JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of a Low-Density Polyethylene Film Containing Butylated Hydroxytoluene on Lipid Oxidation and Protein Quality of Sierra Fish (*Scomberomorus sierra*) Muscle during Frozen Storage

WILFRIDO TORRES-ARREOLA,[†] HERLINDA SOTO-VALDEZ,[‡] ELIZABETH PERALTA,[‡] JOSÉ LUIS CÁRDENAS-LÓPEZ,[†] AND JOSAFAT MARINA EZQUERRA-BRAUER*,[†]

Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Blvd. Luis Encinas y Rosales s/n, Apdo. Postal 1658, Col. Centro, Hermosillo, Sonora, 83000 Mexico, and Centro de Investigación en Alimentación y Desarrollo, A.C., Apdo. Postal 1735, Hermosillo, Sonora, 83000 Mexico

Fresh sierra fish (*Scomberomorus sierra*) fillets were packed in low-density polyethylene films with butylated hydroxytoluene (BHT-LDPE) added. Fillets packed in LDPE with no BHT were used as controls (LDPE). The packed fillets were stored at −25 °C for 120 days in which the film released 66.5% of the antioxidant. The influence of the antioxidant on lipid and protein quality, lipid oxidation, muscle structure changes, and shear−force resistance was recorded. As compared to LDPE films, fillets packed in BHT-LDPE films showed lower lipid oxidation, thiobarbituric acid values (4.20 ± 0.52 vs 11.95 ± 1.06 mg malonaldehyde/kg), peroxide values (7.20 ± 1.38 vs 15.15 ± 1.48 meq/kg), and free fatty acids (7.98 ± 0.43 vs 11.83 ± 1.26% of oleic acid). Fillets packed in BHT-LDPE films showed less tissue damage and lost less firmness than fillets packed in LDPE. A significant relationship between lipid oxidation and texture was detected (R^2 adjusted, 0.70−0.73). BHT-LDPE films may be used not only to prevent lipid oxidation but also to minimize protein damage to prolong the shelf life of sierra fish.

KEYWORDS: Sierra fish; *Scomberomorus sierra*; butylated hydroxytoluene; low-density polyethylene; frozen storage; lipid oxidation; myofibrillar protein denaturation

INTRODUCTION

The commercial success of frozen fish fillets could be limited by losses in either flavor and/or texture (*1*). The deterioration in the flavor of frozen fish is due primarily to lipid oxidation (*2*), particularly in pelagic species rich in dark muscle (*3*) such as sierra fish (*Scomberomorus sierra*).

On the other hand, seafood technologists recognize that protein denaturation and aggregation in fish muscle are normally associated with lipid oxidation (*4*). Studies have demonstrated that breakdown products of lipid oxidation, such as malonaldehyde, can react with the amino groups of myosin (*5*). This reaction could contribute to denaturation and protein modifications in frozen products (*4*).

However, the process of stabilization of lipid oxidation on frozen fish muscle is not well-documented; therefore, the approach based on the addition of antioxidants in fish products is under extensive investigation. Natural and synthetic antioxidants are frequently added to fatty foods, particularly those prone to oxidation. There are two main classes of antioxidants: molecules that prevent the action of catalysts and molecules that are oxidized preferentially to the lipid (*6*). It is the second class that will be addressed in this article. Sacrificial antioxidants are oxidized more easily than the lipids that they are designed to protect. The synthetic molecules used as antioxidants in many foods, permitted as food additives, include butylated hydroxyanisole and butylated hydroxytoluene (BHT) (*7*).

The use of antioxidants in marine products has been evaluated mainly on marine oils and/or by addition directly into fish muscle (*8*). The maintenance of quality and the extension of the shelf life of frozen fish by packaging systems are two routes to marketing success (*9*). Therefore, the application of packaging films containing antioxidants (active packaging) as a medium in contact with food surfaces can delay lipid oxidation and protein denaturation. The release of the antioxidant substance from packaging material to the food surface may prevent exceeding concentrations beyond critical levels established by the Food and Drug Administration (*7*). This beneficial role of antioxidant in packaging films has led to research in the manufacture of antioxidant-added films and their utilization in various food-packaging situations (*10*).

* To whom correspondence should be addressed. E-mail: ezquerra@guayacan.uson.mx.
[†] Universidad de Sonora.
[‡] Centro de Investigación en Alimentación y Desarrollo.

10.1021/jf070418h CCC: $37.00
Published on Web 06/27/2007

During film processing, part of the antioxidant is lost because of its ability to function as a free radical scavenger. Also, it may be lost to the environment because of its high volatility at processing temperatures (*11*). Therefore, to ensure the extension of the shelf life of food susceptible to deterioration by oxidation, active packaging films must be formulated with a higher antioxidant concentration than the common polyolefin films. Studies of migration of BHT from polyolefin films have shown BHT to have high mobility toward fatty foods and into the environment (*12*, *13*). As BHT can be used as an additive for direct addition to food (*7*), its use in active packaging has led to commercial applications, such as in the liners for breakfast cereal packages (*14*).

Huang and Weng (*15*) showed that BHT incorporated into polyethylene films was able to inhibit lipid oxidation in both fish muscle and oil. However, the polyethylene film was not fabricated with the incorporation of the antioxidant during the extrusion process. The present study was conducted to determine if a low-density polyethylene (LDPE) film extruded with BHT delays lipid oxidation and decreases protein denaturation on a fatty fish species. Sierra fish (*S. sierra*) was used due to its economic importance in Mexico, its lipid content (~10%), and its concentration of unsaturated fatty acids, which makes it a suitable oxidation model system.

MATERIALS AND METHODS

Chemicals. BHT (99% purity, TCI America, Portland, United States), LDPE resin (Muehlstein International, Connecticut), sodium dodecyl sulfate, acrylamide, urea, phenyl methyl sulfonide fluoride (PMSF) (Biorad, Guadalajara, Mexico), ethylendiamine tetraacetic acid (EDTA), sodium azide, potassium chloride (KCl), β-mercaptoethanol, bromophenol blue, formaldehyde, epoxy resin, hematoxylin, and eosin (Sigma-Aldrich, Toluca, Mexico) were purchased. Methanol and chloroform (Fluka, Toluca, Mexico), acetonitrile (ACN), high-performance liquid chromatography (HPLC) grade methanol, and water (JT Baker, Xalostoc, Mexico) were also puchased and used.

Instruments. A pilot plant size blow-extrusion machine (Beutelspacher, Mexico D.F., Mexico) was used. A liquid chromatograph (Varian 9012, Mexico) was coupled to a fluorescence detector (Varian 9075, Mexico) with an excitation wavelength of 282 nm and an emission wavelength of 308 nm. A miniprotean III gel electrophoresis unit was purchased from Biorad Laboratory Chemicals (Hercules, CA), and a Gel Image Analysis Systems model GelDoc XR-Quantity One 4.6 was also purchased (Biorad Laboratory Chemicals). Also used were the following instruments: micrometer (model DTT, E.J. Cady & Co., Illinois); oxygen permeability analysis system, Oxtran 2/20 meter (Mocon, Modern Controls, Inc., Minneapolis, MN); thermic sealer NT 300 (Doo-Il R.S. Co., Ltd., South Korea); Texturometer Chatillon 2-3b (Empire Scale Co., Santa Fe Springs, CA); tissue homogenizer M133/1281 (Biospec Products, Bartlesville, OK); tissue processor Tissue-Tek 11 (Mishawaka, IN), HistoEmbedder (Leica Nussloch, Germany), Microtome AG (Scientific Instruments, Buffalo, NY); and an optical microscope (standard microscope 25 ICS attached with polarization equipment, Zeiss MC 80 DX microscope camera, and 35 mm cassette; Carl Zeiss Mikroskopie, Germany).

Antioxidant Films. A LDPE film with 40 mg/g of BHT (BHT-LDPE) added was fabricated by a blow-extrusion process with a pilot plant size blow-extrusion machine. A LDPE film with no BHT was fabricated and used as a control. The film thickness was measured with a micrometer.

Determination of BHT Concentration in the Films. One gram pieces of film (1 cm × 1 cm) were extracted with 100 mL of ACN with constant stirring under reflux at 60 °C for 6 h. After an additional 6 h extraction period to ensure that no BHT remained in the film, the extract was rotary-evaporated to 30 mL at 25 °C. Four replicates were carried out for each sample. BHT quantification was performed by HPLC. A 10 mL sample volume was injected (Rheodyne 7125 injector) into the HPLC and eluted with methanol:water (98:02) at a flow rate of 1 mL/min for 10 min at 25 °C. A C_{18} Omnispher column (150 mm × 4.6 mm) (Varian), protected with C_{18} guard columns (50 mm), was used. A calibration curve for BHT was prepared from 0.5 and 20 ppm solutions in ACN. Results were reported in mg/g of film.

Barrier Properties. The oxygen transmission rate [OTR—$cm^3/(m^2 \cdot 24\ h)$] of each film was measured using an Oxtran 2/20 meter (*16*). Tests were performed at 23 °C. Four replicates were carried out for each sample.

Fish Samples. Sierra fish used in this study were captured with a gill net in the Gulf of California during the autumn season and transported in ice to the Seafood Laboratory at the University of Sonora within 6 h of capture (postrigor state). The fish (200−350 g) were gutted and filleted the same day of catch, and the fillets were packed the next day.

Packaging and Storage of Fresh Sierra Fish. Fillets (120 g) were packed in 30 cm × 10 cm pouches made of BHT-LDPE and LDPE films (600 cm^2 or 4.29 g of film per fillet) and sealed in air with a thermal sealer. The contact of the fillets with the film was assured. The packed fillets were immediately frozen at −40 °C with solid carbon dioxide and acetone solution. The freezing time was established to be 4 min; therefore, a fast frozen system was applied. Frozen fillets were stored in darkness, at −25 °C for 0, 30, 60, 90, and 120 days. Three fillets were sampled, unpacked, and thawed overnight (at 4 °C) for lipid, protein, and histological analyses, whereas 15 fillets were managed in the same way for texture analysis.

Release of BHT from the BHT-LDPE Film. To estimate the migration of BHT from the BHT-LDPE pouches, the films were separated from the fillets at every sampling time. Extraction and quantification of BHT by HPLC were performed as described above. BHT was not quantified in the fish fillets because once it arrives in the tissue, it may be lost when trapping free radicals.

Thiobarbituric Acid Value (TBA). TBA was determined using 10 g of fish flesh (*17*). The TBA was reported as mg malonaldehyde/kg sample.

Peroxide Value (PV) and Free Fatty Acids (FFAs). Lipids were extracted with a water:methanol:chloroform (30:50:100) mixture from a 50 g sample of minced flesh and immediately analyzed. The PV and FFA of the lipid extracts were determined according to Woyewoda (*17*).

Electrophoresis Analysis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) was done by the method of Laemmli (*18*). The stacking gel and the resolving gel contained 4 and 10% acrylamide, respectively. Samples for electrophoresis were prepared by homogenizing 3 g of minced muscle in 30 mL of cold 8 M urea solution (8 M urea, 0.1 mM PMSF, 0.1 mM EDTA, 0.01% sodium azide, and 0.6 M KCl) with a tissue homogenizer for 2 min. The homogenate was diluted 1:3 (w/v) in the sample buffer containing 8% SDS, 25% 0.5 M Tris-HCl (pH 6.8), 20% glycerol, 10% β-mercaptoethanol, 5% deionized water, and 0.03% bromphenol blue (*19*). Images of samples on electrophoresis gels were captured by using GelDoc XR-Quantity One 4.6. Once an image had been acquired, optimization to reduce noise or background was performed. The identification of the main muscle myofibrillar proteins was obtained by signal intensity (OD) of each band in relation to that of a known molecular mass standard.

Histological Observations. Tissues from fresh fish and frozen fillets packed in LDPE and BHT-LDPE films were fixed in 10% formalin. A dehydration process was performed according to Prophet (*20*) by using a tissue processor. After the tissues were embedded in epoxy resin in the HistoEmbedder, thick sections (5 μm thick) were prepared using a microtome. They were stained with hematocylin−eosin and observed under an optical microscope.

Texture. Portions of 15 fillets from fresh (0-day) and frozen fish in contact with BHT-LDPE or LDPE films (30, 60, 90, and 120-days) were subjected to texture analysis. The texture was measured by recording the force required to penetrate the material using a Chatillon 2-3b texturometer with a cylindrical plunger of 0.6 cm diameter. Each sample (1 cm × 1 cm × 1 cm) was placed in a position relative to the cylindrical plunger that would result in shearing across the muscle fiber (*21*).

Statistical Analysis. A completely randomized, two-way analysis of variance statistical design was carried out, using the type of film

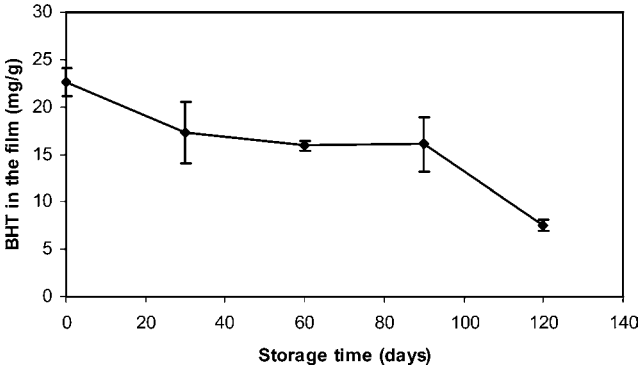

Figure 1. Decrease of BHT from the BHT-LDPE film during storage at −25 °C in contact with sierra fish fillets. Results are means of three replicates. Bars indicate standard deviation.

(two levels) and storage time (five levels) as factors. The difference of PV, TBA, FFA, and texture (shear force) means among factor levels were resolved using the Tukey method. The level of significance was $p < 0.05$. Correlation analysis between lipid oxidation (TBA and PV) and texture was also performed. The computer program used for this analysis was JMP 4.04 (Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

BHT Concentration in the Film. The BHT level remaining after processing of the film was 22.64 ± 1.43 mg/g. Thus, the amount of BHT lost during processing was 43.4%. BHT may be thermally degraded or lost by reaction in performing its function as an antioxidant. Also, it may be lost to the environment because of its high volatility at processing temperatures. It has been reported that 10% of BHT was volatilized during a thermal gravimetric analysis of the antioxidant at 157 °C (*11*). Temperatures up to 165 °C were used in the present work. BHT may also be lost when trapping free radicals from LDPE chains during processing. Other authors reported similar losses for a LDPE film initially fabricated with 0.45 mg/g BHT. After processing, 0.25 mg/g (55.5%) of the antioxidant remained in the film (*12*).

Barrier Property of the Films. The OTR values for the films were 1282 ± 4 and 1872 ± 42 $cm^3/(m^2 \cdot 24\ h)$ for the LDPE and BHT-LDPE, respectively. Meanwhile, the thickness was 193 ± 19 and 226 ± 24 μm for the LDPE and BHT-LDPE films, respectively. The BHT-LDPE film showed a higher OTR as compared to the LDPE film, in spite of being thicker. This could be due to the effect of the small size incorporated antioxidant, which might have decreased the contact among the polymer chains. This effect was also reported for LDPE films added with α-tocopherol (*22*). A value of 7883 $cm^3/(m^2 \cdot 24\ h)$ has been reported for a 33 μm LDPE commercial film with no antioxidants added (*23*). The relatively high OTR value for BHT-LDPE film could promote lipid oxidation in the packed product. That fact was convenient to be able to observe the performance of the film added with the antioxidant. However, it is important to consider that lower OTR values will be expected from the films used in the present work at frozen temperatures, as compared to the experimental results at 23 °C.

Release of BHT from the BHT-LDPE Film. **Figure 1** shows the loss of BHT from the BHT-LDPE film during the frozen storage. The level of the antioxidant decreased from 22.64 ± 1.43 to 17.32 ± 3.23 mg/g during the first 30 days of storage (23.5% of BHT released). This level remained constant from 30 to 90 days. However, at 120 days, only 7.59 ± 0.60 mg/g of BHT was left in the film (66.5% of BHT released). There is not a clear explanation for this steep reduction of BHT in the last month of storage. It will be an interesting topic for future work. Assuming that all BHT released from the BHT-LDPE film migrated to the fillets, figures from 146.0 to 231.7 mg/kg of BHT in the fish could be estimated during the first 90 days of storage. The level would increase to 491.3 mg/kg at the end of the storage. However, it must be considered that not all of the BHT released from the films diffuses into the fillets. Although no reports for migration of BHT at frozen temperatures were found, it might be expected that a portion of the antioxidant would be released to the environment. A solution for this inconvenience is to fabricate BHT-LDPE films coextruded with a different polymer such as polyamide. A rapid decrease of BHT from the LDPE films into different foods and food simulants has been reported at different temperatures. Half of the BHT in a film (0.85 mg/g) in contact with oil was lost to the environment in less than 6 h at 25 °C (*24*). The loss of BHT from an oatmeal packaging (LDPE) occurred via diffusion through the material followed by volatilization from the film surface, by sorption onto the oatmeal or by diffusion into the surrounding atmosphere the final result (*13*). Therefore, the rapid decrease of BHT is due to its relatively small size, rate of evaporation, solubility in oils, and high diffusion ability in the LDPE matrix. Future work must be done to determine the rate of evaporation of BHT from LDPE films at −25 °C.

Effect of the Films on Lipid Stability. The composition of sierra fish fillets was as follows: moisture, 70.94%; protein, 20.64%; fat, 9.45%; and ash, 2.17%. Thus, sierra fish muscle was expected to show high lipid oxidation during the frozen storage. The changes in lipids are directly and indirectly responsible for the quality deterioration in frozen seafood (*2*). During the 120 days of frozen storage, increases in TBA (**Figure 2a**), PV (**Figure 2b**), and FFA (**Figure 2c**) values were observed for sierra fish fillets packed in both films, LDPE and BHT-LDPE. Higher rates of lipid oxidation were observed in fillets packed in LDPE film as compared to BHT-LDPE film.

Both LDPE and BHT-LDPE fillets showed a significant increase in their TBA (after 30 days of frozen storage) and PV (after 90 days of frozen storage) values. However, at 120 days of frozen storage, the PV values of the fillets packed in the LDPE film were higher than those recommended by the Codex Alimentarius (10 mequiv/kg of lipids) (*25*). After 30 days of frozen storage, fillets packed in BHT-LDPE film showed TBA values lower ($p < 0.05$) than the control and did not reach the maximum values of the guidelines. Maximum TBA values quantified in fillets packed in the LDPE and BHT-LDPE films were 11.95 ± 1.06 and 4.20 ± 0.52 mg malonaldehyde/kg, respectively.

After 120 days of frozen storage, fillets packed in BHT-LDPE showed FFA values lower ($p < 0.05$) than control (**Figure 2c**). The formation of FFA during the frozen storage is due mainly to the activity of some endogenous lipases (*1*, *2*). However, during the propagation stage of the lipid oxidation, short-chain FFAs may also be derived from unstable hydroperoxides. Hydroperoxides break down to aldehyde, ketones, alcohol, and short-chain FFA (*2*). Therefore, the migration of BHT from the LDPE may have contributed to the lower level of FFA in these fish fillets.

Lipid oxidation in frozen fish is a net result of interaction of numerous factors such as pH value, amount of unfrozen water, and content of pro- and antioxidant compounds (*26*). The use of packaging is the most effective means of preventing dehydration, and the antioxidants prevent lipid oxidation (*27*). However, the antioxidant usually delays but does not avoid lipid oxidation (*5*), as was observed in this study. Consequently, the use of

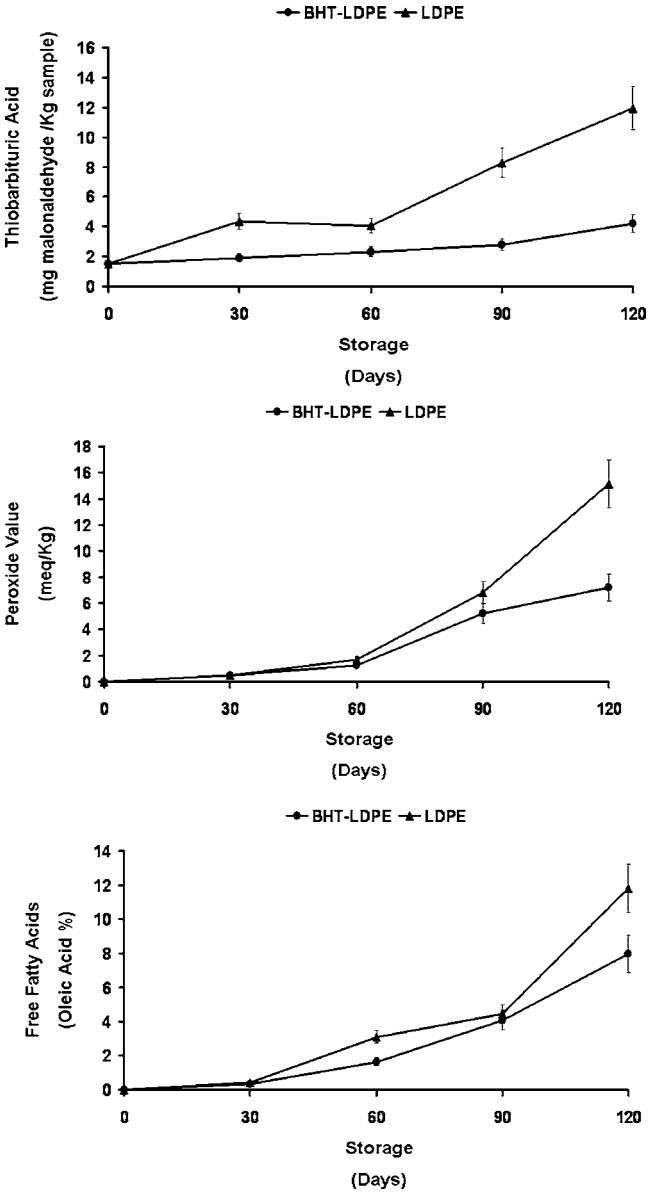

Figure 2. Changes in TBA (**a**), PV (**b**), and FFAs (**c**) of sierra fish fillets packed in LDPE (▲) and BHT-LDPE (●) films for 120 days at −25 °C. Results are means of three replicates. Bars indicate standard deviation.

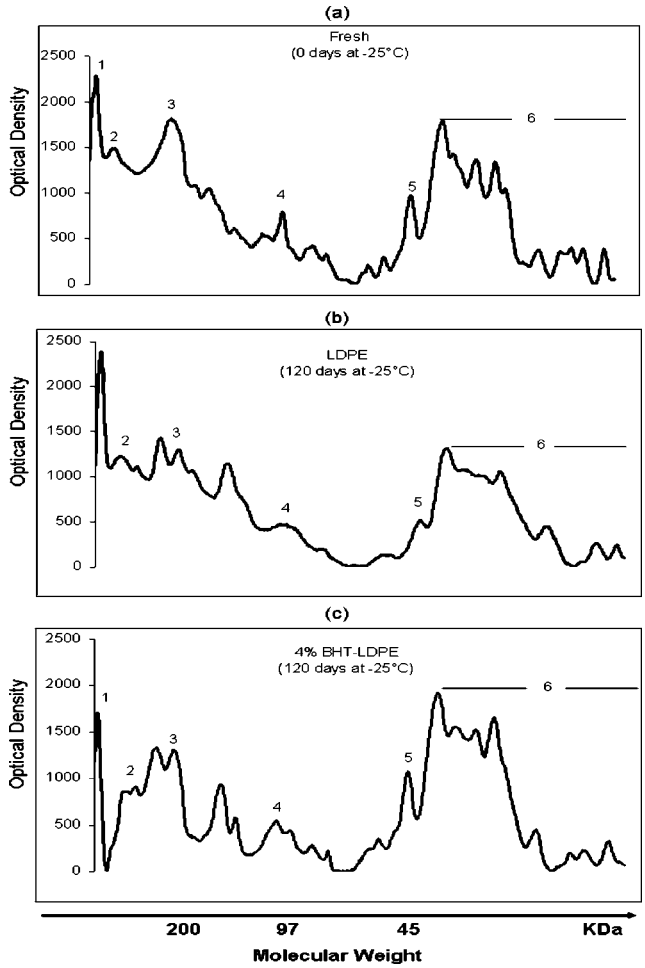

Figure 3. Densitograms of myofibrillar protein extracts from sierra fish fillets packed in LDPE and BHT-LDPE films for 120 days at −25 °C. (**a**) Fresh sierra fish fillet protein extracts, (**b**) 120 days frozen sierra fish fillets packed in LDPE film, and (**c**) 120 days frozen sierra fish fillets packed in BHT-LDPE film. Peak 1, injection point; peak 2, proteins with a molecular mass of 220 kDa; peak 3, myosin (200 kDa); peak 4, paramyosin (97 kDa); peak 5, actin (45 kDa); and zone 6, myosin light chain.

plastic packaging film containing antioxidant decreased the rate of lipid oxidation of sierra fish fillets during 120 days at −25 °C.

Effect of the Films on Fish Muscle Tissue. Previous studies have demonstrated that breakdown products of lipid oxidation produced important structural modifications in the myofibrillar proteins (*4*), and antioxidants like BHT may be used not only to prevent lipid oxidation but also to minimize protein damage (*5*). SDS-PAGE was applied to detect more accurately the protein changes in sierra fish fillets induced by the freezing processes and during storage at −25 °C. Densitograms from SDS-gel electrophoresis of BHT-LDPE and control are shown in **Figure 3**. Densitogram analysis revealed different SDS-PAGE patterns for both muscle tissues, from fillets in contact with LDPE and BHT-LDPE films, during the storage at −25 °C. Concerning the profile of myofibrillar extract of the samples from fillets in contact with the LDPE film, a major decrease in the intensity of two bands (molecular mass 200 and 45 kDa) (**Figure 3b**) was observed, when compared to the fillets in contact with the BHT-LDPE film (**Figure 3c**). Therefore, the antioxidant film retarded protein alterations. These alterations induced more damage to the tissue, as was observed by the optical microscope.

Figure 4 shows optical microscope photographs of fresh and frozen fish muscle packed in LDPE and BHT-LDPE films at different storage times. The cells of fresh muscle showed tight contact with each other, and intracellular materials showed intact features (**Figure 4a**). At 30 days of storage, the muscle cells from fillets packed in the LDPE film began to detach from each other and some fractures were observed in the muscle fibers (**Figure 4b**), whereas the muscle cells from fillets packed in the BHT-LDPE film (**Figure 4c**) showed a similar pattern to those observed for fresh fish.

At 60 and 90 days of storage, the muscle cells from fillets packed in the LDPE film showed severe damage, fracture, and large spacing within fibers (**Figure 4d**,**f**). By the end of the frozen storage (120 days), evident fiber deformation was observed (**Figure 4h**) in the same fillet cells. Meanwhile, the muscle cells from fillets packed in BHT-LDPE films began to show a fracture pattern and interfiber spacing after 90 days at −25 °C (**Figure 4g**). The observed changes in muscle cells are due not only to myofibrillar protein structure changes but also to lipid oxidation (*26*). There is extensive information about

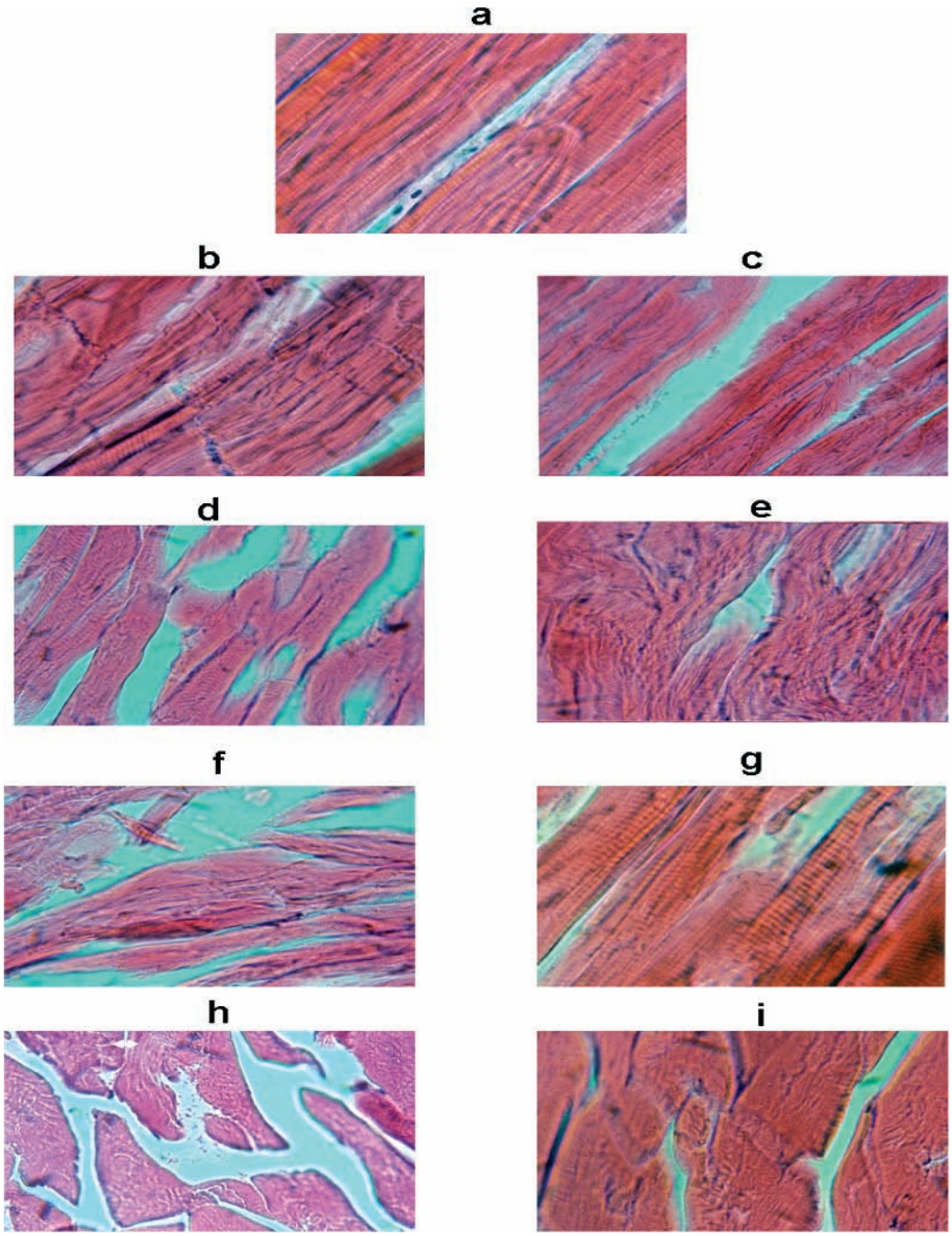

Figure 4. Histological observations of muscle tissue from sierra fish fillets packed in LDPE and BHT-LDPE films for 120 days at −25 °C. (**a**) Fresh sierra fish fillet muscle showing tight contact cells, (**b**) 30 days LDPE, (**c**) 30 days BHT-LDPE, (**d**) 60 days LDPE, (**e**) 60 days BHT-LDPE, (**f**) 90 days LDPE, (**g**) 90 days BHT-LDPE, (**h**) 120 days LDPE, and (**i**) 120 days BHT-LDPE.

the denaturation of myofibrillar proteins during frozen storage of fish (*1*, *2*). Some reports have shown that connective tissue proteins may suffer denaturation during frozen storage (*28*, *29*). Therefore, the disjunction among fish muscle cells observed in this study might be due not only to the loosening of myofibrils but also to disintegration of collagen fibrils. Further studies will be necessary to confirm how the antioxidant prevents fish connective tissue denaturation during frozen storage.

Lipid oxidation produces free radicals, and these are transferred to amino acids and proteins, which result in protein denaturation (*5*). These interactions result mainly in texture changes (*30*). The change in the maximum force (hardness mean value) of the sierra fillets muscle during 120 days of frozen storage is shown in **Figure 5**. Although no statistical differences were detected among the treatments during the first 60 days, after 90 days, fillets packed in LDPE showed a statistically significant decrease ($p < 0.05$) in shear force than those packed in BHT-LDPE.

Tissue toughening is common in many low-fat fish and in some fatty fish species stored at freezing temperatures (*31*). However, our results indicated that the sierra fish fillets become softer after 60 days of frozen storage. A decrease in firmness in salmon during frozen storage was also reported (*32*). These results suggested a firmness decrease during frozen storage of some fish species.

Correlation Analysis between Lipid Oxidation and Texture. The increase in lipid oxidation (TBA and PV) of sierra fish coincided with a decrease in shear force. Correlation analysis showed a significant ($p < 0.05$) relationship (R^2 adjusted 0.70 to 0.73) between TBA, PV, and FFA with texture

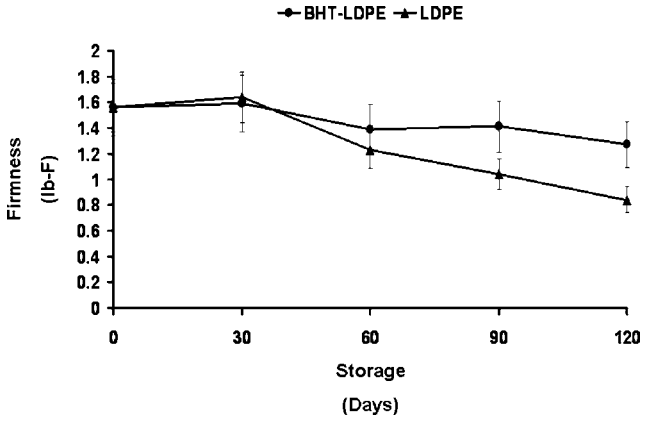

Figure 5. Change in shear–force resistance of fresh sierra fish fillets packed in LDPE (▲) and BHT-LDPE (●) films for 120 days at –25 °C. Results are means of 15 replicates. Bars indicate standard deviation.

Table 1. Correlation Analysis between Lipid Oxidation and Shear Force of Sierra Fish Fillets during 120 Days at –25 °C[a]

	variable		
	TBA:SF	PV:SF	FFA:SF
correlation	–0.87	–0.88	–0.86
R^2	0.76	0.77	0.73
R^2 adjusted	0.73	0.73	0.70
means of response	1.33	1.33	1.33
error	0.13	0.13	0.15
observations	9	9	9

[a] SF, shear force.

(**Table 1**). This result can be associated with the major disjunction observed among fish muscle cells and with the lost of firmness detected in control samples linked to protein denaturation, particularly to myosin heavy chains and actin. Therefore, further studies are needed to understand the kind of alterations occurring during the processing and storage of the sierra fish muscle.

Under the conditions of this work, it was evident that the use of LDPE films containing BHT not only delays the products of lipid oxidation but also prevents the fracture and detachment of sierra fish muscle cells. The role of lipid oxidation in promoting protein denaturation and deterioration in the texture of sierra fish has not been reported previously; therefore, our results might be a first step in the understanding of the alterations occurring during the processing and storage of sierra fish muscle.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; LDPE, low-density polyethylene; TBA, thiobarbituric acid value; PV, peroxide value; FFA, free fatty acid; OTR, oxygen transmission rate; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

ACKNOWLEDGMENT

We thank Reina Castro Longoria, Claudia Elizabeth Galindo-Arcega, Claudia Dinorah Soto-Cantú, and Francisco Javier Valdez Ibarra for their technical assistance.

LITERATURE CITED

(1) Haard, N. F. Biochemical reactions in fish muscle during frozen storage. In *Seafood Science and Technology Proceedings of the International Conference Seafood 2000*; Graham, E., Ed.; Fishing News Books, Blackwell Scientific Publications: Malden, MA, 1991; pp 176−208.

(2) Sikorski, Z.; Kalokowska, A. Freezing of marine food. In *Seafood Resources, Nutrition, Composition and Preservation*; Sikorski, Z., Ed.; CRC Press: Boca Raton, FL, 1990; pp 111−122.

(3) Undeland, I.; Lingnert, H. Lipid oxidation in fillets of herring (*Clupea harengus*) during frozen storage. Influence of prefreezing storage. *J. Agric. Food Chem.* **1999**, *47*, 2075−2081.

(4) Tironi, V. A.; Lopez, L. B.; Pellegrino, N.; Anon, M. C.; Tomas, M. C. Malonaldehyde-induced microstructural modifications in myofibrillar proteins of sea salmon (*Pseudopercis semifasciata*). *J. Food Sci.* **2004**, *69*, 519−523.

(5) Saeed, S.; Fawthrop, S. A.; Howell, N. K. Electron spin resonance (ESR) study on free radical transfer in fish lipid-protein interaction. *J. Sci. Food Agric.* **1999**, *79*, 1809−1816.

(6) Giese, J. Antioxidants: Tools for preventing lipid oxidation. *Food Technol.* **1996**, *50*, 73−74, 76, 78, 80−81.

(7) Code of Federal Regulations. *Food Additives Permitted for Direct Addition to Food for Human consumption, Subpart B-Food Preservatives: 21:172.115*; http://a257.g.akamaitech.net/7/257/2422/10apr20061500/edocket.access.gpo.gov/cfr_2006/aprqtr/pdf/21cfr172.115.pdf. Accessed 2006 November 28, 2006; pp 33.

(8) Tang, S.; Sheehan, D.; Buckley, D. J.; Morrissey, P. A.; Kerry, J. P. Anti-oxidant activity of added tea catechins on lipid oxidation of raw minced red meat, poultry and fish muscle. *Int. J. Food Sci. Technol.* **2001**, *36*, 685−692.

(9) Santos, E. E. M.; Regenstein, J. M. Effects of vacuum-packaging, glazing, and erythorbic acid on the shelf-life of frozen white hake and mackerel. *J. Food Sci.* **1990**, *55*, 64−70.

(10) Chick, J. Antioxidant delivery method. In *Worldpack 2002. Improving the Quality of Life through Packaging Innovation*; CRC Press: Boca Raton, FL, 2002; Vol. 1, pp 144−149.

(11) Vulic, I.; Vitarelli, G.; Zenner, J. Structure-property relationships: Phenolic antioxidants with high efficiency and low color contribution. *Polym. Degrad. Stab.* **2002**, *78*, 27−34.

(12) Wessling, C.; Nielsen, T.; Leufvén, A.; Jägerstad, M. Mobility of α-tocopherol and BHT in LDPE in contact with fatty food simulants. *Food Addit. Contam.* **1998**, *15*, 709−715.

(13) Wessling, C.; Nielsen, T.; Giacin, J. R. Antioxidant ability of BHT- and α-tocopherol-impregnated LDPE film in packaging of oatmeal. *J. Sci. Food Agric.* **2000**, *81*, 194−201.

(14) Yanidis, A. Flexible packaging material containing an antioxidant. U.S. Patent 4,889,696, 1989.

(15) Huang, C. H.; Weng, Y. M. Inhibition of lipid oxidation in fish muscle by antioxidant incorporated polyethylene film. *J. Food Process. Preserv.* **1998**, *22*, 199−209.

(16) ASTM. Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor. Designation: D 3985-81 (Re-approved 1988). *Annual Book of ASTM Standards*; ASTM: Philadelphia, PA, 1988; pp 534−539.

(17) Woyewoda, A. D.; Shaw, S. J.; Burns, B. G. Recommended laboratory methods for assessment of fish quality. *Canadian Technical Report of Fisheries and Aquatic Sciences No. 1448*; Minister of Supply and Services Canada: Halifax, Nova Scotia, Canada, 1986; Cat. no. Fs 97-4/1551, pp 156.

(18) Laemmli, U. Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature (London)* **1970**, *227*, 680−685.

(19) Childs, E. A. Electrophoresis of fish myofibrillar proteins with sodium-dodecyl sulfate-polyacrylamide gel. *J. Fish. Res. Board Can.* **1973**, *30*, 706−707.

(20) Prophet E. B. Fixation. In *Laboratory Methods in Histotechnology*; Prophet, E. B., Mills, B., Arrington, J. B., Sobin, L. H., Eds.; Armed Forces Institute of Pathology (AFIFP): Washington, DC, 1992; pp 27−35.

(21) Dunajski, R. Texture of fish muscle. *J. Texture Stud.* **1974**, *10*, 301−308.

(22) Wessling, C.; Nielsen, T.; Leufvén, A. The influence of α-tocopherol concentration on the stability of linoleic acid and the properties of LDPE. *Packag. Technol. Sci.* **2000**, *13*, 19−28.
(23) Soto-Valdez, H.; Mendoza-Wilson, A. M. Permeabilidad al oxígeno y al vapor de agua de películas plásticas utilizadas en el envasado de productos hortofrutícolas y cereales. II. *Hortic. Mex.* **1998**, *6*, 81−90.
(24) Galindo-Arcega, C. E. Migración del BHT de películas de PEBD y su efecto en la estabilidad del aceite de soya. M.S. Thesis, CIAD, A.C. Hermosillo, Mexico, 2004; p 89.
(25) *The Codex Alimentarius. Section 3, Volume 8: Codex Standard for Named Animal Fat (Codex Stand-211-1999)*; Food Standards Programme/Codex Alimentarius Commission, Food and Agriculture Organization of the United Nations/World Health Organization: Rome, 2001.
(26) Badii, H.; Howell, N. K. Effect of antioxidants and cryoprotectants blended with citric acid on protein solubility and texture of cod (*Gadus morhua*) fillets kept at −10 °C and −30 °C. *J. Agric. Food Chem.* **2002**, *50*, 2053−2061.
(27) Khayat, A.; Schwall, D. Lipid oxidation in seafood. *Food Technol.* **1983**, *37*, 130−40.
(28) Borderias, A. J.; Montero, P. Changes in fish muscle collagen during frozen storage. *Sci. Tech. Froid.* **1985**, *4*, 85−92.
(29) Badii, F.; Howell, N. K. Elucidation of the effect of formaldehyde and lipids on frozen stored cod collagen by FT-Raman spectroscopy and differential scanning calorimetry. *J. Agric. Food Chem*. **2003**, *51*, 1440−1446.
(30) Undeland, I. Lipid-protein interactions in fish during cold storage. *Methods to Determine the Freshness of Fish in Research and Industry*, Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness", AIR3CT94 2283, Nantes, France, Nov 12−14, 1997; pp 258−262.
(31) Saeed, S.; Howell, N. K. Rheological and differential scanning calorimetry studies on structural and textural changes in frozen Atlantic mackerel (*Scomber scombrus*). *J. Sci. Food Agric.* **2004**, *84*, 1216−1222.
(32) Regost, Ch.; Jakobsen, J. V.; Rora, A. M. B. Flesh quality of raw and smoked fillets of Atlantic salmon as influenced by dietary oil sources and frozen storage. *Food Res. Int.* **2004**, *37*, 259−271.

Received for review February 12, 2007. Revised manuscript received April 17, 2007. Accepted April 20, 2007. This study was supported by FOMIX-Sonora (2004-C03-011) and Qualyplast, S.A. de C.V.

JF070418H