Effect of Chlorine Dioxide Treatment on Lipid Oxidation and Fatty Acid Composition in Salmon and Red Grouper Fillets

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ABSTRACT: Chlorine dioxide (ClO_2) has been explored as a potential substitute for aqueous chlorine to clean seafood products. In an attempt to understand the interaction of ClO_2 with organic compounds, duplicate fillets of Atlantic salmon (*Salmo salar*) and red grouper (*Epinephelus morio*) were treated for 5 min with freshly prepared aqueous ClO_2 at 20, 40, 100, and 200 ppm total available ClO_2 in 3.5% brine. Thiobarbituric acid (TBA) values and fatty acid composition were determined. ClO_2-treated salmon and red grouper showed a dose-related increase in TBA; the 100 and 200 ppm groups had significantly ($P < 0.05$) greater TBA values than controls and the 20 ppm group. Treated red grouper and salmon did not differ in percentage monounsaturated and polyunsaturated fatty acids compared to controls, although differences occurred with some individual fatty acids. Thus, ClO_2 treatment did not greatly affect fatty acid composition of treated fillets.
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KEY WORDS: Chlorine, chlorine dioxide, fatty acid composition, omega-3 fatty acid, oxidation, polyunsaturated fatty acids, red grouper, salmon, seafood, thiobarbituric acid.

As people are becoming more aware of the relationship between diet and health, consumption of seafood has increased because fish provides long-chain omega-3 polyunsaturated fatty acids (PUFA), which have been demonstrated to reduce platelet aggregation (1) and cardiovascular risk (2). Seafood fatty acids contain larger amounts of 20- and 22-carbon and more highly unsaturated fatty acids than plants and animals (3).

Aqueous chlorine has been used in the food industry to clean products, including seafood, containers, and equipment. However, owing to health concerns regarding trihalomethanes and other chlorination reaction products, generated during interaction of organics with aqueous chlorine, efforts have been made to explore alternatives. Chlorine dioxide (ClO_2) is a good candidate because it has a bactericidal efficacy that is equivalent to seven times higher than the concentration of aqueous chlorine in poultry processing chiller water (4). Furthermore, less potentially toxic reaction products are produced during treatment of organic matter with ClO_2. Although ClO_2 has been tested with seafood and vegetables to enhance freshness and extend the shelf life of products, little is known about its reaction with organic matter and its effect on nutrient content, including fatty acid composition.

ClO_2 is a potent oxidizer and an effective chlorinating agent. Therefore, the double bonds of fatty acids may undergo oxidation and halogen addition in the presence of ClO_2. The objective of this study was to investigate the effect of ClO_2 solutions at 20, 40, 100, and 200 ppm total available chlorine dioxide (TACD) in brine (3.5% NaCl solution) on oxidation and fatty acid compositions of red grouper and salmon fillets.

EXPERIMENTAL PROCEDURES

Fresh fillets of Atlantic salmon (*Salmo salar*) and red grouper (*Epinephelus morio*) were purchased from a local seafood store in Gainesville, Florida. Fillets from red grouper and salmon, weighing 1.8 and 2.7 kg, respectively, were cut into about 4-cm sections from anterior to posterior. The sections were further cut into cubes ($4 \times 4 \times 4$ cm). Fish cubes were mixed and randomly sampled for treatment with aqueous ClO_2 solutions or brine. Duplicate samples were used for each treatment group. The experiment was repeated once.

Chlorine-demand-free water (CDF water) was prepared by the method of Ghanbari *et al.* (5) by passing distilled water through two successive Barnstead deionizing units (Dubuque, IA) and then a glass column with Porapak® Q (Supelco, Bellefonte, PA). This water was used to prepare all reagents. Aqueous solutions of ClO_2 were freshly prepared for each experiment from Oxine® Concentrate (Bio-Cide International, Norman, OK). It contained 2% ClO_2 and 98% inert ingredients. A stock solution of 2,000 ppm TACD was prepared by first reacting Oxine® concentrate for 5 min at room temperature with 85% phosphoric acid at a 20:1 (vol/vol) ratio in a brown flask sealed with a glass stopper. The solution was then diluted with 9 vol of ice-cold CDF water. This stock solution was used to prepare various working solutions (20, 40, 100, and 200 ppm TACD) in 3.5% ice-cold brine. Both the stock and working ClO_2 solutions were freshly prepared on the day of the experiment. Aqueous ClO_2 concentrations were determined by iodo-

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metric titration and then by the *N,N′*-diethyl-*p*-phenylenediamine (DPD) ferrous titration method (6).

Duplicate fish fillets (25 g each) were treated separately with stirring for 5 min with ice-cold ClO_2 solutions in 3.5% brine at 20, 40, 100, and 200 ppm in beakers at a ratio of 1:5 (wt/vol). After extra liquid was drained, the fillets were used for estimating thiobarbituric acid-reactive substances (TBARS) molar value (µmol malonaldehyde/kg fish) by distillation-colorimetric method (7) for lipid oxidation analysis. Controls included nontreated fillets and those treated with 3.5% brine. The TBARS values of the samples were calculated from a tetraethoxypropane (TEP) standard curve (absorbance vs. µmoles TEP). For lipid extraction, the procedure of Folch *et al.* (8) as modified by Christie (9) was followed.

Fatty acid methyl esters (FAME) were prepared in screw-cap tubes by the method of Maxwell and Marmer (10). A Sigma 3B gas chromatograph (Perkin Elmer, Norwalk, CT) with split injector (1:40 ratio) and flame-ionization detector was equipped with a DB-Wax (30 m × 0.25 mm i.d., 0.25 µm film) column (J&W Scientific, Folsom, CA), which was operated with helium as a carrier gas at a linear flow velocity of 25 cm/s. The oven temperature was held at 195°C for 12 min, then programmed at 2°C/min to 241°C. The injector and detector temperatures were set at 300°C. Fatty acids were identified by comparing their retention times with standards (Nu-Chek-Prep, Elysian, MN) and a cod liver oil reference (11). Individual fatty acid was reported as area percentage. Each sample was injected twice for analysis.

Analysis of variance was performed by using the general linear models procedure of the Statistical Analysis System (12). Duncan's multiple range test was used for pairwise comparisons at a significance level of $P = 0.05$.

RESULTS AND DISCUSSION

Of the various components that affect quality attributes of fish, the lipids are most important. Lipids may undergo hydrolysis and oxidation reactions during processing and storage that adversely affect flavor, color, and texture. The formation of carbonyl components and changes in fatty acid composition can serve as criteria for evaluation of oxidative rancidity in fish. Treatment of salmon and red grouper fillets for 5 min with ClO_2 solutions caused a dose-related increase in TBARS values (Fig. 1). Those treated with 100 and 200 ppm ClO_2 had significantly ($P < 0.05$) greater TBARS values than the two controls and the 20 ppm group. Red grouper treated with 40 ppm ClO_2 also had a significantly ($P < 0.05$) greater TBARS value than nontreated and brine-treated samples. Ke *et al.* (13) studied lipid oxidation in various parts of frozen mackerel. The TBARS values in the white and dark muscles from mackerel increased from 2.4 and 4.2 µmol/kg fish to 6.8 and 8.2 µmol/kg fish, respectively, after storage at −15°C for 2 mon. Skin sample (subcutaneous fat) of mackerel increased from 5.8 to 58.9 µmol/kg fish. Compared to these data, the TBARS molar values of red grouper and salmon after treatment with ClO_2 solutions were only moderately elevated, even though the treatment caused a dose-related increase in TBARS values. Since we did not examine frozen storage of the treated fillets, it is unclear how the treatment would affect subsequent oxidation during storage.

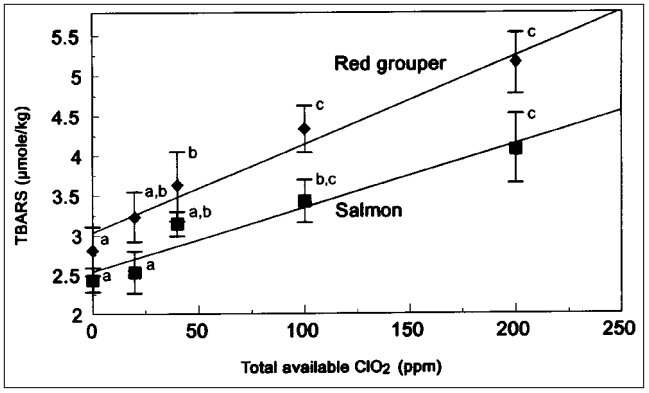

FIG. 1. Effect of treatment with aqueous ClO_2 solutions on thiobarbituric acid-reactive substances (TBARS) values of fish fillet. Means ± standard deviation followed by different letters was significantly ($P < 0.05$) different from each other for each fish species. The brine-treated salmon (■) and red grouper (◆) had respective TBARS values of 2.43 ± 0.62 and 2.81 ± 0.31 µmole malonaldehyde/kg fish, respectively.

Least square means of fatty acid composition of red grouper and salmon following treatment with increasing levels of ClO_2 were compared (Tables 1 and 2). Lipids of salmon and red grouper fillets were highly unsaturated, as expected. High levels of 20:5ω3, 22:5ω3, and 22:6ω3 were found in both red grouper and salmon fillets. Atlantic salmon had higher ω-3 PUFA than red grouper. There were no obvious differences in fatty acid compositions that appeared to be a result of ClO_2 treatment (Tables 1 and 2). Highly unsaturated fatty acids (20:5ω3, 22:6ω3, and 20:4ω6) that would be expected to be sensitive to oxidizing conditions were not significantly ($P > 0.05$) affected by treatments in either red grouper or salmon. Several of the fatty acids analyzed did appear to differ significantly owing to treatment (in salmon, 17:0, 18:0, 18:2ω6, 18:3ω3, 18:4ω3, 20:4ω6, 20:4ω3, and 21:5ω3; in red grouper, 18:0, 20:2ω6, 22:4ω6, and 22:5ω3). However, there was no clear dose response in fatty acid composition, and the differences in fatty acid composition between controls and treatment were minor. These results are probably attributable to experimental error in the fatty acid analysis and the unavoidable variations in fatty acid composition among the cubed composite fish samples exposed to the various treatments. There appeared to be no effect of ClO_2 treatment on fatty acid composition in salmon or red grouper under the experimental conditions examined.

TBARS were significantly ($P < 0.05$) but modestly elevated by ClO_2 treatment. The treated red grouper and salmon fillets did not differ in the percentage monounsaturated and PUFA compared with nontreated controls, although differences in individual fatty acids occurred. These differences most likely were a result of experimental error because there is no easily understood way that ClO_2 would oxidize or add

TABLE 1
Least Square Means of Fatty Acid Compositions of Red Grouper Fillets Following Treatment with Aqueous Chlorine Dioxide[a]

Fatty acid	CT	BR	20	40	100	200	SEM	Effect of TRT (-*P*-)
14:0	2.12	2.21	1.82	1.98	1.70	1.64	0.206	0.2953
15:0	0.57	0.61	0.56	0.56	0.52	0.53	0.031	0.3748
16:0	22.2	22.8	23.6	22.9	21.9	22.0	0.664	0.4449
16:1ω7	5.76	5.19	4.80	4.84	4.58	4.15	0.397	0.1133
16:2ω4	0.97	0.95	0.95	1.04	0.95	0.94	0.042	0.5581
17:0	0.79	0.80	0.78	0.79	0.82	0.77	0.041	0.9648
17:1ω8	0.72	0.70	0.67	0.70	0.65	0.66	0.057	0.9434
18:0	6.41	6.70	6.62	6.90	6.48	6.73	0.101	0.0171
18:1ω9	14.6	14.1	14.1	14.4	13.1	13.1	0.473	0.1188
18:1ω7	2.23	2.03	1.95	2.14	1.90	1.78	0.114	0.0923
18:2ω6	1.22	1.32	1.31	1.25	1.21	1.33	0.054	0.4156
18:3ω3	0.56	1.28	1.41	1.11	1.22	1.31	0.198	0.0565
20:1ω9	0.70	0.86	0.65	0.76	0.58	0.61	0.076	0.1217
20:2ω6	0.74	1.78	1.45	1.25	1.23	1.09	0.131	0.0001
20:4ω6	6.89	6.67	7.28	7.51	7.75	7.93	0.379	0.1609
20:5ω3	2.80	2.56	2.43	2.56	2.97	2.52	0.129	0.0522
22:0	0.67	0.63	0.64	0.31	0.37	0.55	0.127	0.2365
22:4ω6	2.18	1.84	1.95	2.28	2.21	2.11	0.094	0.0178
22:5ω6	1.83	1.73	1.90	1.93	2.05	2.27	0.127	0.0737
22:5ω3	2.38	2.05	1.99	1.95	2.13	1.99	0.082	0.0068
22:6ω3	15.7	13.9	15.2	16.3	16.7	17.3	0.893	0.1058

[a]CT: nontreated control; BR: brine-treated control; SEM: standard error mean; Effect of TRT (-*P*-): effect of treatment on *P* values.

TABLE 2
Least Square Means of Fatty Acid Compositions of Salmon Fillets Following Treatment with Aqueous Chlorine Dioxide[a]

Fatty acid	CT	BR	20	40	100	200	SEM	Effect of TRT (-*P*-)
14:0	4.06	3.79	4.29	3.75	3.88	4.12	0.151	0.1075
15:0	0.46	0.44	0.47	0.44	0.43	0.45	0.011	0.2214
16:0	16.0	16.2	15.1	16.6	15.5	15.6	0.353	0.0561
16:1ω7	6.31	5.67	6.71	5.89	5.94	6.23	0.241	0.0556
16:2ω4	1.04	1.00	1.06	1.03	1.01	1.03	0.024	0.4651
17:0	0.37	0.36	0.36	0.40	0.36	0.37	0.008	0.0241
17:1ω8	0.67	0.63	0.73	0.66	0.65	0.69	0.027	0.1690
18:0	4.16	4.11	4.04	4.25	4.09	4.14	0.058	0.0482
18:1ω9	20.2	19.2	21.3	19.6	20.3	20.6	0.544	0.1263
18:1ω7	3.71	3.80	3.58	3.99	3.78	3.74	0.100	0.1383
18:2ω6	2.39	2.28	2.52	2.27	2.36	2.45	0.061	0.0418
18:3ω3	0.62	0.61	0.64	0.59	0.62	0.63	0.009	0.0173
18:4ω3	0.84	0.82	0.92	0.77	0.83	0.86	0.026	0.0067
20:1ω9	2.65	2.52	2.74	2.57	2.66	2.66	0.082	0.5231
20:4ω6	0.86	0.89	0.81	0.86	0.87	0.84	0.018	0.0314
20:4ω3	1.12	1.10	1.16	1.05	1.12	1.13	0.023	0.0464
20:5ω3	7.33	7.47	7.20	7.01	7.25	7.20	0.140	0.3392
22:0	0.87	0.83	0.91	0.83	0.88	0.89	0.025	0.1719
21:5ω3	0.40	0.38	0.43	0.36	0.40	0.41	0.014	0.0255
22:4ω6	0.26	0.25	0.26	0.23	0.27	0.25	0.010	0.0985
22:5ω6	0.29	0.30	0.29	0.30	0.31	0.30	0.010	0.5875
22:5ω3	4.33	4.33	4.50	4.13	4.48	4.42	0.104	0.1599
22:6ω3	15.4	17.3	14.0	16.1	16.2	15.1	0.910	0.2024

[a]See Table 1 footnote.

Cl to 18:1ω7 but not 18:1ω9. The differences in fatty acid composition within the fillets would likely also have contributed to the differences observed. According to Coppock *et al.* (14), the treatment of flour with 280 ppm ClO_2 does not cause any immediate effect on fatty acid composition; the content of linolenic or arachidonic acid was not significantly ($P > 0.05$) changed. However, the use of too high a dose of ClO_2 may cause a color change in the flour by oxidizing the lipids (15,16).

Therefore, the results from our experiments indicate that

ClO_2 treatment for 5 min did not affect the fatty acid composition of salmon and red grouper and resulted in little lipid oxidation.

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