JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Role of the Raw Composition of Pelagic Fish Muscle on the Development of Lipid Oxidation and Rancidity during Storage

Rodrigo Maestre,* Manuel Pazos, and Isabel Medina

Instituto de Investigaciones Marinas (CSIC), Eduardo Cabello 6, E-36208 Vigo, Spain

ABSTRACT: The muscle composition of a pelagic fish species, Atlantic mackerel (*Scomber scombrus*), has been studied to determine the relationship with its susceptibility to develop lipid oxidation during chilled storage. For such an aim, the initial concentrations of the major components (water, total lipids, protein, and PUFAs) and minor pro-oxidant and antioxidant components (ascorbic acid, α-tocopherol, hemoglobin, total iron, LMW-iron, copper, and zinc) of different batches of mackerel were characterized. For the study, several batches of mackerel were caught during the spring and summer periods. The different batches were subjected to chilled storage, and the onset of lipid oxidation was statistically related with the initial muscle composition. Results showed significant compositional differences among the mackerel lots, especially for the muscle lipid content (2.83−9.50%). In a first step, a Pearson correlation test was used to check the influence of each component on the progress of lipid oxidation. Results showed a significant relationship between shelf life and water and total lipid contents. Multiple regression was performed to reveal the contribution of each component to the susceptibility to lipid oxidation. The model obtained combines the content of PUFAs, total iron, hemoglobin, and ascorbic acid. An accurate prediction of shelf life in terms of rancidity was achieved by the model created (R^2 = 0.9975). These results establish that the levels of endogenous pro-oxidants and antioxidants present in fish muscle together with the polyunsaturated lipids are relevant factors affecting the shelf life of mackerel muscle.

KEYWORDS: lipid oxidation, mackerel, Pearson correlation, multiple regressions, shelf life

INTRODUCTION

Pelagic fish is extremely rich in essential ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (20:5ω-3) and docosahexanoic acid (22:6ω-3). Such fatty acids have shown potential benefits for human health.[1,2] However, the oxidative degradation of unsaturated fatty acids is the major cause of quality deterioration in pelagic fish species, leading to the development of rancid off-odors and a reduction of the nutritional value.

Lipid oxidation in fish muscle based foods is influenced by external factors such as storage temperature, time, precooking, processing, or packaging.[3,4] Internal factors related to the composition of muscle also play a key role in the oxidative reaction. According to the radical nature of the reaction, lipid oxidation is sensitive to substances able to catalyze or inhibit the formation of free radicals. In pork, beef, and chicken, the total content of PUFAs together with the presence of pro-oxidant heme proteins and free ionic iron has been suggested to play an important role in lipid oxidation.[5] The different sources of iron and their concentrations have also demonstrated a relative impact on meat lipid oxidation.[6] In these studies the balance between the total antioxidant/pro-oxidant capacities of meat seemed to be significant for determining the onset of lipid oxidation. Food processing also affects largely the state of intrinsic components. Meat thermal treatments are described to disrupt membranes, release iron, and accelerate the diffusion of reactants, leading to the propagation of oxidation.[7]

Studies performed on the oxidation of fish muscle based foods reveal a more complex situation, mainly attributed to a higher proportion and variety of PUFAs[8] and a major pro-oxidant activity of fish hemoglobins.[7] Additionally, pelagic fish species are characterized by huge variations in compositional parameters depending on season and food availability.[9−11] Such variability affects the content of fat and the levels of antioxidants and pro-oxidants. Various attempts using model systems have been made to investigate the role of lipids and promoters of oxidation on the development of lipid oxidation. The elevated susceptibility of fatty fish species to undergo lipid oxidation is often attributed to their intrinsic amount of PUFAs.[12] However, Undeland et al.[13] reported that the incorporation of 15% fish triacylglycerols to washed cod mince did not accelerate the formation of rancid off-odors. In different washed fish mince model systems, the total content of lipids showed a lower contribution than Hb to the development of oxidation.[14] Phospholipids have been denoted the primary substrate for fish lipid oxidation due to their high proportion of PUFAs.[15] Consequently, there is not a clear conclusion about the real influence of fat content on fish oxidation, and which are the decisive components that govern lipid oxidation in fish. Redox active metals such as iron and copper have been also described to enhance lipid oxidation in fish muscle due to their capacity to generate free radicals via Fenton-type reaction or hydroperoxides decomposition.[16]

The endogenous antioxidant system of fish that includes lipophilic and hydrophilic compounds has been also shown to influence the onset of fish lipid oxidation.[14] The loss of endogenous α-tocopherol seems to mark the onset of the propagation phase of lipid oxidation.[17] Other endogenous antioxidants such as ascorbic acid, which is lost in the early post-mortem stages, are important for maintaining of α-tocopherol in its original reduced

Received: February 18, 2011
Revised: May 2, 2011
Accepted: May 2, 2011
Published: May 02, 2011

dx.doi.org/10.1021/jf200686z | *J. Agric. Food Chem.* 2011, 59, 6284–6291

state.[18] The depletion order for endogenous antioxidants agrees with their "pecking order", which predicts a faster consumption of ascorbic acid than of α-tocopherol on the basis of the one electron reduction potential.[19] This must be considered in studies aimed to determine the oxidative susceptibility of fish muscle. Accordingly, the relationship between endogenous antioxidants and pro-oxidants of fish muscle is important to maintain the redox balance and must be considered in studies aimed to prevent lipid oxidation.[17,20]

This work aimed to evaluate the contribution of the initial composition of fish muscle on the development of lipid oxidation occurring during post-mortem storage. The experiments were performed directly in fish muscle because it provides more realistic information than model systems as all components are collectively studied at their original concentrations. Compositional variations of Atlantic mackerel (*Scomber scombrus*) caught at different seasonal periods were investigated and, then, related to their oxidative susceptibility during chilled storage. The influence of either major components (water, lipids, PUFAs, and protein) or minor components (ascorbic acid, α-tocopherol, hemoglobin, total iron, nonheme iron, copper, and zinc) on the progress of lipid oxidation was first investigated by lineal univariate regressions. The effect of raw composition on lipid oxidation was then evaluated by multiple regressions to gain knowledge of the relationship between several compositional variables and the shelf life of pelagic fish muscle in terms of oxidation.

MATERIALS AND METHODS

Reagents. Metaphosphoric acid, ascorbic acid (AA), dehydroascorbic acid (DHAA), iodine, 4,5-dimethyl-*o*-phenylenediamine (DMPD), bovine hemoglobin (Hb), sodium heparin, sodium chloride, tris-[hydroxymethyl]aminoethane (Tris), sodium dithionate, dibasic sodium phosphate, monobasic sodium phosphate potassium chloride, sodium dodecyl sulfate (SDS), DL-*all-rac*-α-tocopherol, ferrous chloride ($FeCl_2 \cdot 4H_2O$), ferric chloride ($FeCl_3 \cdot 6H_2O$), and streptomycin sulfate were purchased from Sigma-Aldrich (Steinheim, Germany). Standards of methyl fatty acids were also obtained from Sigma-Aldrich. Potassium iodide was obtained from Fluka (New-Ulm, Switzerland) and sodium thiosulfate from BDH (Poole, U.K.). Isobutanol was obtained from Merck (Darsmtadt, Germany). All chemicals and solvents used were of either analytical or HPLC grade. Water was purified using a Milli-Q system (Millipore, Billerica, MA). Carbon monoxide (99.97% purity) was provided by Air Liquide (Porriño, Spain).

Fish Supply. Seven different batches of fresh Atlantic mackerel (*S. scombrus*) were supplied by Vigo local markets during March 2009−July 2010. Each batch contained 8−10 kg of fish (20−30 individuals). Mackerel presented an extra quality of freshness according to EU regulations.[21]

Chilled Minced Mackerel. After arrival at the laboratory, each batch of fish was deboned and eviscerated, and the white muscle without skin was separated. The white muscle was chopped with a grinder machine, hole of 5 mm, to obtain homogeneous minced fish muscle. Streptomycin sulfate (200 ppm) was added to inhibit microbial growth during chilled storage. For each batch, initial compositional data were directly obtained in triplicate from the fresh minced muscle. For the lipid oxidation experiments, minced fish muscle was aliquotted in portions of 10 g and stored in 50 mL Erlenmeyer flasks at 4 °C. In each sampling time, lipid hydroperoxides and sensorial analysis were performed in triplicate.

Water Determination. The water content was determined according to the AOAC official method.[22]

Protein Determination. The AOAC Kjeldahl procedure was employed to estimate the protein content.[23]

Lipid Extraction. Lipids were extracted from fish muscle according to the method of Bligh and Dyer[24] and quantified gravimetrically.[25]

Fatty Acid Analysis. Fatty acids were methylated according to the method of Lepage and Roy,[26] and fatty acid 19:0 was added as an internal standard. The fatty acid methyl esters (FAME) were analyzed using a capillary gas chromatograph equipped with an autoinjector coupled to a flame ionization detector (Perkin-Elmer, Waltham, MA). The oven temperature started at 140 °C and reached 205 °C at an increment of 1 °C/min. The carrier gas was nitrogen, and splitless injection (1 μL) was done at 275 °C. The detector temperature was 260 °C. Fatty acids were identified by comparison of retention times with a mixture of standards. Results are expressed both as milligrams per kilogram of sample and as a percentage of the total fatty acids.

Determination of α-Tocopherol Concentration. α-Tocopherol (α-TOH) was extracted from fish muscle by adaptation of the procedure of Burton et al.[27] as described by Pazos et al.[28] The analysis of α-TOH was performed by HPLC according to the method of Cabrini et al.;[29] the chromatograph was coupled to a fluorescence detector (Exc 292/Em 328). α-TOH levels were expressed in milligrams per kilogram of muscle.

Determination of Ascorbate Concentration. Ascorbate ($AscH^-$) was acidically extracted from the minced fish muscle, oxidized, derivatized with DMPD, and analyzed by HPLC coupled to a fluorescence detector according to the method of Iglesias et al.[30]

Extraction and Quantification of Heme Proteins. Heme proteins were extracted from mackerel muscle according to the method of Larsson et al.[31] Briefly, samples were homogenized in a 50 mM Tris buffer (pH 8.0) containing 80 mM KCl and 1 mM EDTA. The homogenate was centrifuged at 37000*g* for 40 min at 4 °C, and the supernatant was filtered through a filter paper. Quantification of heme protein was done in duplicate on each sample extract according to the method by Brown[32] as modified by Richards and Hultin.[33] Bovine Hb was used to prepare the standard curve, and the results were expressed as micromoles of Hb per kilogram of sample (w/w).

Content of Total Iron, Copper, and Zinc. The total levels of the redox-active transition metals iron, copper, and zinc were determined as described by AOAC official method.[34] Briefly, samples were digested in nitric acid and hydrogen peroxide in a microwave oven and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES). The emission lines employed for iron, copper, and zinc were 238.204, 324.752, and 206.200 nm, respectively. The nonheme iron or low molecular weight iron (Fe-LMW) was determined as the difference between total iron and heme iron.

Sensory Analysis. Sensory detection of rancid odors was evaluated by an expert panel formed by four trained specialists in descriptive analysis of fishy off-odors. The determination was made in a room designed for such a purpose after the samples had been held during 10 min at room temperature. Approximately 10 g was placed in separate sterile polystyrene Petri dishes and put on a tray of ice. Panelists concentrated on detecting rancidity/painty odors using a structured scale, from 8 (absolutely fresh) to 1 (putrid).[35]

Peroxide Value. The formation of the primary lipid oxidation products, lipid peroxides, in chilled fish muscle was determined at the different sampling times by using the ferric thiocyanate method[36] and expressed as milliequivalents of oxygen per kilogram of muscle.

Shelf Life. A refusal value of hydroperoxides, corresponding to 0.70 mequiv oxygen/kg muscle, was fixed by the expert panel according to the appearance of the incipient rancid off-odors in mackerel muscle. This shelf life shows the useful commercial period of the fish minced before it provokes refusal for the customer due to rancidity. It was graphically calculated for each experiment (Figure 1).

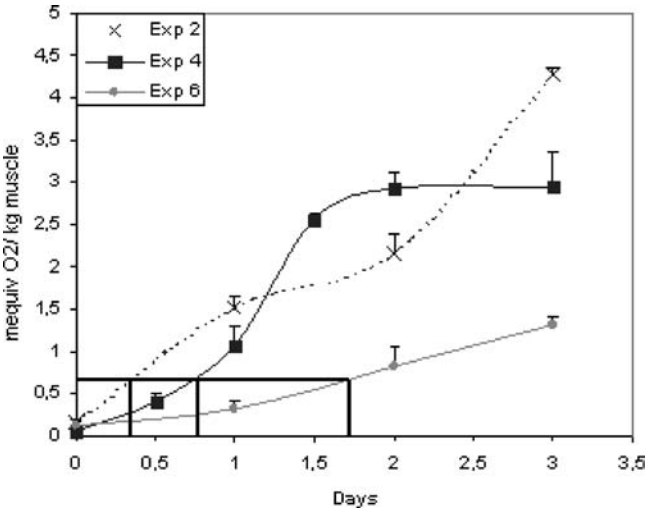

Figure 1. Kinetics of the formation of lipid peroxides during chilled storage for oxidation experiments 2, 4, and 6, and calculation of the shelf life referred to the formation of rancid odors. Mackerel muscle of experiments 2, 4, and 6 contained, respectively, fat levels of 78.2, 45.2, and 28.7 g fat/kg muscle.

Statistical Analysis. All analyses were performed in triplicate, and data are reported as the mean ± standard deviation. Simple correlations among the compositional parameters and the lipid oxidation vulnerability were evaluated by Pearson coefficients. Multiple regressions were established by using the forward stepwise addition technique in which variables are sequentially added to an "empty" model.[37] Statistical analyses were performed with the software Statistica 6.0. (StatSoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Initial Compositional Data. Table 1 shows the average levels of both major components (proteins, total lipids, water, and PUFAs) and minor components (ascorbic acid, α-tocopherol, hemoglobin, total iron, LMW-iron, copper, and zinc) corresponding to the mackerel batches caught during spring and summer and potentially involved in the progress of lipid oxidation. In addition, Pearson correlations are shown to illustrate the relationship between these factors (Table 2).

The contents of minor and major components of the different batches of mackerel exhibited wide variability. The percentage of water and total lipids in white muscle displayed high variations from 69.1 to 77.00% and from 28.3 to 95.0 mg/g, respectively (Table 1). However, the total protein content showed a lower variability, with values ranging between 18.5 and 20.8%. A significant inverse correlation was found between the water and fat contents ($p < 0.05$), consistent with the lowest water content found for the fattiest muscle (Table 2). The differences in fat content are probably a result of the energy requirements of migrations that commonly precede or accompany sexual maturation and spawning of mackerel.[38] Therefore, the extensive lipid variation is principally attributable to changes in the triglyceride content because the phospholipid fraction remains moderately constant.[39]

Analysis of the fatty acid profile detected significant differences among the lots of mackerel (Table 3). The results showed a high percentage of the monounsaturated fatty acids 22:1ω-11 and 20:1ω-9 in batches 1 and 4. These batches correspond to the first units of mackerel captured in 2009 and 2010. This indicates that

Table 1. Initial Compositional Data of the Mackerel Mince Used in Each Experiment and Time for Detection of Incipient Rancid Odors[a]

batch	water (%)	protein (%)	lipid content (g/kg muscle)	ascorbic acid (mg/kg muscle)	TOH (mg/kg muscle)	Hb (μmol/kg muscle)	total Fe (mg/kg muscle)	Fe-LMW (mg/kg muscle)	Cu (mg/kg muscle)	Zn (mg/kg muscle)	PUFA (g/kg muscle)	shelf life (days)	
1	76.9 ± 0.1	18.79 ± 0.30	28.3 ± 0.31	5.28 ± 0.67	4.5 ± 0.19	9.5 ± 0.27	2.41 ± 0.27	0.282	0.478 ± 0.011	3.02 ± 0.04	16.32 ± 0.40	2.11	
2	71.02 ± 0.62	20.23 ± 0.47	78.2 ± 0.65	7.78 ± 0.45	5.64 ± 0.41	9.22 ± 0.31	2.63 ± 0.11	0.565	0.413 ± 0.008	3.19 ± 0.05	30.47 ± 0.24	0.35	
3	73.8 ± 0.5	20.8 ± 0.7	51.1 ± 4.23	10.59 ± 0.85	6.05 ± 0.63	8.5 ± 0.40	2.42 ± 0.12	0.370	0.422 ± 0.007	3.36 ± 0.04	19.04 ± 0.8	1.697	
4	74.4 ± 0.19	19.9 ± 0.56	45.2 ± 4.5	2.89 ± 0.11	0.93 ± 0.03	10.89 ± 1.00	4.44 ± 0.34	2.000	0.290 ± 0.02	3.28 ± 0.06	9.11 ± 1.68	0.76	
5	71.6 ± 0.37	18.5 ± 0.16	75.4 ± 6.33	10.34 ± 1.47	5.06 ± 0.20	8.89 ± 0.16	2.33 ± 0.04	0.350	0.381 ± 0.004	3.02 ± 0.09	26.32 ± 0.374	0.95	
6	77.0 ± 0.81	18.9 ± 0.31	28.7 ± 0.24	10.14 ± 0.40	3.4 ± 0.40	6.18 ± 0.74	3.88 ± 0.37	2.490	0.240 ± 0.03	4.07 ± 0.09	11.28 ± 0.93	1.73	
7	69.1 ± 0.42	19.7 ± 0.61	95.0 ± 0.97	2.40 ± 0.42	1.91 ± 0.62	6.20 ± 0.32	3.03 ± 0.02	1.640	0.396 ± 0.066	2.54 ± 0.09	30.31 ± 0.82	0.82	
range	69.1–77.0	18.5–20.8	28.3–95.0	2.40–10.59	0.93–6.05	6.18–10.89	2.33–4.44	0.282–2.490	0.240–0.478	2.54–4.07	9.11–30.47	0.35–2.11	

[a] Analyses were done in triplicate; results are expressed as the mean ± standard deviation.

dx.doi.org/10.1021/jf200686z |J. Agric. Food Chem. 2011, 59, 6284–6291

Table 2. Correlation Coefficients between the Initial Compositional Parameters of Mackerel Mince Shown in Table [1,a]

	lipid	AA	TOH	Hb	total Fe	Fe-LMW	PUFAs	water	protein	Cu	Zn
fat	1.00	−0.20	0.01	−0.21	−0.32	−0.19	0.89*	−1.00	0.43	0.26	−0.69
AA	−0.20	1.00	0.77*	−0.17	−0.39	−0.30	0.01	0.23	0.13	−0.07	0.58
TOH	0.01	0.77*	1.00	0.05	−0.81*	−0.78*	0.37	0.01	0.28	0.54	0.12
Hb	−0.21	−0.17	0.05	1.00	0.03	−0.40	−0.25	0.16	0.23	0.23	−0.12
total Fe	−0.32	−0.39	−0.81*	0.03	1.00	0.91*	−0.65	0.29	−0.16	−0.85*	0.43
Fe-LMW	−0.04	−0.37	−0.79*	−0.52	0.83*	1.00	−0.34	0.04	−0.21	−0.78*	0.28
PUFAs	0.89*	0.01	0.37	−0.25	−0.65	−0.47	1.00	−0.86*	0.30	0.54	−0.66
water	−1.00*	0.23	0.01	0.16	0.29	−0.09	−0.86*	1.00	−0.49	−0.26	0.70
protein	0.43	0.13	0.28	0.23	−0.16	−0.08	0.30	−0.49	1.00	0.19	−0.16
Cu	0.26	−0.07	0.54	0.23	−0.85*	−0.88*	0.54	−0.26	0.19	1.00	−0.66
Zn	−0.69	0.58	0.12	−0.12	0.43	0.43	−0.66	0.70	−0.16	−0.66	1.00

[a] Parameters marked with an asterisk (*) showed significant correlation ($p < 0.05$ and $n = 7$).

Table 3. Fatty Acid Profiles for All Batches of Mackerel Muscle Employed in the Oxidation Experiments[a]

	percentage of fatty acids						
	batch 1	batch 2	batch 3	batch 4	batch 5	batch 6	batch 7
14:00	5.68 ± 0.30	3.20 ± 0.07	3.11 ± 0.11	7.85 ± 0.04	3.38 ± 0.10	2.11 ± 0.21	2.95 ± 0.30
16:00	16.04 ± 0.39	23.12 ± 0.08	22.93 ± 0.12	15.44 ± 0.18	23.43 ± 0.06	20.95 ± 0.45	21.08 ± 1.07
16:1ω-7	2.90 ± 0.09	3.95 ± 0.07	4.01 ± 0.08	3.4 ± 0.01	5.49 ± 0.03	5.43 ± 0.22	6.10 ± 0.27
18:00	3.59 ± 0.22	5.09 ± 0.03	5.01 ± 0.10	3.17 ± 0.08	6.16 ± 0.07	5.94 ± 0.03	5.86 ± 0.04
18:1ω-9	10.09 ± 0.70	19.11 ± 0.13	19.38 ± 0.48	8.89 ± 0.06	19.81 ± 0.51	14.61 ± 0.39	20.40 ± 0.30
18:1ω-7	2.29 ± 0.07	3.84 ± 0.04	3.90 ± 0.05	1.97 ± 0.03	5.29 ± 0.13	5.39 ± 0.07	5.64 ± 0.03
18:2ω-6	1.58 ± 0.11	1.30 ± 0.02	1.30 ± 0.04	1.81 ± 0.03	0.87 ± 0.03	0.86 ± 0.02	0.80 ± 0.04
18:3ω-3	0.69 ± 0.02	1.20 ± 0.01	1.20 ± 0.05	0.96 ± 0.00	0.63 ± 0.03	0.42 ± 0.03	0.53 ± 0.01
20:1ω-9	12.05 ± 0.56	1.70 ± 0.01	1.68 ± 0.04	13.06 ± 0.11	1.78 ± 0.05	2.07 ± 0.13	2.54 ± 0.11
18:4ω-3	1.73 ± 0.07	2.71 ± 0.06	2.71 ± 0.14	2.56 ± 0.03	1.54 ± 0.06	0.84 ± 0.02	1.27 ± 0.04
20:4ω-6	0.82 ± 0.04	1.22 ± 0.01	1.21 ± 0.02	0.79 ± 0.03	1.33 ± 0.03	1.48 ± 0.09	1.08 ± 0.05
22:1ω-11	22.27 ± 1.38	0.88 ± 0.03	0.81 ± 0.05	25.47 ± 0.17	0.41 ± 0.03	2.92 ± 0.18	2.76 ± 0.22
22:1ω-9	1.43 ± 0.05	0.40 ± 0.01	0.41 ± 0.01	1.28 ± 0.01	0.39 ± 0.01	0.42 ± 0.05	0.55 ± 0.06
20:4ω-3	0.61 ± 0.02	1.46 ± 0.01	1.48 ± 0.05	0.62 ± 0.02	0.89 ± 0.03	0.56 ± 0.06	0.63 ± 0.01
20:5ω-3	4.09 ± 0.22	9.46 ± 0.08	9.47 ± 0.03	3.85 ± 0.02	10.76 ± 0.19	13.24 ± 0.95	11.62 ± 0.55
24:1ω-9	1.95 ± 0.05	1.03 ± 0.02	1.50 ± 0.04	0.72 ± 0.85	0.76 ± 0.03	0.48 ± 0.05	0.21 ± 0.01
22:5ω-3	0.94 ± 0.04	1.81 ± 0.03	1.80 ± 0.01	0.87 ± 0.00	2.27 ± 0.03	2.21 ± 0.21	2.53 ± 0.21
22:6ω-3	11.22 ± 0.89	18.50 ± 0.09	18.10 ± 0.39	7.30 ± 0.27	14.81 ± 0.38	20.05 ± 0.30	13.46 ± 0.93
saturated	25.31 ± 0.91	31.41 ± 0.18	31.01 ± 0.33	26.7 ± 0.30	32.97 ± 0.23	28.66 ± 0.69	29.89 ± 1.41
monounsaturated	52.99 ± 2.89	30.92 ± 0.31	31.64 ± 0.75	54.79 ± 1.24	33.94 ± 0.79	31.33 ± 1.06	38.19 ± 1.01
polyunsaturated	21.70 ± 1.41	37.62 ± 0.31	37.26 ± 0.74	18.76 ± 0.40	33.10 ± 0.79	39.67 ± 1.68	31.91 ± 1.84

[a] Analyses were done in triplicate; results are expressed as the mean ± standard deviation.

the high percentages of 22:1ω-11 and 20:1ω-9 may be a seasonal parameter caused by dietary factors,[40] sexual maturation,[38] or differences in water temperature.[41,42] As a general observation, palmitic acid (16:0) and docosahexaenoic acid (22:6ω-3) followed by eicosapentaenoic acid (20:5n-3) were the most abundant saturated and polyunsaturated fatty acids, respectively, for all of the experiments (Table 3). The content of PUFAs exhibited a significant positive correlation with the lipid concentration.

The content of pro-oxidants and antioxidants also displayed significant variations among the different mackerel batches (Table 1). The initial concentrations of α-tocopherol (α-TOH) varied from 0.928 to 6.055 mg TOH/kg muscle. These values were in agreement with those previously reported for mackerel light muscle.[43] Although TOH is a lipophilic antioxidant, no correlation was found with the total lipid content. Fish is unable to synthesize α-TOH, and it is accumulated from the diet,[44,45] being influenced by factors such as seasonal period[46] and the age of the fish. As for the fish gender, no differences of α-TOH content have been previously reported between males and females of rainbow trout.[47] The concentration of ascorbic acid (AA) ranged between 2.40 and 10.59 mg AA/kg muscle, and these levels agreed with previous values found in the white muscle of mackerel.[48,49] The levels of AA are also influenced by fish feeding.[50] Iglesias et al.[48] have reported that fatty species contained ratios of ascorbic acid (AA)/dehydroascorbic acid (DHAA) of <1, being significantly higher in muscle from lean fish species. The authors hypothesized that the greater proportion of DHAA, the primary oxidized product of AA, in pelagic fish

Table 4. Linear Correlations between the Initial Composition of Mackerel Muscle and Its Shelf-Life Referred to the Formation of Rancid Odors[a]

	lipid	AA	TOH	Hb	total Fe	Fe-LMW	PUFA	water	protein	Cu	Zn
Pearson correlation	−0.76*	0.29	0.22	−0.18	−0.16	−0.09	−0.53	0.78*	−0.29	0.13	0.36

[a] Parameters marked with an asterisk (*) showed significant correlation ($p < 0.05$ and $n = 7$).

species can be attributed to their higher content in oxidizable substrates (PUFAs) and pro-oxidant substances as heme proteins. The levels of the endogenous antioxidants TOH and AA were found to be directly correlated. This correlation has been described previously in herring[51] and could be attributed to the nutritional status of the fish.

The initial concentrations of hemoglobin and redox transition metals such as iron (Fe), copper (Cu), and zinc (Zn), which are potential initiators of lipid oxidation in fish muscle, have been also determined. The metals studied have been postulated as important elements involved in oxidative stress, as well as pro-oxidant and antioxidant agents. Pro-oxidant action of iron and copper occurs through multiple mechanisms as the Fenton-type reaction[52] and peroxidase activity.[53] The antioxidant activity is essentially associated with the activity of the enzymes catalase (Fe) and superoxide dismutase (Fe, Zn, Cu). The concentration of hemoglobin varied largely from 6.18 to 10.89 μmol Hb/kg muscle, in accordance with previous determinations in mackerel and herring light muscle.[31,54] These levels of hemoglobin were found to be approximately 10−400 times higher than those found in lean fish species such as cod (0.03−0.23 μmol Hb/kg muscle).[31] The total content in Fe also displayed significant differences (2.41−4.44 mg Fe/kg muscle) among mackerel batches. Previous works showed an inverse relationship between size and iron content in fish species.[55] The low molecular weight iron (LMW-Fe), which was determined by difference between total iron and heme iron, represented around 10−66% of total iron found in mackerel muscle. These results were in concordance with previous results by Decker and Hultin in mackerel muscle.[56] A relevant feature is the negative relationship between TOH and the concentration of both total and free iron, suggesting that the pro-oxidant iron may act to decrease TOH levels. Iron and AA exhibited also a negative correlation, although it was not statistically significant. The total amount of copper was significantly lower and less variable than that of iron. Zinc ranged from 2.54 to 4.07 mg/kg muscle. Previous investigations on mackerel composition have shown a correlation between zinc content and age.[57]

Relationship between Composition and Lipid Oxidation. With the aim of study the relationship of compositional parameters with the onset of lipid oxidation, a data matrix was obtained from an array of chilled experiments. These results were analyzed by Pearson correlations and are shown in Table 4. For each experiment, the progress of oxidation was followed through the formation of the primary lipid oxidation products, lipid hydroperoxides, and the sensory detection of rancid odors. All chilled experiments developed oxidation in a variable time period ranging between 0.35 and 2.11 days. In all samples analyzed, the peroxide value of 0.70 mequiv/kg muscle granted the sensory detection of the incipient rancid off-odors and was used to determinate the shelf life period (Figure 1). Table 4 indicates the degree of linear relationship between the appearance of the incipient rancid off-odors expressed as shelf life (in days) and the constituents of mackerel muscle. Linear regressions have been used before to study fish oxidation during chilled storage. Undeland et al.[58] found an almost linear relationship between the primary oxidation products, peroxides, and rancid odors during ice storage of herring fillets.[58] The same authors reported that linear models fit better than exponential models and were also able to explain high variation in the data corresponding to loss of α-tocopherol and other endogenous antioxidants present in herring muscle.

The onset of lipid oxidation showed a significant dependence on total lipid and water contents ($p < 0.05$). Figure 1 shows the lipid oxidation kinetics of three batches differing in fat content. The total amount of lipids and shelf life in terms of oxidation exhibited a negative linear relationship, and therefore, samples of mackerel mince having the highest values of total lipid reached the threshold value of peroxides earlier than samples with low values of total tipids. It seems that the lipid substrate is a crucial factor involved in the development of lipid oxidation. The onset of oxidation and water content showed a positive linear relationship, which can be explained by the strong inverse relationship between total lipid and water content (collinear variables) indicated above (Table 3). The total amount of PUFAs and protein also showed a strong linear correlation with shelf life, although it was not significant. In previous studies, Undeland et al.[13] found that added triacylglycerols did not accelerate hemoglobin-mediated lipid oxidation in washed cod model system. These authors suggested that lipid oxidation in fatty fish may be more related to the quantity and type of the aqueous pro-oxidant and the membrane lipids (mainly formed by PUFAs), than to variations in total fat content. These conclusions were achieved in a model fish mince system in which total fat content was modulated by the addition of fish oil. The incorporation of these added triacylglycerols and their bioaccesibility to O_2 and pro-oxidant compounds present in fish muscle may differ from the state in the entire fish muscle. The same authors also suggested later that the use of fattier fish than cod could be more useful for washed minced model systems aimed at the study of oxidation kinetics of such species.[31]

Several works in muscle-based foods have shown that the kinetics of oxidation processes were closely associated with the pro-oxidative [31,58,59] and antioxidative endogenous activities during ice storage.[5,31,60] On the basis of linear regression analyses, the depletion of ascorbic acid was the chemical parameter most strongly correlated with the development of rancid odors in refrigerated fillets of herring.[58] Min et al.[5] compared the susceptibility of meats from different animal species to lipid oxidation during storage at 4 °C. They concluded that the contents of myoglobin and the endogenous antioxidant capacities (ferric ion reducing capacity and radical scavenging activity) play the primary role for the differences in susceptibility of raw meats to lipid oxidation. In the present study, neither the initial concentration of ascorbic acid nor that of tocopherol calculated in the different batches of mackerel showed a significant linear correlation with the onset of lipid oxidation during refrigerated storage. With regard to the pro-oxidants, the initial

Table 5. Coefficients of the Multiple-Regression Model[a]

	β	std error	regression coeff	std error	p level
intercept			8.305044	0.306585	0.001360
PUFAs	−1.45552	0.054058	−0.106385	0.003951	0.001377
total iron	−1.20174	0.056867	−0.934113	0.044204	0.002232
Hb	−0.56379	0.039025	−0.208919	0.014461	0.004757
AA	−0.26506	0.043024	−0.048015	0.007794	0.025350

[a] The standardized regression coefficients (β) resulted from analyses in which all variables have been scaled to have a mean of 0 and a standard deviation of 1. The nonstandardized coefficients represent the regression parameters obtained from the analyses.

contents of hemoglobin or metals present in mackerel muscle were not linearly correlated with the appearance of off-odors during the chilled tests. Accordingly, ascorbic acid and tocopherol showed positive coefficients in agreement with their positive influence on the retardation of oxidation.

Multiple Regressions. Attempts to explain the onset of lipid oxidation considering the whole composition of mackerel were then performed by multivariate regression models. Multiple regression is a powerful tool commonly used for selecting the significant variables that explain the highest proportion of variance in many processes.[61] Forward stepwise multivariate techniques were aimed to reveal the variables that could explain the susceptibility of the different groups of mackerel to lipid oxidation.

Table 5 shows the results of the forward stepwise multivariate regression. Coefficients were considered to be significantly different from zero at $p < 0.05$. The model contained variables having higher contribution to explain the susceptibility to lipid oxidation: the content of PUFAs followed by the contents of total iron, hemoglobin, and ascorbic acid, respectively. Although these independent variables were not significantly correlated with the shelf-life across Pearson coefficients, their combination resulted in outstanding values of $R^2 = 0.9975$, $F = 202.43$, and $p = 0.0049$. The resultant model obtained for the regresion developed is described by the following equation:

$$\text{shelf life} = 8.305 - 1.06 \times \text{PUFAs} - 0.934 \times \text{total iron} - 0.209 \times \text{Hb} - 0.048 \times \text{AA}$$

The regression technique introduced first the content in PUFAs, indicating its higher contribution as a predictor of shelf life. The amount of double bonds or PUFAs has shown a direct correlation with the oxidation rate.[16] Total iron content was parameter with the second highest contribution in the model followed by hemoglobin. Ascorbic acid exhibited the lowest significant effect in the multicomponent model. The presence of this antioxidant in the model instead of α-tocopherol is in contrast with previous studies that observed a strong correlation of α-tocopherol degradation with the progress of lipid oxidation. This means that ascorbic acid is a more sensitive parameter for the first stages of oxidation. Previous works have shown that ascorbic acid was consumed in much earlier post-mortem stages.[17,62]

Regression analysis is also widely used to estimate the conditional expectation of the dependent variable given by the independent variables. Multiple-regression equations have been proposed for predicting the shelf life of frozen fish muscle.[63] This study established a multiple-regression model that fits reasonably with the development of TBARs. The model was based on the potential of fish muscle lipids to be autoxidized at 60 °C (very related to the amount of PUFAs) and the content of pro-oxidants: total iron and myoglobins or hemoglobins. The incorporation of the fat content into the model did not improve the regression coefficients obtained. These results are in agreement with ours, which revealed the importance of PUFA, ascorbic acid, and pro-oxidants in the susceptibility of chilled mackerel to oxidation.

Table 6. Predicted versus Observed Shelf-Life Period of Chilled Minced Mackerel

expt	predicted	observed	% dev
1	2.079 ± 0.215	2.11	1.74
2	0.307 ± 0.182	0.35	14.00
3	1.734 ± 0.148	1.67	3.69
4	0.774 ± 0.232	0.76	1.81
5	0.975 ± 0.154	0.95	2.56
6	1.703 ± 0.225	1.73	1.58
7	0.839 ± 0.230	0.82	2.26

Table 6 shows the experimentally observed versus predicted shelf lives by the linear regression model for the seven groups of chilled mackerel. The model resulted in shelf life deviations from predicted to experimental values ranging from 1.58 to 14%. Thefereore, the model can be used to predict the chilled shelf life of minced mackerel with considerable accuracy.

In conclusion, these results emphasize the important role of the total content of polyunsaturated lipids in the susceptibility of a pelagic fish species such as mackerel to oxidation. Together with unsaturated lipids, the content of aqueous pro-oxidants, iron and hemoglobin, and the content of the ascorbic acid were the components showing major contributions to the explanation of such susceptibility. The accurate study of the kinetics of lipid oxidation in fish species together with multiple regression techniques offers a useful tool for predicting the shelf life of fish. Further work in this research will include the use of more complex, nonlinear modeling.

AUTHOR INFORMATION

Corresponding Author

*E-mail maestre@iim.csic.es. Phone: +34 986 231930. Fax: +34 986 292762.

Funding Sources

This investigation has been supported by the Spanish Ministry of Science (AGL2009-12374-C03-01). The Spanish Ministry of Science and Xunta de Galicia are also gratefully acknowledged for the doctoral fellowship to R.M. and the postdoctoral contract "Eduardo Parga Pondal" to M.P., respectively.

REFERENCES

(1) Ackman, R. G. *Marine Biogenic Lipids, Fats and Oils*: CRC Press: Boca Raton, FL, 1989; Vol. II.

(2) Lee, K. W.; Lip, G. Y. H. The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease. *QJM* **2003**, *96* (7), 465–480.

(3) Min, B.; Ahn, D. Factors in various fractions of meat homogenates that affect the oxidative stability of raw chicken breast and beef loin. *J. Food Sci.* **2009**, *74* (1), C41–C48.

(4) Kilic, B.; Richards, M. P. Lipid oxidation in poultry Döner kebab: pro-oxidative and anti-oxidative factors. *J. Food Sci.* **2003**, *68* (2), 686–689.

(5) Min, B.; Nam, K. C.; Cordray, J.; Ahn, D. U. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *J. Food Sci.* **2008**, *73* (6), C439–C446.

(6) Yancey, E. J.; Grobbel, J. P.; Dikeman, M. E.; Smith, J. S.; Hachmeister, K. A.; Chambers, E. C., IV; Gadgil, P.; Milliken, G. A.; Dressler, E. A. Effects of total iron, myoglobin, hemoglobin, and lipid oxidation of uncooked muscles on livery flavor development and volatiles of cooked beef steaks. *Meat Sci.* **2006**, *73* (4), 680–686.

(7) Chen, C. C.; Pearson, A. M.; Gray, J. I.; Fooladi, M. H.; Ku, P. K. Some factors influencing the nonheme iron content of meat and its implications in oxidation. *J. Food Sci.* **1984**, *49* (2), 581–584.

(8) Ackman, R. G.; Gunnlaugsdottir, H. Seafoods and fishery byproducts. Natural and unnatural environments for longer chain omega-3 fatty acids. In *Lipid Oxidation in Food*; Angelo, A. J. S., Ed.; ACS Symposioum Series; American Chemical Society: Washington, DC, 1992; pp 209−230.

(9) Hamre, K.; Lie, O.; Sandnes, K. Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring-spawning herring (*Clupea harengus* L.). *Food Chem.* **2003**, *82* (3), 441–446.

(10) Szlinder-Richert, J.; Usydus, Z.; Wyszynski, M.; Adamczyk, M. Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus* membras. *J. Fish Biol.* **2010**, *77* (3), 585–599.

(11) Ozden, O. Micro, macro mineral and proximate composition of Atlantic bonito and horse mackerel: a monthly differentiation. *Int. J. Food Sci. Technol. 45* (3), 578−586.

(12) Tres, A.; Bou, R.; Codony, R.; Guardiola, F. Influence of different dietary doses of n-3- or n-6-rich vegetable fats and α-tocopheryl acetate supplementation on raw and cooked rabbit meat composition and oxidative stability. *J. Agric. Food Chem.* **2008**, *56* (16), 7243–7253.

(13) Undeland, I.; Hultin, H. O.; Richards, M. P. Added triacylglycerols do not hasten hemoglobin-mediated lipid oxidation in washed minced cod muscle. *J. Agric. Food Chem.* **2002**, *50*, 6847–6853.

(14) Richards, M. P.; Hultin, H. O. Rancidity development in a fish model system as affected by phospholipids. *J. Food Lipids* **2001**, *8*, 215–230.

(15) Wilson, B. R.; Pearson, A. M.; Shorland, F. B. Effect of total lipids and phospolipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. *J. Agric. Food Chem.* **1976**, *24*, 7–11.

(16) Frankel, E. N. *Lipid Oxidation*; Oily Press: Dundee, Scotland, 1998.

(17) Pazos, M.; Sánchez, L.; Medina, I. α-Tocopherol oxidation in fish muscle during chilling and frozen storage. *J. Agric. Food Chem.* **2005**, *53*, 4000–4005.

(18) Packer, J. E.; Slater, T. F.; Willson, R. L. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* **1979**, *278* (5706), 737–738.

(19) Buettner, G. R. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol and ascorbate. *Arch. Biochem. Biophys.* **1993**, *300* (2), 535–543.

(20) Chen, Y. C.; Nguyen, J.; Semmens, K.; Beamer, S.; Jaczynski, J. Effects of dietary α-tocopheryl acetate on lipid oxidation and α-tocopherol content of novel omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets. *LWT−Food Sci. Technol.* **2008**, *41* (2), 244–253.

(21) European Union Council Regunlation 103/76 of January 1976.

(22) AOAC. Method 950.46. Moisture content in meat. In *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, 2002.

(23) AOAC. Method 928.08. Protein content. In *Official Methods of Analysis*, 17th ed.; Association of Offical Analytical Chemists: Gaithersburg, MD, 2002.

(24) Bligh, E. G.; Dyer, W. J. A rapid method of total lipidextraction and purification. *J. Biochem Physiol.* **1959**, *37*, 911–917.

(25) Herbes, S. E.; Allen, C. P. Lipid quantification of freshwater invertebrates: method modification for microquantitation. *Can. J. Fish. Aquat. Sci.* **1983**, *40* (8), 1315–1317.

(26) Lepage, G.; Roy, C. C. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **1986**, *27* (1), 114–120.

(27) Burton, G. W.; Webb, A.; Ingold, K. U. A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. *Lipids* **1985**.

(28) Pazos, M.; González, M. J.; Gallardo, J. M.; Torres, J. L.; Medina, I. Preservation of the endogenous antioxidant system of fish muscle by grape polyphenols during frozen storage. *Eur. Food Res. Technol.* **2005**, *220*, 514–519.

(29) Cabrini, L.; Landi, L.; Stefanelli, C.; Barzanti, V.; Anna Maria, S. Extraction of lipids and lipophilic antioxidants from fish tissues: a comparison among different methods. *Comp. Biochem. Physiol. Part B: Comp. Biochem.* **1992**, *101* (3), 383–386.

(30) Iglesias, J.; González, M. J.; Medina, I. Determination of ascorbic and dehydroascorbic acid in lean and fatty fish species by high-performance liquid chromatography with fluorometric detection. *Eur. Food Res. Technol.* **2006**, *223*, 781−786.

(31) Larsson, K.; Almgren, A.; Undeland, I. Hemoglobin-mediated lipid oxidation and compositional characteristics of washed fish mince model systems made from cod (*Gadus morhua*), herring (*Clupea harengus*), and salmon (*Salmo salar*) muscle. *J. Agric. Food Chem.* **2007**, *55* (22), 9027–9035.

(32) Brown, W. D. Chromatography of myoglobin on diethylaminoethyl cellulose columnas. *J. Biol. Chem.* **1961**, 2238–2240.

(33) Richards, M. P.; Hultin, H. O. Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. *J. Agric. Food Chem.* **2000**, *48*, 3141–3147.

(34) AOAC. Official method 999.10. Lead, cadmium, zinc, copper and iron in foods. Atomic absorption spectrophotometry after microwave digestion. *Official Methods of Analysis*, 17th ed.; Association of Offical Analytical Chemists: Gaithersburg, MD, 2003; Vol. 1−2.

(35) Richards, M. P.; Kelleher, S. D.; Hultin, H. O. Effect of washing with or without antioxidants on quality retention of mackerel fillets during refrigerated and frozen storage. *J. Agric. Food Chem.* **1998**, *46*, 4363–4371.

(36) Chapman, R. A.; Mackay, K. The estimation of peroxides in fats and oils by ferric thiocyanate method. *J. Am. Oil Chem. Soc* **1949**, *26*, 360–363.

(37) Neter, J.; Wasserman, W.; Kutner, M. H. Applied linear regression models. Ed R Irwin: IL, 1983.

(38) Wallace, P. D. Seasonal variation in fat content of mackerel (*Scomber scombrus* L.) caught in the western English Channel. *Fish. Res. Tech. Rep.* **1991**, 91.

(39) Weihrauch, J.; Son, Y.-S. Phospholipid content of foods. *J. Am. Oil Chem. Soc.* **1983**, *60* (12), 1971–1978.

(40) Csengeri, I.; Farkas, T.; Majoros, F.; Olah, J.; Szalay, M. Effect of feeds on the FA composition of carp (*Cyprinus carpio* L). *Aquacult. Hung.* **1978**, *1*, 24–34.

(41) Lovell, R. T. Nutrition of aquaculture species. *J. Anim. Sci.* **1991**, *69* (10), 4193–4200.

(42) Henderson, R. J.; Tocher, D. R. The lipid composition and biochemistry of freshwater fish. *Prog. Lipid Res.* **1987**, *26*, 281–347.

(43) Pazos, M.; Sanchez, L.; Medina, I. α-Tocopherol oxidation in fish muscle during chilling and frozen storage. *J. Agric. Food Chem.* **2005**, *53* (10), 4000–4005.

(44) Bell, J. G.; McEvoy, J.; Tocher, D. R.; Sargent, J. R. Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *J. Nutr.* **2000**, *130* (7), 1800–1808.

(45) Bai, S. C.; Gatlin, D. M. Dietary vitamin E concentration and duration of feeding affect tissue α-tocopherol concentrations of channel catfish (*Ictalurus punctatus*). *Aquaculture* **1993**, *113* (1−2), 129–135.

(46) Deshapende, S. S.; Deshapende, U. S.; Salunkhe, D. K. Nutritional and health aspects of food antioxidants. In *Foods Antioxidants. Technological, Toxicological and Health Perspectives*; Madhavi, D. V., Deshapende, S. S., Eds.; Dekker: New York, 1996; pp 351−470.

(47) López, M. C.; Satué, M. T.; González, M. L.; Agramont, A. α-Tocopherol content in trout oil. *Food Chem.* **1995**, *53* (1), 67–70.

(48) Iglesias, J.; González, M. J.; Medina, I. Determination of ascorbic and dehydroascorbic acid in lean and fatty fish species by

high-performance liquid chromatography with fluorometric detection. *Eur. Food Res. Technol.* **2006**, *223*, 781–786.

(49) Petillo, D.; Hultin, H. O.; Krzynowek, J.; Autio, W. R. Kinetics of antioxidant loss in mackerel light and dark muscle. *J. Agric. Food Chem.* **1998**, *46*, 4128–4137.

(50) Sealey, W. M.; Gatlin, D. M., III. Dietary vitamin C and vitamin E interact to influence growth and tissue composition of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) but have limited effects on immune responses. *J. Nutr.* **2002**, *132* (4), 748–755.

(51) Hamre, K.; Lie, Ø.; Sandnes, K. Seasonal development of nutrient composition, lipid oxidation and colour of fillets from Norwegian spring-spawning herring (*Clupea harengus* L.). *Food Chem.* **2003**, *82* (3), 441–446.

(52) Stohs, S. J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biol. Med.* **1995**, *18* (2), 321–336.

(53) Fang, W.-C.; Kao, C. H. Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Plant Sci.* **2000**, *158* (1−2), 71–76.

(54) Richards, M. P.; Hultin, H. O. Contribution of blood and blood components to lipid oxidation in fish muscle. *J. Agric. Food Chem.* **2002**, *50*, 555–564.

(55) Cowx, I. G. Concentrations of heavy metals in the tissues of trout *Salmo trutta* and char *Salvelinus alpinus* from two lakes in North Wales. *Environ. Pollut. Ser. A, Ecol. Biol.* **1982**, *29* (2), 101–110.

(56) Decker, E. A.; Hultin, H. O. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci.* **1990**, *55*, 947–950.

(57) Papadopoulou, C.; Kanias, G. D.; Moraitopoulou Kassimati, E. Zinc content in otoliths of mackerel from the Aegean. *Mar. Pollut. Bull.* **1978**, *9* (4), 106–108.

(58) Undeland, I.; Hall, G.; Lingnert, H. Lipid oxidation in fillets of herring (*Clupea harengus*) during ice storage. *J. Agric. Food Chem.* **1999**, *47* (2), 524–532.

(59) Undeland, I.; Ekstrand, B.; Lingnert, H. Lipid oxidation in herring (*Clupea harengus*) light muscle, dark muscle, and skin, stored separately or as intact fillets. *J. Am. Oil Chem. Soc.* **1998**, *75* (5), 581–590.

(60) Kishowar, J.; Alistair, P.; Corinne, M. S. Fatty acid composition, antioxidants and lipid oxidation in chicken breasts from different production regimes. *Int. J. Food Sci. Technol.* **2004**, *39* (4), 443–453.

(61) Krener, P. G.; Abramowitz, S. I. Prediction of videotelemetry productivity from clinical screening parameters. *J. Am. Acad. Child Psychiatry* **1985**, *24* (5), 597–602.

(62) Iglesias, J.; Pazos, M.; Andersen, M. L.; Skibsted, L. H.; Medina, I. Caffeic acid as antioxidant in fish muscle: mechanism of synergism with endogenous ascorbic acid and α-tocopherol. *J. Agric. Food Chem.* **2009**, *57* (2), 675–681.

(63) Kurade, S. A.; Baranowski, J. D. Prediction of shelf-life of frozen minced fish in terms of oxidative rancidity as measured by TBARS number. *J. Food Sci.* **1987**, *52* (2), 300–302.