Lipid and protein deterioration during the chilled storage of minced sea salmon (*Pseudopercis semifasciata*)

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Abstract: Sea salmon is a very appreciated seafood. The aim of this work was to analyze changes in lipid and protein fractions of minced muscle during chilled storage (1 ± 1 °C). Lipid oxidation was important during the first 6 days of storage according to 2-thiobarbituric acid (TBA) values determined, decreasing mainly ω3 22:6 fatty acid content. Lipid hydrolysis was evident after 9 days of storage. Interaction compounds between oxidation products and other cellular components were analyzed by fluorescence measurements. The results obtained showed evidence of the formation of interaction products involved, mainly polar components such as proteins. Decreases in myosin and actin thermal stability and myosin denaturation were recorded by differential scanning calorimetry. Solubility of total and myofibrillar proteins decreased after 6 days of storage. The electrophoretic profile of soluble fractions showed an increase in the intensity of bands corresponding to low-molecular-weight polypeptides and a decrease in high-molecular-weight species. Available lysine content did not change during chilled storage.

Keywords: sea salmon; minced muscle; chilling; lipid oxidation; protein modifications

INTRODUCTION

Microbial activity is one of the main deleterious factors during chilled storage of fish. Endogenous enzymes cause other types of deterioration which can occur before or at the same time as the microbiological process.[1] Fish muscle proteins can undergo different types of modification during chilled storage due to the presence of some different proteases, such as collagenases, lysosomal cathepsins and calpains, which can produce changes in texture, myofibrillar proteins and connective tissue degradation.[1,2] Proteolysis can be associated with tissue softening and low-molecular-weight peptide formation, which generates in addition organoleptic changes and accelerates microbial growth in some cases.[3]

Fish lipids are very sensitive to the oxidation process because of their high content of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[4] Oxidation of these fatty acids produces volatile compounds (2-pentenal, 2-hexenal, 4-heptenal, 2,4-heptadienal, 2,4,7-decatrienal), which can elicit flavor deterioration. This is an important cause of quality loss during fish processing and storage, specially in fatty fish. Meanwhile, primary oxidation products – free radicals and hydroperoxides – and secondary ones such as aldehydes, dialdehydes and epoxides oxidation products can react with other cellular components such as proteins, peptides, free amino acids, phospholipids and nucleic acids.[4,5] These interactions have very important consequences in food quality of seafood. Thus, carbonylic compounds can react with amine groups forming Schiff base type products. Final conjugated Schiff bases present a high color intensity (by non-enzymatic browning mechanism) and a characteristic fluorescence spectrum.[5] Thus, several studies have demonstrated the existence of different groups of fluorophores according to their excitation/emission maxima (Exc/Em).[6]

Fluorescent compound determination of muscle foods can be performed on different types of samples. Organic and aqueous phases obtained by a Bligh and Dyer[7] extraction have been extensively used in fluorescent compound determination to evaluate fish quality. Initially formed fluorescent compounds shift their fluorescence maxima due to a progressive formation of Schiff bases, increasing their molecular weight and unsaturated link number.[5] This wavelength shift can be measured in both phases as the fluorescence intensity at two-excitation/emission maxima ratio, being correlated with other quality indices during fish processing.[8,9]

Oxidized lipid–protein interactions affect the nutritional and sensorial quality of foods. It is possible to observe a decrease in nutritional value of proteins due to reaction with the ε-amine groups of lysine, oxidation of -SH group of methionine and other amino acid changes.[10,11] Sensorial degradation is related to flavor and color (browning) changes and texture modifications due to the cross-linking of polypeptide chains.[5,12]

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Sea salmon (*Pseudopercis semifasciata*) is a demersal non-fatty fish species from the southwest Atlantic ocean, being a very appreciated seafood as a local resource. Levels of capture have presented a considerable increase in the last five years, destined for external and internal purposes (fresh, chilled and frozen).[13] However, little research has been carried out, especially in relation to biological and taxonomic aspects,[14,15] or concerning microbiological deterioration in chilled fillets.[16] Because lipid oxidation can affect the product quality, involving organoleptic, functional and nutritional modifications, the objective of the present work was to analyze the quality changes of sea salmon muscle during chilled storage, mainly related to the lipid and protein fractions and their possible deteriorative interaction. Minced sea salmon muscle would be a potential interesting raw material for the formulation of some products. In addition, the oxidation process is possibly accelerated in mince due to the increased surface area compared to fillets or whole fish, thus making the minced fish more interesting for the purposes of this study.

MATERIALS AND METHODS

Materials

Sea salmon was caught by commercial vessels in the southwest Argentinean sea and kept in ice until arrival at the laboratory (48–72 h after catching). Fish were externally washed and filleted. Fillets were washed by water immersion and minced. Three independent experiments were performed using mince from at least two specimens (1.5–4.0 kg per specimen) in each one. Samples of minced sea salmon (30–50 g) were packed in polyethylene bags and stored at 1 ± 1 °C. At different storage times (0–9 days) two samples were used for analytical measurements.

Bovine albumin, trinitrobenzensulfonic acid (TNBS) and electrophoretic grade chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were of analytical grade.

Proximal composition of muscle

Total protein content (Kjeldahl method), total lipid content (Bligh and Dyer method),[7] water content (drying at 105 °C −24 h) and ash content (550 °C) were determined in minced sea salmon muscle. For each lot of minced muscle, measurements were performed at least in duplicate.

Lipid fraction composition

Triglyceride (TG) and phospholipid (PL) fractions were separated from the total lipid extract[17] by preparative thin-layer chromatography (TLC) (silica gel G plates, Merck, Darmstadt, Germany) using a hexane–ethylic ether–acetic acid (80:20:2) solvent system. Lipids were extracted from the silica by three successive treatments with chloroform–methanol–acetic acid–water (50:39:1:10). PL and TG were obtained after treatment with ammoniacal water[17] and acidic compounds, such as free fatty acids (FFA), after acidic treatment. Methyl esters (ME) were prepared according to Christie,[18] extracted with methanol–chloroform–water (1:1:0.9) and dried under nitrogen. Samples were purified by TLC using hexane–ether (95:5), being identified by 0.5 g/kg dichlorofluorescein in methanol and ultraviolet light and eluted with water–methanol–hexane (1:1:1). Fatty acid profiles were analyzed in a Varian gas chromatograph (Varian, Sunnydale, CA, USA) using a polar stationary phase of 10% SP2330 (Supelco, Bellefonte, PA, USA) and nitrogen as a carried gas (30 mL min^{-1}). A temperature gradient (155–230 °C, 5 °C min^{-1}) was applied. Detection was performed using two flame ionization detectors. Peak identification was carried out according to the retention times and quantification by electronic integration (Varian workstation) using a standard mixture of fatty acid methyl esters from Sigma Chemical Co.

Images from TLC plates were analyzed to compare compound intensities corresponding to different storage times. For this, specific zones of each one were identified and trimmed. Images (in RGB space) were converted to grey-scale values using MATLAB 6.5 software. Intensity values were then normalized (i.e., zero corresponded to white and one to black). Finally, mean intensity value was calculated for each compound and storage time.

2-Thiobarbituric acid assay (TBA) number

Minced muscle (2 g) was homogenized with 16.0 mL of 5% w/v trichloroacetic acid (TCA). After 30 min in ice, extracts were filtered and 2.0 mL of filtrate was mixed with 2.0 mL of 0.5% w/v TBA solution in closed tubes. After reaction (30 min, 70 °C), absorbance at 532 nm was determined using a Beckman DU 650 spectrophotometer (Fullerton, CA, USA). TBA number was calculated ($\varepsilon = 3.6 \times 10^5$ L mol^{-1} cm^{-1}).[19] Two independent extracts were obtained, each one being analyzed in duplicate.

Determination of fluorescent compounds

Fluorescent compound determinations were carried out in a PerkinElmer LS 50 B spectrofluorometer (Waltham, MA, USA) with Flwinlab software on the organic and aqueous extracts obtained from 5.00 g of sea salmon muscle by Bligh and Dyer extraction.[7,20] Excitation/emission wavelength maxima were determined, and were 325 nm/412 nm and 388 nm/460 nm, respectively. Emission spectra at each excitation maxima were recorded as a function of chilled storage time, measuring fluorescence intensity F at the maximum. Relative fluorescence (RF) was calculated according to[21]

$$\mathrm{RF} = (F \times V)/(F_{\mathrm{st}} \times m)$$

where F is sample fluorescence intensity; V is extract volume, F_{st} is standard fluorescence intensity (350 nm/450 nm), and m is sample mass. Quinine

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sulfate ($1\,\mu g\,mL^{-1}$) in $0.05\,mol\,L^{-1}$ H_2SO_4 solution was used as standard. For each sample three independent extractions were carried out.

Differential scanning calorimetry (DSC)

DSC studies were performed in a Polymer Laboratories calorimeter (Rheometric Scientific Ltd, Church Stretton, UK) with Plus V 5.41 software. The equipment was calibrated at a heating rate of $10\,^{\circ}C\,min^{-1}$ using indium as standard. Samples (18–22 mg wet weight) were placed in DSC hermetic pans, ensuring good contact between the sample and the capsule bottom, and scanned from 15 to 100 °C at $\beta = 10\,^{\circ}C\,min^{-1}$ in triplicate. As reference, a capsule containing distilled water was used. Denaturation enthalpies (ΔH_{myosin}, ΔH_{actin} and ΔH_{total}) were estimated by measuring the corresponding areas under the DSC transition curve. Specific areas (partial area/total mg of dry sample) were calculated.

Muscle protein extractability

Minced muscle (2.50 g) was homogenized with 50.0 mL of $0.03\,mol\,L^{-1}$ Tris-HCl, $0.6\,mol\,L^{-1}$ KCl (pH = 7) buffer solution. After centrifugation ($1500 \times g$, 30 min, 4 °C) the supernatant was separated (total extractable proteins, TP). Extractable myofibrillar protein (MP) fractions were obtained from 10.0 mL TP extract, mixed with 90.0 mL distilled water and stored at 4 °C (16–24 h). They were then centrifuged ($2100 \times g$, 30 min, 4 °C) and the supernatant was removed. The pellet containing myofibrillar proteins was suspended with 5.0 mL of $0.03\,mol\,L^{-1}$ Tris-HCl, $0.6\,mol\,L^{-1}$ KCl (pH = 7) solution. Protein concentration in the extracts was determined using a modified Biuret method in duplicate.[22]

Electrophoresis of extractable fractions

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of soluble fractions was performed. Samples were treated with $8\,mol\,L^{-1}$ urea, 0.3% SDS (pH 9.0) buffer, with 2-mercaptoethanol (2-ME) and a similar amount of protein (30 µg) was loaded onto each lane. Slab SDS-PAGE was carried out using the Laemmli discontinuous buffer system[23] (Hercules, CA, USA) in a Mini Protean II Dual Slab Cell (BIO-RAD). A 3–15% gradient gel was used. Gels were stained with Coomasie Brilliant Blue R-250 (0.2% w/v) and analyzed using Molecular Analyst software (BIO-RAD).

Determination of available lysine

Available lysine was determined on a protein pellet obtained from the muscle by an indirect technique using TNBS (Contreras, unpublished). Minced muscle (1 g) was treated with 6.4 mL absolute ethanol. After centrifugation ($1500 \times g$, 10 min, 4 °C) and separation of the supernatant, the pellet was treated with 5.0 mL acetone, centrifuged under the same conditions and the acetone supernatant separated, repeating this treatment three times. Finally, acetone was completely eliminated by drying. For available lysine determination, around 20 mg of pellet (exactly measured) was treated with 1.5 mL methanol, 1.5 mL of $0.5\,mol\,L^{-1}$ borate (pH = 9.2) buffer and 0.2 mL trinitrobenzensulfonic acid (TNBS) ($20\,mg\,mL^{-1}$), agitating during 1 h at room temperature. Reactive blank tubes (RB) (without sample) and blank tubes (B) were also prepared. After agitation, 0.2 mL saturated solution of alanine was added to all tubes except the B one (replaced by 0.2 mL water). After 20 min of agitation, 2.0 mL of 10% w/v trichloroacetic acid (TCA) were added. Centrifugation ($1500 \times g$, 10 min, 15 °C) was performed to precipitate proteins. Absorbance at 345 nm was determined in the supernatant (dilution 1/20) and available lysine content in the pellet was calculated as follows:

$$\text{g lysine/kg pellet} : (\text{RB} - S) \times m_{\text{TNBS}} \times M_{\text{lysine}} \times 1000/(RB \times \text{M}_{\text{TNBS}} \times \text{m}_{\text{p}})$$

where RB = (absorbance of reactive blank tube − absorbance of tube B), S = (absorbance of sample tube absorbance − absorbance of tube B), m_{TNBS} = mass of TNBS (4 mg), M_{lysine} = molecular weight of lysine, M_{TNBS} = molecular weight of TNBS, and m_p = mass of pellet.

Available lysine % was expressed with respect to the protein content (g lysine/100 g protein) determining the pellet protein content by Kjeldhal's method.

Statistical analysis

Data were analyzed by means of analysis of variance (ANOVA) according to the general linear model procedure. When differences were significant ($P < 0.05$) mean values were evaluated by least significant difference (LSD) by the Fisher test using a SYSTAT statistical package.[24]

RESULTS AND DISCUSSION

Proximal composition of sea salmon muscle

Main components of sea salmon muscle were determined in samples from specimens captured at different months of the year, in both sexes and at different ages (different size). The results yielded a protein content (183 ± 7 g/kg muscle) which did not show any significant difference among the muscles analyzed. However, lipid content presented important variations as a function of the capture time and the size of fish (0.28–1.08%).

Lipid fraction modifications during the chilled storage

Lipid fraction composition

Sea salmon muscle lipid fraction analyzed (0.32–0.38%) presented the following composition: triglycerides (TG) = 16.8% w/w, phospholipids (PL) = 83.2% w/w (principal PL components were phosphatidylcholine (PC) and phosphatidylethanolamine (PE)). Table 1 shows fatty acid

class ratios of total lipid; TG and PL fractions were determined from the fatty acid profiles obtained by gas chromatography (GC). It is possible to see that PUFAs represented more than 55.0% of total fatty acids, the $\omega 3$ fraction being the major one (44.3% of total fatty acids). In the TG fraction, PUFAs represented more than 41.0% of total fatty acids (with 27.5% of $\omega 3$ PUFAs) and 61% in the PL fraction ($\omega 3$ fraction 47.8%). These results showed that a high ratio of $\omega 3$ PUFAs should be constituents of the PL fraction. With respect to the fatty acid constituents of each PUFA fraction, the major one in the $\omega 3$ fraction was docosahexaenoic acid (DHA) ($\omega 3$ 22:6) (339 ± 5 g/kg total fatty acids in the total lipid fraction), followed by the eicosapentaenoic acid (EPA) ($\omega 3$ 20:5) (75 ± 3 g/kg). The $\omega 6$ fraction presented as main components 20:4 (58 ± 3 g/kg in the total lipid fraction) and 22:5 (43 ± 2 g/kg) fatty acids.

After 6 days of chilled storage, TLC plate analysis did not present any differences in the profile with respect to $t = 0$ (Fig. 1). However, after 9 days of storage a very important increase (430%) in the intensity of the band corresponding to free fatty acids (FFA), as well as an increment in lysophosphatidylcholine (LPC) (110%), were evident, with a small decrease of PC and PE bands (around 10%). In addition, TG band intensity did not decrease. These results suggest an important phospholipid hydrolysis process producing the increased FFA content. This fact is in agreement with previous works indicating that phospholipids are the main source of FFA in other muscle types, such as chicken.[25] Studies using other lean fish species (blue whiting) have demonstrated a significant increment in the FFA content at long chilled storage time (13 days),[9] while in hake FFA formation has been shown to be a good freshness index.[26]

Figure 2(A) presents the fatty acid classes as a function of chilled storage of sea salmon muscle.

Table 1. Fatty acid profiles in lipid fractions of sea salmon minced muscle (*Pseudopercis semifasciata*)

	% of total fatty acids		
Fraction	Total lipids	TG	PL
SFAs	25.8 ± 0.7	25.8 ± 0.6	26.5 ± 1.8
MUFAs	17.4 ± 0.3	31.5 ± 0.4	12.9 ± 0.6
$\omega 3$-PUFAs	44.3 ± 0.7	27.5 ± 0.2	47.8 ± 2.2
22:6	33.9 ± 0.5	13.8 ± 0.2	38.7 ± 1.1
22:5	1.8 ± 0.1	3.5 ± 0.2	2.4 ± 0.2
20:5	7.5 ± 0.3	6.1 ± 0.1	5.7 ± 0.3
18:3	1.0 ± 0.1	4.1 ± 0.4	0.9 ± 0.01
$\omega 6$-PUFAs	11.3 ± 0.5	12.3 ± 1.3	10.2 ± 0.2
22:5	4.3 ± 0.2	3.4 ± 0.1	3.0 ± 0.1
20:4	5.8 ± 0.3	3.2 ± 0.2	5.8 ± 0.01
18:2	1.2 ± 0.1	3.0 ± 0.7	1.1 ± 0.1

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; $\omega 3$ and $\omega 6$ PUFAs, polyunsaturated fatty acids. Each value is represented as the mean $\pm$ SD ($n = 3$). Lipid fractions were obtained from muscles with 3.2–3.8 g lipids kg^{-1} muscle.

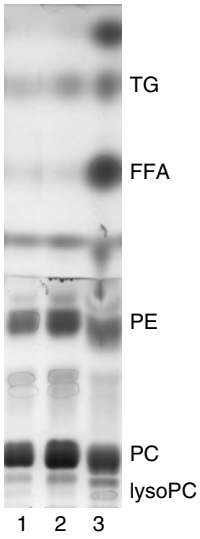

Figure 1. TLC of the total lipid fraction from minced sea salmon muscle as a function of chilled storage time: (1) 0, (2) 6, and (3) 9 days, respectively.

It is possible to observe a significant decrease ($P < 0.05$) of $\omega 3$ PUFA ratio after 6 days of storage. DHA was the main component affected, while $\omega 6$ PUFAs did not present changes during chilled storage (Fig. 2(B)).

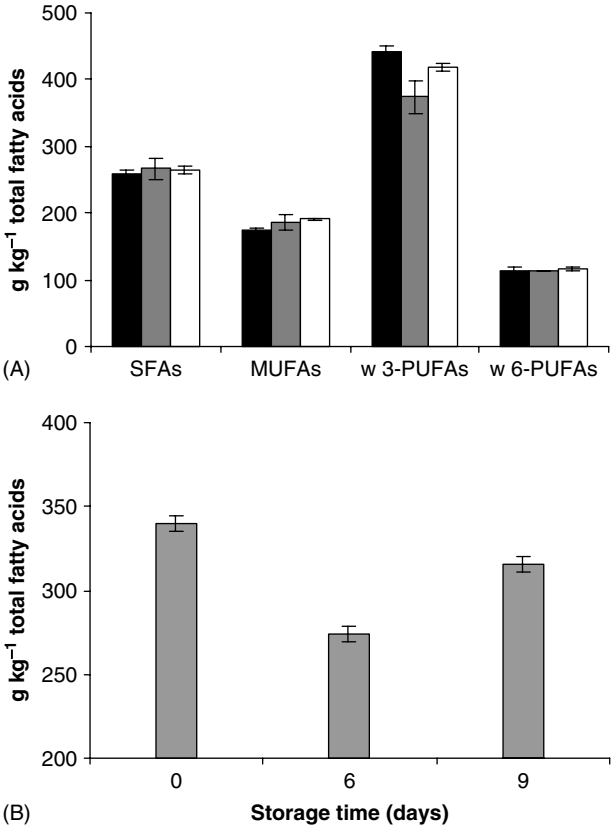

Figure 2. (A) Fatty acid classes in sea salmon minced muscle at different times of storage: 0 (□), 6 (▒), and 9 (■) days. (B) $\omega 3$–22:6 fatty acid content as a function of the chilled storage time of sea salmon minced muscle.

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Lipid oxidation

TBA number was determined during chilled storage of sea salmon mince with different lipid contents. This parameter increased significantly ($P < 0.05$) as a function of storage time up to 6 days; between 6 and 9 days, TBA values did not change (Fig. 3). This behavior was similar for all the samples assayed, although the maximum value achieved was different according to the fish lipid content (Fig. 3). TBA values found were higher than those presented by blue whiting (0.50–0.75% of lipids);[9] differences could be attributed to the different lipid fraction composition of muscles and/or previous handling of samples (mince). However, the evolution of this parameter in blue whiting muscle was similar to that of sea salmon, with an increment after 6 days of storage. TBA value evolution in sea salmon mince showed a good correlation with changes in PUFA fraction, especially with the decrease in DHA after 6 days of storage.

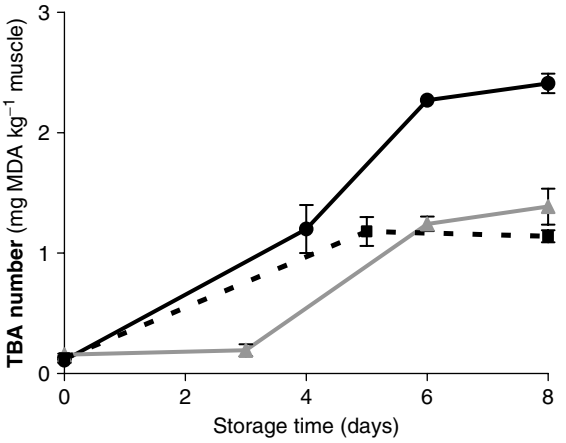

Figure 3. TBA number as a function of chilled storage time of sea salmon muscle. Muscles with different lipid content: 1% (—), 0.49% (—), 0.32% (·····).

Protein fraction modifications during chilled storage of sea salmon muscle

Differential scanning calorimetry

Sea salmon muscle presented two endothermic transitions at T_{max} $50.6 \pm 0.9\,^{\circ}C$ and $76.6 \pm 1.0\,^{\circ}C$, respectively (Fig. 4(A), curve a). The first transition could be related to myosin denaturation, with a small contribution from sarcoplasmic proteins. The second one is mainly associated with actin denaturation.[27] Denaturation enthalpy values determined were: $\Delta H_{mio} = 8.8 \pm 1.6\,J\,g^{-1}$; $\Delta H_{act} = 1.6 \pm 0.7\,J\,g^{-1}$; $\Delta H_{total} = 10.4 \pm 1.6\,J\,g^{-1}$.

Thermal profiles presented some changes during chilled storage of sea salmon muscle. After 7 days of storage it was possible to observe a shoulder at 54–55 °C and a decrease in actin denaturation temperature ($72.9 \pm 0.4\,^{\circ}C$) (Fig. 4(A), curve b). After 9 days of storage, myosin denaturation presented two transition ($49.9 \pm 0.3\,^{\circ}C$ and $54.0 \pm 0.3\,^{\circ}C$), and a denaturation temperature of actin at $67.7 \pm 0.4\,^{\circ}C$) (Fig. 4(A), curve c). These thermal profiles were similar to those obtained for isolated sea salmon myofibrillar proteins,[28] suggesting a breakdown of the myofilament structure.

Myosin denaturation enthalpy decreased significantly after 7 days storage ($P < 0.05$), and this protein remained at 40% without structural modification (Fig. 4(B)). These changes in myosin were more pronounced as storage time increased and achieved more than 80% denaturation; however, actin did not show changes in denaturation enthalpy.

Protein extractability

Total extractable proteins (TP) – including sarcoplasmic proteins (SP), myofibrillar proteins (MP) and other soluble nitrogen compounds – and extractable myofibrillar proteins (MP) were analyzed as a function of chilled storage. After 6 days, protein concentration of the TP fraction decreased, with a slight increase

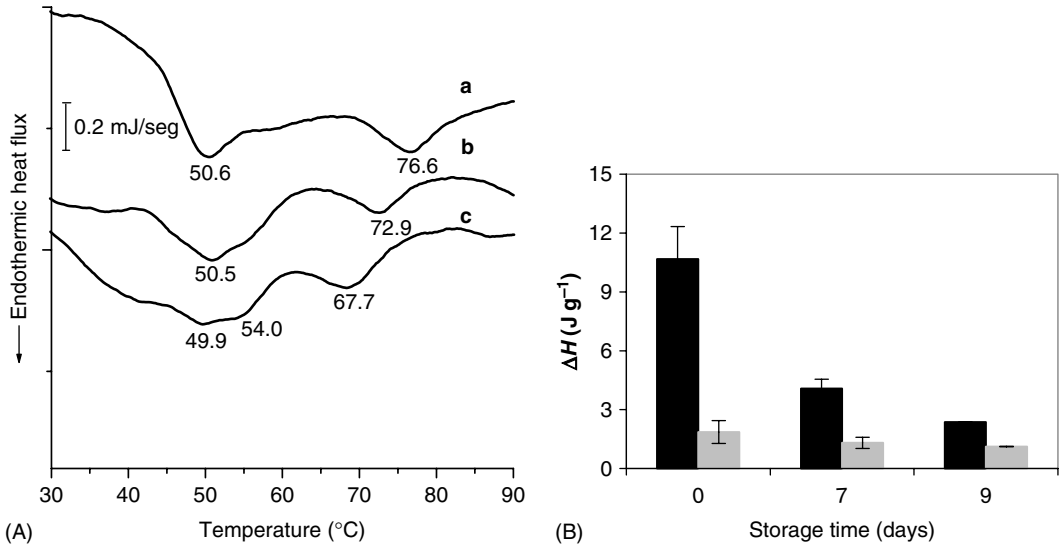

Figure 4. DSC of sea salmon muscle during chilled storage. (A) thermal profiles: (a) day 0, (b) day 7, (c) day 9. (B) Denaturation enthalpies of myosin (■) and actin (▭) as a function of the storage time.

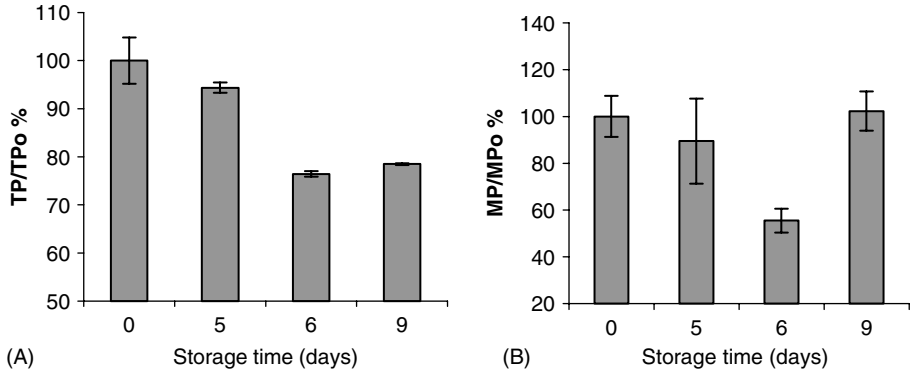

Figure 5. Protein extractability as a function of chilled storage. (A) Total extractable proteins (TP). (B) Extractable myofibrillar proteins (MP). TP and MP evolution are expressed as the relative ratio respect to their starting values (TP_0 and MP_0, respectively).

after this time (Fig. 5(A)). MP showed an important decrease at 6 days of storage, increasing markedly after that and achieving higher extractability values than the starting extract (Fig. 5(B)).

Results suggested an aggregation of sarcoplasmic and myofibrillar proteins during the first 6 days of storage, the latter being most affected. Myofibrillar protein insolubilization could be related to conformational changes in myosin recorded by DSC. The increase in myofibrillar protein extractability ($t = 9$ days) was in agreement with detected thermal profile changes (see Fig. 4(A), curve c). Results presented differences with respect to studies carried out in other species. Hake muscle did not show changes in myofibrillar protein extractability during storage at 0 °C ($t = 11$ days);[29] meanwhile Atlantic salmon muscle presented a diminution in solubility of water-soluble proteins at 9 days of chilled storage, without changes in the myofibrillar fraction.[30] On the other hand, in fatty species such as sardine, myofibrillar protein solubility diminished after 20 h of storage, increasing anew after 9 days.[31]

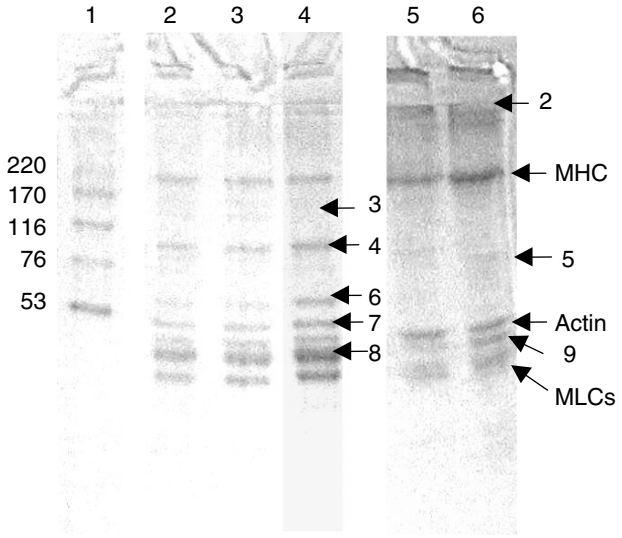

Figure 6. SDS-PAGE of the extractable fractions (with 2-ME). Lane 1: high-molecular-weight marker; lanes 2, 3 and 4: PT extract, 0, 6 and 9 days of chilled storage, respectively; lanes 5 and 6: MP extract, 0 and 9 days of chilled storage, respectively. 30 μg protein was loaded in each lane.

Electrophoresis of extractable fractions

TP and MP fractions were analyzed by SDS-PAGE. Figure 6 shows the electrophoretic profiles corresponding to samples treated with SDS, urea and 2-ME. After 6 days of chilled storage, the TP fraction did not present any changes on its electrophoretic profile with respect to initial time (Fig. 6, lanes 2 and 3). MP content was similar to that corresponding to the starting extract, suggesting that all of these species would participate in insoluble aggregates determined by extractability assays. At 9 days of storage, the TP fraction presented some changes, such as a decrease in the intensity of band 3 (140 kDa), an increase in band 4 (90 kDa) and in other bands of very low molecular weight (Fig. 6, lane 4). All these protein species correspond to the sarcoplasmic protein fraction, due to the fact that they did not appear in myofibrillar protein profiles (Fig. 6, lanes 5 and 6). At the same storage time, the MP fraction showed a decrease in band 2 (high molecular weight) and band 5 (81 kDa), and increased intensity of myosin heavy chain (MHC) (205 kDa), actin, band 9, myosin light chains (MLCs) and other lower-molecular-weight species: 80–200 kDa (Fig. 6, lane 6).

Extractability and electrophoretic results suggest that at longer times of chilled storage high-molecular-weight proteins suffered a breakdown in their constituent polypeptides. There was no evidence of an enzymatic proteolytic process since a new protein band did not appear; however, we cannot discount that some proteolysis could occur, generating small fragments running from the gels. Another factor influencing the protein conformation is pH. Increments of pH above 7 can promote a decrease of thermal stability and denaturation of myofibrillar proteins.[32,33] In this case, pH (initial value: 6.4) increased significantly after 7 days of storage, exceeding a value of 7 at 9 days.

Determination of available lysine

It was previously mentioned that lipid oxidation products can react with ε -amino groups of lysine. In a previous study in our laboratory, a diminution in

Table 2. Fluorescent compounds in organic and aqueous phases obtained from sea salmon minced muscle (*Pseudopercis semifasciata*) as a function of chilled storage time

	Organic phase			Aqueous phase			
Time (days)	$RF_{325/412}$	$RF_{388/460}$	δF_{org}	$RF_{325/412}$	$RF_{388/460}$	δRF_{aq}	$\delta F_{org}/\delta F_{ac}$
0	0.03 (0.01)a	0.01 (0.002)a	0.46 (0.002)a	0.43 (0.02)a	0.06 (0.002)a	0.13 (0.002)a	3.42 (0.02)a
5	0.04 (0.01)a	0.02 (0.01)ab	0.55 (0.18)a	0.51 (0.07)a	0.07 (0.003)a	0.13 (0.01)a	4.21 (1.02)a
8	0.09 (0.01)b	0.03 (0.002)b	0.39 (0.01)a	0.54 (0.01)a	0.08 (0.003)a	0.15 (0.01)a	2.59 (0.17)b
12	0.14 (0.02)c	0.06 (0.01)b	0.45 (0.12)a	0.65 (0.05)b	0.46 (0.28)b	0.69 (0.38)b	0.82 (0.62)c

Each value is represented as the mean ± SD ($n = 3$). Different letters (a, b, c) in the same column indicate significant differences ($P < 0.05$). RF, relative fluorescence (see 'Material and methods'). δ F, $RF_{388/460}/RF_{325/412}$.

available lysine content of sea salmon myofibrillar proteins due to reaction with malonaldehyde in a model system was demonstrated.[34] Taking into account this fact, available lysine content was evaluated during the storage of minced muscle, as another means to evidence lipid oxidation product–protein interaction and also as nutritional information. However, available lysine content of muscle proteins of sea salmon did not present significant differences during the period of chilled storage studied (74 ± 10 g lysine/kg of proteins). According to this, it was not possible using the present method to evidence any interaction between oxidation products and free amino groups of lysine.

Fluorescent interaction compounds

Fluorescent interaction compounds were evaluated during chilled storage of sea salmon muscle, obtaining both phases using a Bligh and Dyer procedure.[7] Excitation/emission maxima previously determined were 325/412 and 388/460. Table 2 shows the fluorescence intensity in the maxima as a function of storage time. In the organic phase, both fluorescence maxima presented a significant increase ($P < 0.05$) at 8 days storage; meanwhile an increase occurred after a long time in storage in the aqueous phase.

As was previously mentioned, the time evolution of interaction compounds can be associated with a shifting of the fluorescence maxima to higher wavelengths, which was calculated as $\delta F = RF_{388/460}/RF_{325/412}$ for both phases; as well as with a more hydrophilic character of the fluorescent compounds, which can be measured by the $\delta F_{org}/\delta F_{ac}$ ratio. During chilled storage δF_{org} and δF_{aq} values were constant for up to 8 days. It is remarkable that δF_{aq} increased significantly ($P < 0.05$) after this time (12 days). In addition, $\delta F_{org}/\delta F_{ac}$ showed a decrease after 5 days of storage (Table 2). Similar results had been reported in other non-fatty species such as blue whiting (*Micromesistius poutassou*).[9] Results obtained suggest a more polar character of the interaction compounds present in sea salmon muscle as a function of chilled storage. Polar compounds could be related to the interaction of carbonyl compounds and amine groups of proteins, peptides or free amino acids, which can be measured in the aqueous phase. In this phase, fluorescence values increased at longer times of storage than those determined by TBA number.

CONCLUSIONS

Sea salmon is a non-fatty species. However, evolution of the lipid oxidation process could be demonstrated, especially up to 6 days of storage. Results suggest that TG was the main fraction involved in this process. $\omega 3$ PUFAs, mainly docosahexaenoic acid (DHA), was the compound most affected. Phospholipid hydrolysis was evident at long times of storage (9 days).

Lipid oxidation is a deteriorative process to be considered during chilled storage of products containing minced sea salmon muscle in order to protect health benefits related to $\omega 3$ fatty acid intake. At extended times of chilled storage, only oxidized lipid–protein interactions were recorded; this interaction does not seem to be related to protein structural modifications and extractability patterns. Protein alterations could be associated with other deteriorative events in sea salmon muscle such as changes in pH level. However, other reaction pathways between lipid oxidation products (primary and secondary) and proteins have not been discounted.

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