Control of Lipid Oxidation in Cooked and Uncooked Refrigerated Carp Fillets by Antioxidant and Packaging Combinations

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The effect of several commercial antioxidants on lipid oxidation of common carp fillets stored at ~ 5 °C for 16 days was evaluated. Also, different packaging conditions were used to study their effects on lipid oxidation and rancid odor. Antrancine 350 was found to be the best antioxidant in controlling lipid oxidation throughout the refrigerated storage. This antioxidant was highly effective at a concentration of 200 ppm and dipping time of 45 min. Vacuum packaging was more effective ($P \leq 0.05$) in retarding oxidative rancidity than was packaging without vacuum. The cooked fillets, packaged either with or without vacuum, had higher ($P \leq 0.05$) TBA values than did the uncooked fillets. Also, hot packaging gave better protection against lipid oxidation compared to cold and ambient temperature packaging. The sensory evaluation test revealed that the uncooked fillets stored in nylon−polyester bags was the most acceptable treatment throughout the refrigerated storage, while fillets packaged in polyethylene bags without vacuum were unacceptable by the end of the 16 day storage.

Keywords: *Lipid oxidation; rancid odor; carp fillets; antioxidant; vacuum packaging*

INTRODUCTION

It is well recognized that oxidation of the lipid fraction of fish muscle is a major cause of deterioration of fatty fish (Stansby, 1990; Brannan and Erickson, 1996) due to the high degree of unsaturation in fish lipids (Mai and Kinsella, 1979) and the high concentration of metals in fish (Khayat and Schwall, 1983). Lipid oxidation has a detrimental effect on the quality of fish, as it leads to the development of off-odors and off-flavors (Yinci et al., 1995; Brannan and Erickson, 1996). The formation of malonaldehyde in food has been associated with oxidative rancidity causing mutagenic and carcinogenic effects to humans (Mukai and Goldstein, 1976; Siu and Draper, 1978). The free radical reaction of peroxidation is often a major problem for food manufacturers whose interests include maintenance of nutritional qualities and shelf life of lipid-containing foods (Loliger, 1991; Hettiarachchy et al., 1996). The use of various antioxidants in controlling lipid oxidation in fish systems has been reported (Benedict et al., 1975; Kelleher et al., 1992; Ramanathan and Das, 1992, 1993; Koning and Merwe, 1996). These antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, *tert*-butylhydroxyquinone (TBHQ), tocopherols, which act as free radical terminators, citric acid, which acts as a metal chelating agent, and ascorbic acid, which acts as an oxygen scavenger and reducing agent. Also, it has been reported by Ahn et al. (1992) that packaging of cooked meat immediately after cooking was very effective in prevention of lipid oxidation by reducing oxygen contact with meat. Nolan et al. (1989) found that cooked meat samples stored in carbon dioxide and nitrogen were less oxidized than those stored in air but were more oxidized than vacuum packaged meat. The present study was an attempt to control lipid oxidation by antioxidant and different packaging conditions. The objectives of the present study were (1) to evaluate the effect of different commercial antioxidants on lipid oxidation of common carp fillets stored at refrigerated condition (~5 °C), (2) to determine the optimum concentration as well as dipping time for antioxidant to reduce or inhibit lipid oxidation, and (3) to compare the quality status and oxidative rancidity of uncooked carp fillets with those of cooked and packaged fillets under hot, ambient, and cold conditions either with or without vacuum.

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MATERIALS AND METHODS

Common carp fish (*Cyprinus carpio*) was obtained from Barsiek farm (Kafr El-Dwar, Behera, Egypt) and transported in iced containers to the laboratory. Commercial antioxidants used in this study, pristene RO (natural rosemary extract soluble in lipid), pristene RW (natural rosemary extract soluble in water), pristene 180 (70% concentrate of natural mixed tocopherols), pristene 181 (35% natural mixed tocopherols and 8% ascorbyl palmitate), pristene 189 (38% natural mixed tocopherols and 5% citric acid), sustane HW-4 (20% BHT and 20% BHA), and sustane 20 (20% TBHQ and 10% citric acid), were obtained from UOP Food Products and Processes (Des Plaines, IL). Antrancine 350 (propyl gallate, TBHQ, and citric acid) was obtained from Jan Dekker (Wormerveer, Netherlands). The above-mentioned antioxidants are generally regarded as safe (GRAS) for use in foods and have been approved by the U.S. FDA (Giese, 1996).

Thiobarbituric acid (TBA) was obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of analytical grade and were obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of Samples. Common carp fish (about 1 kg each) were filleted, deskinned, and deboned. Head and tail were removed. The fillets were divided into uniformly sized pieces (thickness ~ 1.5 cm, width ~ 6 cm, and length ~ 10 cm) of ~100 g each prior to the treatment with antioxidants.

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Total lipid contents of each piece were quite similar to each other (5.64 ± 0.23 g/100 g of wet sample, preliminary study). Nine groups were made for control and antioxidant treatments. Stock solutions of the above-mentioned antioxidants were prepared by dissolving each antioxidant in 50% ethanol except for pristene RW, which was completely dissolved in water. The fillets were dipped into each of the antioxidant solutions containing 200 ppm for 30 min and then packaged in polyethylene bags [thickness = 25 μm, oxygen transmission rate = 2500 $cm^3/(m^2 \times 24$ h atm)] and stored at ~5 °C for 16 days. Control treatment was prepared by dipping the fillets in distilled water for 30 min. Samples were withdrawn at 4 day intervals for thiobarbituric acid (TBA) analysis.

The best antioxidant was chosen for further experiment to be tested for the optimum concentration of the antioxidant as well as dipping time. Experimental treatments from the selected antioxidant for the fillets consisted of six levels (50, 100, 150, 200, 250, and 300 ppm), and the dipping time was 30 min. To determine the optimum dipping time, the fillets were dipped in the best antioxidant solution at optimum concentration for different dipping times (15, 30, 45, 60, 75, and 90 min). The best antioxidant treatment and concentration as well as dipping time were chosen for cooking and packaging studies. The fillets were treated with antrancine 350 at a concentration of 200 ppm and dipping time of 45 min. To examine the effect of cooking and packaging conditions either with or without vacuum on lipid oxidation and quality, the carp fillets were divided into two sets of samples. The first set was aerobically packaged in polyethylene bags [thickness = 25 μm, oxygen transmission rate = 2500 $cm^3/(m^2 \times 24$ h atm)]. The other set was vacuum packaged in a nylon−polyester bag [thickness = 100 μm, oxygen transmission rate = 18 $cm^3/(m^2 \times 24$ h atm)]. Each set was divided into four portions. One portion was packaged without cooking, and the other three portions were cooked on preheated (180 °C) Farberware skillets (model B3000, Kidde, Inc., Bronx, NY). The carp fillets were cooked for 10 min on one side and then turned and cooked for 10 min (internal temperature of 70 °C). After cooking, one portion was packaged immediately (hot packaging), while the second portion was packaged after cooling at ambient temperature for 2 h (ambient packaging). The third portion was chilled in a refrigerator for 45 min and then packaged (cold packaging). Samples were taken at specific time intervals for TBA analysis.

TBA. TBA was determined spectrophotometrically according to the procedure described by Siu and Draper (1978). Fish sample (10 g) was homogenized in 25 mL of distilled water and then mixed with 25 mL of 10% trichloroacetic acid. The mixture was vortex mixed and filtered. One milliliter of 0.06 M thiobarbituric acid was added to 4 mL aliquots of the filtrate and heated in a boiling water bath (10 min) for color development. The absorbance was measured at 532 nm using a Spectronic 2000 spectrophotometer. The TBARS values were expressed as milligrams of malonaldehyde per kilogram of dry sample. Triplicate analyses were performed on all fillets, and two determinations were conducted per replicate.

Sensory Properties. Sensory evaluation of stored fish samples was conducted to determine the presence of rancid odor. Evaluation was made by eight panelists from the staff of the University of Menofiya, Shibin El-Kom, Egypt, who had been trained to detect odor and degree of spoilage in fatty fish. Panelists were provided with a set of eight randomly coded samples. Cooked fish samples were reheated at 100 °C for 10 min, while uncooked samples were cooked on preheated (180 °C) Farberware skillets as described above. Evaluations were conducted in individual sensory evaluation booths away from the preparation area. Sensory scores were recorded utilizing a 6-point descriptive odor score. Descriptive terms used were absent, very slight, slight, moderate, strong, and very strong. Numerical values ranged from 0 (absent) to 6 (very strong).

Statistical Analysis. Data were analyzed using randomized complete blocks factorial design (Montgomery, 1984). An analysis of variance (SAS, 1988) was conducted. When a significant main effect was detected, the means were separated with the Student−Newman−Keuls test. The predetermined acceptable level of probability was 5% ($P \leq 0.05$) for all comparisons.

Table 1. Effect of Different Commercial Antioxidants on TBA Values (Milligrams of Malonaldehyde per Kilogram of Dry Matter) of Raw Common Carp Fillets Stored at 5 °C for 16 Days

	storage time					
treatment	0 days	4 days	8 days	12 days	16 days	mean[a]
control	0.38	0.66	1.05	2.22	3.38	1.518[h]
pristene RO[b]	0.36	0.56	0.76	1.09	1.49	0.852[f]
pristene RW[b]	0.36	0.50	0.66	0.91	1.25	0.736[d]
pristene 180[b]	0.35	0.52	0.75	1.01	1.38	0.802[e]
pristene 181[b]	0.35	0.55	0.77	1.11	1.45	0.846[f]
pristene 189[b]	0.35	0.58	0.86	1.21	1.62	0.924[g]
sustane HW-4[b]	0.34	0.41	0.54	0.66	0.81	0.552[b]
sustane 20[b]	0.34	0.47	0.59	0.76	0.98	0.628[c]
antrancine 350[b]	0.34	0.35	0.45	0.52	0.62	0.456[a]
mean[c]	0.352[a]	0.500[b]	0.714[c]	1.054[d]	1.442[e]	

[a] Means in the same column with different letters are significantly different ($P \leq 0.05$), LSD = 0.027. [b] Pristene RO = natural rosemary extract soluble in lipid; pristene RW = natural rosemary extract soluble in water; pristene 180 = 70% concentrate of natural mixed tocopherols; pristene 181 = 35% natural mixed tocopherols and 8% ascorbyl palmitate; pristene 189 = 38% natural mixed tocopherols and 5% citric acid; sustane HW-4 = 20% BHT and 20% BHA; sustane 20 = 20% TBHQ and 10% citric acid; antrancine 350 = propyl gallate; TBHQ, and citric acid. [c] Means in the same row with different letters are significantly different ($P \leq 0.05$), LSD = 0.020.

RESULTS AND DISCUSSION

TBA values of common carp fillets were significantly ($P \leq 0.05$) affected by the type of antioxidants and storage periods (Table 1). TBA values of fish treated with commercial antioxidants were consistently lower ($P \leq 0.05$) than control throughout the storage period. Antrancine 350 had the lowest TBA value among commercial antioxidants followed by sustane HW-4 and sustane 20. Pristene 189, pristene RO, and pristene 181 were less effective than other tested antioxidants in controlling lipid oxidation of common carp fillets. Treated fish with antioxidants as well as control exhibited a gradual increase ($P \leq 0.05$) in lipid oxidation as the storage time increase. Control fish had a higher TBA value than treated fish with antioxidants. These results are in good agreement with those obtained by Ramanathan and Das (1992) and Mai and Kinsella (1979), who reported an increase in TBA values in stored fish treated with phenolic antioxidants. The increase in the extent of lipid oxidation associated with storage temperature has been reported and attributed to the increase in the rate of propagation and decomposition of alkyl peroxides (Troller and Christian, 1978).

It is important to note that antrancine 350, which had the strongest effect in controlling the extent of lipid oxidation of common carp fillets, consisted of a combination of propyl gallate, TBHQ, and citric acid. Also, Sustane HW-4, which consisted of a combination of BHA and BHT, had less effect than antrancine 350. TBHQ has been proved to be effective in preventing the formation of TBA reactive materials in fish and also seemed to be slightly more effective than BHA (Mai and Kinsella, 1979). TBHQ had been reported to be the best phenolic antioxidant for fish lipids (Sweet, 1973). Kelleher et al. (1992) reported that minced Atlantic mackerel treated with propyl gallate had lower TBA value than mackerel treated with BHT and BHA. It has been generally known that lipid oxidation in fish is affected

Table 2. Effect of Different Concentrations of Antrancine 350 on TBA Values (Milligrams of Malonaldehyde per Kilogram of Dry Matter) of Raw Common Carp Fillet Stored at 5 °C for 16 Days

	storage time					
treatment	0 days	4 days	8 days	12 days	16 days	mean[a]
control	0.35	0.70	0.98	2.09	3.15	1.454[e]
antrancine 350[b]						
50 ppm	0.35	0.59	0.79	0.93	1.09	0.750[d]
100 ppm	0.35	0.50	0.67	0.77	0.92	0.642[c]
150 ppm	0.35	0.48	0.58	0.73	0.83	0.594[b]
200 ppm	0.35	0.39	0.50	0.59	0.68	0.502[a]
250 ppm	0.35	0.38	0.51	0.58	0.67	0.498[a]
300 ppm	0.35	0.39	0.50	0.60	0.68	0.504[a]
mean[c]	0.350[a]	0.490[b]	0.647[c]	0.898[d]	1.146[e]	

[a] Means in the same column with different letters are significantly different ($P \leq 0.05$), LSD = 0.014. [b] Antrancine 350 = propyl gallate, TBHQ, and citric acid. [c] Means in the same row with different letters are significantly different ($P \leq 0.05$), LSD = 0.012.

Table 3. Effect of Dipping Time in Antrancine 350 on TBA Values (Milligrams of Malonaldehyde per Kilogram of Dry Matter) of Raw Common Carp Fillets Stored at 5 °C for 16 Days

	storage time					
treatment	0 days	4 days	8 days	12 days	16 days	mean[a]
control	0.36	0.69	1.04	2.18	3.29	1.512[d]
antrancine 350[b]						
15 min	0.36	0.50	0.62	0.79	0.95	0.644[c]
30 min	0.36	0.46	0.54	0.64	0.74	0.548[b]
45 min	0.36	0.36	0.47	0.56	0.62	0.474[a]
60 min	0.36	0.36	0.46	0.57	0.63	0.476[a]
75 min	0.36	0.37	0.47	0.58	0.63	0.482[a]
90 min	0.36	0.37	0.47	0.58	0.64	0.484[a]
mean[c]	0.360[a]	0.444[b]	0.581[c]	0.843[d]	1.071[e]	

[a] Means in the same column with different letters are significantly different ($P \leq 0.05$), LSD = 0.013. [b] Antrancine 350 = propyl gallate, TBHQ, and citric acid. [c] Means in the same row with different letters are significantly different ($P \leq 0.05$), LSD = 0.011.

by metal ions such as heme and non-heme iron (Khayat and Schwall, 1983; Kelleher et al., 1992). Citric acid has been found to have iron binding capabilities and may reduce or eliminate its catalytic effects on lipid oxidation (Ahn et al., 1993).

The above-mentioned reports of TBHQ, propyl gallate, and citric acid support our findings on the effectiveness of antrancine 350 over other commercial antioxidants in controlling lipid oxidation of carp fillets.

TBA values were significantly ($P \leq 0.05$) affected by the concentration of antioxidant and storage period (Table 2). Increasing the concentration of antioxidant from 50 to 200 ppm resulted in a significant ($P \leq 0.05$) decrease in TBA values. On the other hand, TBA values were not affected by concentrations >200 ppm. TBA values were progressively increased ($P \leq 0.05$) throughout the storage period. Control treatment showed a rapid increase in TBA values, while fish treated with different levels of antrancine 350 increased with a slower rate throughout the storage period.

TBA values of raw carp fillets were significantly ($P \leq 0.05$) affected by dipping time and storage period (Table 3). Increasing the dipping time up to 45 min resulted in a significant ($P \leq 0.05$) decrease in TBA values. However, TBA values were not affected by dipping time >45 min. TBA values were significantly ($P \leq 0.05$) increased as the storage period progressed. The rate of increase in TBA values of the control samples was higher than that of the treated fish with antrancine 350 throughout the storage period. The results indicated that the extent of lipid oxidation in common carp fillets was inhibited throughout the refrigerated storage with antrancine 350 treatment at a concentration of 200 ppm and dipping time of 45 min. Therefore, this antioxidant will be used for the packaging experiment and sensory evaluation test.

The effects of cooking and packaging conditions on TBA values of common carp fillets treated with antrancine 350 either with or without vacuum packaging during refrigerated storage are shown in Table 4. Cooked carp fillets showed higher ($P \leq 0.05$) TBA values than uncooked carp fillets during the refrigerated storage either with vacuum or without vacuum packaging condition. Oxidative rancidity usually occurs more rapidly in cooked ground fish than in raw fish (Ramanathan and Das, 1992). Lee and Toledo (1977) also found that cooking significantly raised the TBA values of minced mullet (*Mugil* spp.) during refrigerated storage. The cooking process probably disrupts the muscle membrane system, thereby exposing the lipid components to oxygen and/or other reaction catalysts such as iron (Sato and Hegarty, 1971; Love and Pearson, 1976). Vacuum packaging was more effective ($P \leq 0.05$) than the absence of vacuum packaging in controlling lipid oxidation of common carp fillets both in uncooked or in cooked conditions. This was in agreement with the results of Hwang and Regenstein (1988), who showed

Table 4. Effect of Various Packaging Conditions and Storage Periods on TBA Values (Milligrams of Malonaldehyde per Kilogram of Dry Matter) of Cooked and Uncooked Carp Fillets Treated with Antrancine 350 (at Concentration of 200 ppm and Dipping Time of 45 min)

	storage time					
treatment	0 days[a]	4 days	8 days	12 days	16 days	mean[b]
without vacuum packaging[c]						
uncooked	0.36	0.37	0.47	0.55	0.61	0.472[b]
cooked hot packaging	0.47	0.49	0.59	0.75	0.94	0.648[f]
cooked ambient packaging	0.48	0.54	0.67	0.83	1.08	0.720[h]
cooked cold packaging	0.48	0.52	0.61	0.79	0.98	0.676[g]
with vacuum packaging[d]						
uncooked	0.36	0.36	0.39	0.41	0.43	0.390[a]
cooked hot packaging	0.47	0.47	0.51	0.55	0.57	0.514[c]
cooked ambient packaging	0.48	0.52	0.57	0.62	0.65	0.568[e]
cooked cold packaging	0.47	0.49	0.54	0.58	0.61	0.538[d]
mean[e]	0.446[a]	0.470[b]	0.543[c]	0.635[d]	0.734[e]	

[a] Zero day samples were analyzed 2 h after treatment. [b] Means in the same column with different letters are significantly different ($P \leq 0.05$), LSD = 0.022. [c] Packaged in polyethylene bags. [d] Packaged in nylon−polyester bags. [e] Means in the same row with different letters are significantly different ($P \leq 0.05$), LSD = 0.017.

Table 5. Effect of Various Packaging Conditions and Storage Periods on Rancid Odor[a] of Cooked and Uncooked Carp Fillets Treated with Antrancine 350 (at Concentration of 200 ppm and Dipping Time of 45 min)

	storage time					
treatment	0 days	4 days	8 days	12 days	16 days	mean[b]
without vacuum packaging[c]						
uncooked	0.58	0.71	1.16	2.33	3.04	1.564[e]
cooked hot packaging	0.66	0.79	1.87	2.96	4.37	2.132[f]
cooked ambient packaging	0.70	0.96	2.29	3.54	4.91	2.473[h]
cooked cold packaging	0.70	0.87	2.12	3.25	4.71	2.263[g]
with vacuum packaging[d]						
uncooked	0.58	0.66	0.75	0.96	1.46	0.884[a]
cooked hot packaging	0.66	0.71	1.00	1.46	1.87	1.140[b]
cooked ambient packaging	0.70	0.87	1.21	1.87	2.37	1.404[d]
cooked cold packaging	0.66	0.79	1.12	1.66	2.16	1.264[c]
mean[e]	0.655[a]	0.792[b]	1.440[c]	2.213[d]	3.103[e]	

[a] Means based on a 6-point scale: 0 = absent; 1−1.5 = very slight; 1.5−3 = slight; 4 = moderate; 5 = strong; and 6 = very strong. [b] Means in the same column with different letters are significantly different ($P \leq 0.05$), LSD = 0.079. [c] Packaged in polyethylene bags. [d] Packaged in nylon−polyester bags. [e] Means in the same row with different letters are significantly different ($P \leq 0.05$), LSD = 0.063.

the efficiency of vacuum packaging in retarding oxidative rancidity during frozen storage of menhaden mince. With regard to packaging conditions of cooked carp fillets either with or without vacuum, hot packaged fillets had lower ($P \leq 0.05$) TBA values than ambient and cold temperature packaged fillets throughout the storage period. Changes in TBA values of hot-packaged fillets under vacuum from 0 day to 16 day were not much different compared to other packaging treatments. The results indicated that the uncooked fillets treated with antioxidant and vacuum packaging had the lowest ($P \leq 0.05$) TBA values throughout the storage period. However, the combination of antioxidant and hot vacuum packaging gave the best protection against lipid oxidation among other cooked fillets with different packaging conditions.

The effect of various packaging conditions and storage period on panel scores of cooked and uncooked carp fillets treated with antrancine 350 are shown in Table 5. There was a significant ($P \leq 0.05$) difference in panel scores among packaging conditions of cooked and uncooked fillets packaged either with vacuum or without vacuum. The results indicated that the uncooked fillets packaged with vacuum received lower ($P \leq 0.05$) panel scores than other treatments. Also, hot packaging with vacuum had lower rancid odor compared to ambient and cold temperature packaging. In general, fillets packaged in polyethylene bags without vacuum had a stronger rancid odor development than those in nylon−polyester bags with vacuum. Also, cooked fillets either with or without vacuum packaging were found to be more rancid than uncooked fillets. Rancid odor was significantly ($P \leq 0.05$) increased as the storage period progressed. At the end of the storage period carp fillets packaged in nylon−polyester bags under vacuum had scores ranging from very slight to slight rancid odor, while those packaged in polyethylene bags without vacuum had scores ranging from moderate to strong rancid odor. Uncooked fillets and hot-packaged fillets stored in vacuum bags were the most acceptable treatments throughout the storage period.

CONCLUSION

It could be recommended to keep treated carp fillets with antioxidant in the uncooked form with vacuum packaging and then to apply the cooking process after refrigerated storage. This might prevent the development of oxidative rancidity, which leads to off-odor and qualitative deterioration in fish muscle.

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