activated ClO_2 were not objectionable and, in fact, improved product appearance, yet this difference was only evident in comparison with nonexposed controls.

All samples of mahi-mahi were affected by the exposure to water or test solutions. Obviously, the exposures to water and test solutions caused color changes in red muscle from the preferred ruby-red to a darker reddish brown. This color continued to darken for all exposed fillets. The noted bleaching effect of white tissue in samples exposed to 7.6−25.7 ppm activated ClO_2 was only discernible in side-by-side comparisons with nonexposed controls. The degree of bleaching was not judged objectionable. The immediate red muscle color change was objectionable and would reduce the value of the fillets.

Although the activated ClO_2 concentrations used for shrimp treatment were higher than those for scallops, the treatment resulted in no discernible differences for all exposure times for the first 2 days of storage. Compared to the controls that showed obvious spoilage after 5 days of refrigeration, shrimp exposed to ClO_2 was less objectionable after the same storage conditions and time. The least objectionable shrimp after 5-day refrigeration was the product previously exposed to 34.9 ppm activated ClO_2 for over 60 min, even though the initial spoilage odors were obvious. Overall, exposure of shell-on shrimp in activated ClO_2 did not influence the sensory attributes of the product immediately or during subsequent refrigerated storage.

SUMMARY

The extracts from the fish treated with solutions of 200 ppm aqueous chlorine showed weak mutagenic activity toward TA 100. Such treatment with aqueous ClO_2 did not show any mutagenic activity. Additionally, the reaction products obtained from fish cubes following treatment with tested chlorine and ClO_2 solutions were not mutagenic. The treated seafood products at a dose level of ≤30 ppm, in most cases, showed no discernible differences in quality attributes to tap water treated controls. However, the ClO_2-treated shrimp showed better quality than the control group after 5 days of refrigerated storage. Although low concentrations of chlorate were detected in ClO_2-treated sea scallop, mahi-mahi, and shrimp, this is not expected to cause a health concern to consumers since it will be converted to chloride during cooking. Chlorite residues were not found in any of the ClO_2-treated seafoods.

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