Original article

Lipid and protein changes in chilled sea salmon (*Pseudopercis semifasciata*): effect of previous rosemary extract (*Rossmarinus officinalis* L.) application

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Summary The aim of this work was to analyse the effect of rosemary extract application (200 and 500 ppm) on lipid oxidation, colour and protein modifications during the chilled storage (1.0 ± 0.7 °C) of sea salmon (*Pseudopercis semifasciata*). Lipid oxidation and ω3-22:6 fatty acid content modification were prevented by the addition of rosemary extract. Analysis of interaction between lipid oxidation products and proteins by fluorescence showed no relationship between their temporal changes in the aqueous phase and the lipid oxidation evolution since a similar behaviour was observed in both absence and presence of antioxidant. Protein extractability, sodium dodecyl sulphate-polyacrylamide gel electrophoresis, differential scanning calorimetry and lysine content determinations revealed no differences between muscle untreated or treated with rosemary. Fluorescent compounds evolution in organic phase would be in relation with the appearance of lipid oxidation products. In addition, rosemary extract partially prevented the loss of red colour in chilled muscle. Although protein alterations could not be prevented, rosemary extract shows to be a promissory antioxidant in sea salmon muscle.

Keywords Chilling, lipid oxidation, protein changes, rosemary extract, sea salmon.

Introduction

Fish lipids are very sensitive to the oxidation process because of their high content of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic and docosahexaenoic acid (DHA) (Frankel, 1998). Oxidation of these fatty acids produces volatile compounds (2-pentenal, 2-hexenal, 4-heptenal, 2,4-heptadienal, 2,4,7-decatrienal) resulting in flavour deterioration. This is an important cause of quality loss during fish processing and storage. Free radicals, primary and secondary oxidation products can react with other cellular components such as proteins, peptides, free amino acids, phospholipids and nucleic acids (Frankel, 1998; Aubourg, 1999). These interactions have very important consequences on food quality; reactions with proteins are especially relevant in foods with high content of proteins such as seafood.

The biological value of proteins decreases because of reactions with the ε-amine groups of lysine, the oxidation of methionine, and other amino acids changes. Sensorial degradation is related to flavour and colour (browning) changes and also to texture modifications because of cross-linking polypeptide chains (Pokorny *et al.*, 1993; Aubourg, 1999). Carbonyl compounds can react with amine groups forming Schiff base-type products. The final conjugated Schiff bases present a high colour intensity (browning) and characteristic fluorescence spectra (Aubourg, 1999). Organic and aqueous phases obtained by a Bligh & Dyer (1959) extraction has been extensively used for fluorescent compounds determination to evaluate fish quality (Aubourg, 1999). Fluorescent compounds formed at the beginning of the interaction transform into other ones with higher wavelength maxima, according to a progressive formation of Schiff bases that leads to an increase in their molecular weight and number of unsaturated bonds (Aubourg, 1999). This wavelength shift can be measured in both phases as a ratio between fluorescence intensity at two excitation/emission maxima, being correlated with other quality indices during fish processing (Aubourg *et al.*, 1997, 1998).

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For preventing the negative consequences of lipid oxidation on food quality as well as on the consumer health (Shahidi, 1997), antioxidants are incorporated during the food processing. Synthetic antioxidants [butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ)] have been used, but various recent studies have demonstrated their carcinogenic activity (Lindberg Madsen & Bertelsen, 1995; Shahidi, 1997). Thus, natural antioxidants are being extensively studied, and spices and herbs have been identified as important sources of these compounds. Diverse studies have evaluated the antioxidant properties of the rosemary (*Rosmarinus officinalis* L.) (Lindberg Madsen & Bertelsen, 1995). This spice contains certain compounds such as carnosol and carnosic acid, with high antioxidant activity, and others (rosmanol, rosmarinic acid, rosmariquinone, etc.) that present a minor activity (Bracco *et al*., 1981; Offord *et al*., 1997). These substances would act by a hydrogen atom – donating mechanism (Frankel, 1998).

Sea salmon (*Pseudopercis semifasciata*) is an appreciated sea food because of the quality of its meat. It is caught by bottom trawl and hooklines and preserved in ice or by freezing on board and shore. It is commercialised in the internal (fresh and frozen, whole round and fillet) and the external market (frozen, headed and gutted and fillet). Other possible way of commercialisation is as minced products such as patties. The aim of the present study was to analyse the course of lipid oxidation during chilled storage of sea salmon muscle and to evaluate the effect of the application of a commercial rosemary extract on this process and its consequences on fish quality.

Materials and methods

Materials

Sea salmon (*P. semifasciata*) (1.5–4.0 kg per fish) was caught by commercial vessels in the Southwest Argentinean Sea. Bovine albumin, trinitrobenzene sulfonic acid (TNBS) and electrophoretic grade chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals were of analytical grade.

Sample preparation

Fish were kept in ice up to arrival to the laboratory (about 48 h), externally washed, filleted and minced. A representative portion of minced muscle was treated with *Guardian* Rosemary extract 09 (DANISCO, Copenhagen, Denmark). This antioxidant, classified as a GRAS additive in United States (21 CFR 182.20), was applied as an aqueous dispersion (1/10 wt/wt) by spraying and then, treated minced muscle was homogenised (treated sample, T). Two concentration levels of rosemary extract were evaluated: 200 (T200) and 500 (T500) ppm (final concentrations in the meat) respectively. Two independent experiments for each rosemary concentration (and the corresponding untreated muscles UT) were performed. Each experiment was carried out using mince pooled from at least two fish. Total protein content (Kjeldahl method) and total lipid content[14] of each pooled minced muscle were determined. Samples (30 or 50 g according to the different experiments) of minced muscle treated (T) or untreated (UT) with rosemary extract were packed in polyethylene bags and stored at 1.0 ± 0.7 °C for different times before being analysed. Two samples corresponding to each treatment condition were used for analytical measurements at each sampling time.

Lipid composition

Fatty acids profiles of the total lipid fractions obtained by Bligh & Dyer (1959) method were analysed. For that, methyl esters (ME) were prepared according to Christie (1982), extracted with methanol:chloroform:water (1:1:0.9) and dried under nitrogen. ME were purified by thin layer chromatography using hexane:ether (95:5), being identified by 0.05% dichlorofluorescein in methanol and ultraviolet light and eluted with water:methanol:hexane (1:1:1). Analysis was performed in a Varian gas chromatograph (Varian, Sunnydale, CA, USA) using a polar stationary phase 10% SP2330 (Supelco, Bellefonte, PA, USA) and nitrogen as a carried gas (30 mL min^{-1}). A gradient of temperature (155–230 °C, 5 °C min^{-1}) was applied. Detection was performed by two flame ionisation detectors. Peaks identification was carried out according to the retention times and quantification by electronic integration (Varian Workstation) using an standard mixture of fatty acids ME from Sigma Chemical Co.

Lipid oxidation

Thiobarbituric acid value determination

The course of lipid oxidation during the chilled storage was followed by the thiobarbituric acid (TBA) number. For that, 2 g of minced muscle were homogenised with 16.0 mL of 5% w/v trichloroacetic acid (TCA), kept 30 min in ice, and filtered. Two millilitre of filtrate were mixed with 2.0 mL of 0.5% w/v TBA solution. After reaction (30 min, 70 °C), absorbance at 532 nm was measured in a Beckman DU 650 spectrophotometer (Beckman Coults, Fullerton, CA, USA). TBA number was calculated ($\epsilon = 3.6 \times 10^5$ M^{-1} cm^{-1}) (Botsoglou *et al*., 1994). Two independent extracts were obtained for each sample and the TBA reaction was performed by duplicate for each one.

Determination of fluorescent compounds
Interactions between lipid oxidation products and cell components were analysed by the analysis of fluorescent compounds in organic and aqueous phase from a Bligh & Dyer (1959) extraction. Excitation/emission wavelength maxima (325/412 and 388/460) had been previously determined (Tironi *et al.*, 2007) and relative fluorescence (RF) in the maxima respect to a standard solution of quinine sulphate (1 μg mL^{-1} in 0.05 M H_2SO_4) was calculated in each case according to (Aubourg *et al.*, 1992):

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

where: F, sample fluorescence intensity; V, extract volume; F_{st}, standard fluorescence intensity (350 nm/450 nm); and m, sample mass. For each sample three independent extractions were carried out. As was previously mentioned in the Introduction, 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 δForg/δFaq ratio.

Protein alteration

Protein changes in chilled muscle UT and T (200 and 500 ppm) were analysed by different techniques.

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

Protein extractability
Two and a half grams (2.50 g) of minced muscle were homogenised with 50.0 mL of 0.03 M Tris–HCl, 0.6 M KCl, pH = 7.0 buffer solution. After centrifugation (1500 × ***g***, 30 min, 4 °C) the supernatant was separated (total extractable proteins, TP) (Awad *et al.*, 1969). To obtain *extractable myofibrillar proteins* fractions 10.0 mL of TP extract were mixed with 90.0 mL of distilled water and stored at 4 °C (16–24 h). The mixture was centrifuged (2100 × ***g***, 30 min, 4 °C) and the supernatant was removed. The pellet containing myofibrillar proteins was suspended with 5.0 mL of 0.03 M Tris–HCl, 0.6 M KCl, (pH = 7) solution. Protein concentration in extracts was determined by duplicate by a modified Biuret method (Robson *et al.*, 1968). At least two extracts per sample were analysed.

SDS-PAGE of the extractable fractions
Samples were treated with sample buffer (8 M urea, 0.3% sodium dodecyl sulphate, pH 9.0, 5% 2-mercaptoethanol) and a similar protein mass (30 μg) was loaded onto each lane. Slab SDS-polyacrylamide gel electrophoresis (PAGE) was carried out by the Laemmli discontinuous buffer system (Laemmli, 1970) in a Mini Protean II Dual Slab Cell (Bio-Rad, Hercules, CA, USA). A 3–15% gradient gel was used. Gels were stained with Coomasie Brilliant Blue R-250 (0.2% w/v) and analysed with a MOLECULAR ANALYST Software (Bio-Rad).

Available lysine content
Available lysine was determined on a protein pellet obtained from the muscle by an indirect technique using TNBS (Tironi *et al.*, 2007). Minced muscle was treated with absolute ethanol. After centrifugation (1500 × ***g***, 10 min, 4 °C) and separation of the supernatant, pellet was treated with acetone, centrifuged at the same conditions and acetone supernatant separated, repeating this treatment three times. Finally, acetone was completely eliminated by dryness. Twenty milligrams of pellet (exactly measured) were treated with 1.5 mL of methanol, 1.5 mL of 0.5 M borate buffer (pH = 9.2) and 0.2 mL of TNBS (20 mg mL^{-1}) (agitation 1 h at room temperature).Tubes corresponding to the reaction blank (RB, without sample) and blank (B, without sample or alanine) were also prepared. After agitation, 0.2 mL of saturated solution of alanine was added to all the tubes except tube B (replacing by 0.2 mL of water). After 20 min of agitation, 2.0 mL of 10% w/v TCA was added. Centrifugation (1500 × ***g***, 10 min and 15 °C) was performed to sediment proteins. Absorbance at 345 nm was measured in the supernatant (dilution 1/20). Available lysine in the pellet was determined as:

$$\%\text{Lysine}(\text{gper100 gpellet}) = [(RB - S) \times m_{TNBS} \times M_{lysine} \times 100]/(RB \times M_{TNBS} \times m_p)$$

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; m_p = mass of pellet. Lysine % (g per 100 g protein) was obtained from the % lysine previously shown, determining the pellet protein content by Kjeldahl (f = 6.25).

Colour determination

Colour parameters on the CIELAB system a^*, b^* and L^* were determined using a Minolta CR300 colorimeter (Minolta, Osaka, Japan). Five determinations were performed on different sample locations, taking two samples for each time-rosemary extract concentration condition.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) according to the General Linear Model Procedure. When the ANOVA showed differences to be significant, mean values were evaluated by Least Significant Differences by the Fisher test using a SYSTAT statistical package (Wilkinson, 1990).

Results and discussion

Proximal composition of the sea salmon muscle

Protein content of samples from specimens captured at different months of the year – both sexes and different ages (different size) – was 18.26 ± 0.72 g per 100 g muscle, with no significant differences among the muscles analysed. However, lipid content showed important variations as a function of the capture time and the size of fishes, ranging from 0.32 ± 0.08 and 1.08 ± 0.15 g per 100 g muscle.

Lipid fraction modifications during chilled storage

Lipid oxidation

In a previous work, we have informed that during chilled storage of sea salmon muscle, TBA number increases significantly up to 6 days and then remains constant (Tironi *et al*., 2007). Application of 200 or 500 ppm rosemary extract significantly reduced ($P < 0.05$) production of secondary oxidation products. Figure 1a shows the time course of TBA reactive substances accumulation in minced sea salmon muscle with a lipid content of 0.49 ± 0.09% in the absence (UT) or presence of 500 ppm rosemary extract (T500). In T500 muscle, TBA number did not change during an 8-day storage period. Although maximal TBA number achieved by UT sea salmon muscles was dependent on lipid content (Tironi *et al*., 2007), for T500 muscle, TBA number behaviour was similar to that shown in Fig. 1a – changes during the storage

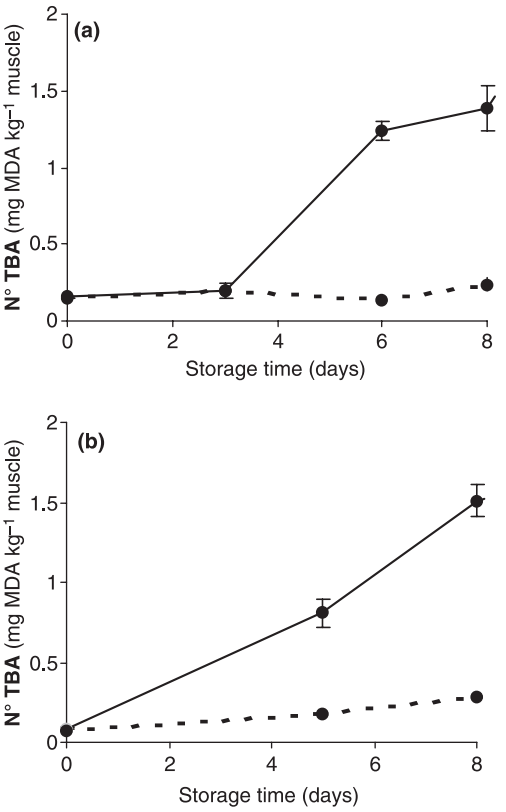

Figure 1 Thiobarbituric acid number as a function of the chilled storage time of sea salmon minced muscle without (UT) ——, and with rosemary extract (T) – – –: (a) T500, (b) T200. TBA number is expressed as the mean ± SD (n = 4).

time were not detected – for samples with different lipid contents (between 0.32 ± 0.08% and 1.08 ± 0.15%).

When 200 ppm rosemary extract was applied (T200), a small and progressive increase in TBA number was observed over time (Fig. 1b), but the final TBARS values were very low.

Secondary lipid oxidation products, such as malonaldehyde (MDA) and others, did not develop in the presence of rosemary extract. This may be as a result of the ability of the antioxidants in rosemary extract to inhibit formation of primary oxidation products via hydrogen atom donation or to prevent break down of lipid hydroperoxides (Frankel, 1998). Both rosemary extract concentrations were effective, and action was independent of the muscle lipid content (between 0.32% and 1.08%) and the oxidation level in the corresponding UT samples.

Lipid fraction composition

The effect of rosemary extract on total lipid fraction composition was analysed in comparison with UT samples. Fatty acid profiles obtained by gas chromato-

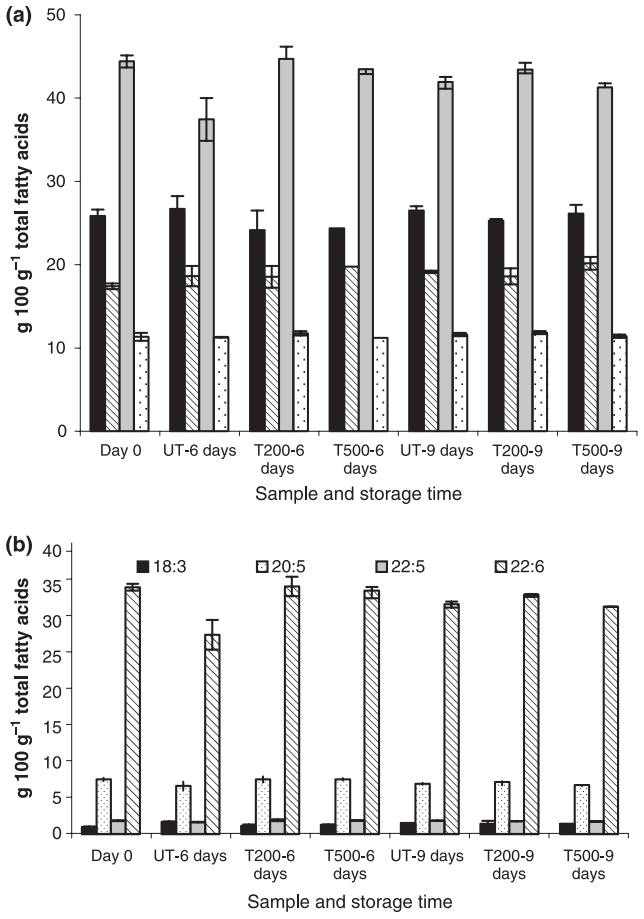

Figure 2 (a) Fatty acid classes proportion in total lipid fraction of sea salmon muscle as a function of chilled storage time and rosemary extract application: ■ saturates, ⬚ monounsaturates, ▨ ω3-polyunsaturates and ▧ ω6-polyunsaturates. (b) ω3-fatty acids proportion in total lipid fraction of sea salmon muscle as a function of chilled storage time and rosemary extract application. Results are expressed as the mean ± SD (n = 2).

graphy (GC) are shown in Fig. 2a. No changes in the fatty acid ratio were recorded in T200 and T500 muscles after 6 days of storage, whereas the ω3-PUFA (ω3-PUFA) ratio decreased in UT muscle (Tironi *et al.*, 2007). In contrast, after 9 days of storage, the ω3-PUFA ratio was similar in all samples. Without rosemary extract, there was a slight decrease in the DHA (22:6) ratio in the ω3-PUFA fraction after 6 days of storage (Fig. 2b). However, no changes were observed in T200 and T500 muscles. The ω6-PUFA fraction was not significantly affected by application of rosemary extract for any of the storage times studied (data not shown). These results support the protective effect of rosemary extract on the lipid fraction of sea salmon muscle and demonstrate that the lipid oxidation process affects mainly the ω3 22:6 PUFA fraction.

Fluorescent interaction compounds

Lipid oxidation products can react with amine groups to generate interaction compounds with fluorescent properties (Frankel, 1998). Figure 3a depicts the evolution of fluorescence maxima for T200 muscles compared with UT samples to analyse the effect of rosemary application on these parameters. During a 12-day storage period, rosemary extract application had no effect ($P > 0.05$) on the fluorescence intensity of the organic phase when measured at wavelengths of 325/412. At 388/460, T200 did not present significant changes during the storage period; however, fluorescence increased after 8 days in absence of antioxidant ($P < 0.05$). The fluorescence intensity of the aqueous phase from T200 and UT muscles did not differ significantly ($P > 0.05$). The δForg, δFaq and δForg/δFaq parameters were also not significantly ($P > 0.05$) affected by 200 ppm antioxidant treatment (Fig. 3b). Fluorescent development of T500 samples was comparable with the previously described for T200 samples, with not significant differences ($P < 0.05$) respect to the UT samples (data not shown).

A hypothesis for the origin of fluorescent compounds present in sea salmon muscle can be formulated based on these results. No antioxidant-related differences were detected in the aqueous phase. However, significant increases ($P < 0.05$) in 325/412, 388/460 and δFaq were observed in UT, T200 and T500 samples after several days of storage, suggesting that these changes are not related to the formation of interaction compounds that involve lipid oxidation products. Other processes that occur during long-term chilled storage of muscle may be associated with this fluorescence increase. Other authors (Aubourg *et al.*, 1998) have reported a high correlation between δFaq and total volatile basic nitrogen (TVB-N) in muscle of chilled whiting (*Micromesistius poutassou*). Plotting δFaq vs. TVB-N values yielded a strong linear correlation ($r^2 = 0.91$). Thus, taking into account the lack of rosemary effect, fluorescence changes detected in the aqueous phase during chilled storage likely reflect other deteriorative changes in the muscle, such as protein degradation. However, δForg changes are likely associated with lipid oxidation because UT and rosemary-treated muscles evolution differed.

Effects of rosemary extract application on protein fraction alterations

In agreement with previous work (Tironi *et al.*, 2007), sea salmon muscle proteins are greatly modified during chilled storage. These alterations can be attributed to diverse causes, including interaction with lipid oxidation products. To assess the importance of the oxidation process on protein modification, we studied protein fractions in the presence of rosemary extract. Total and myofibrillar protein extractability was reduced after

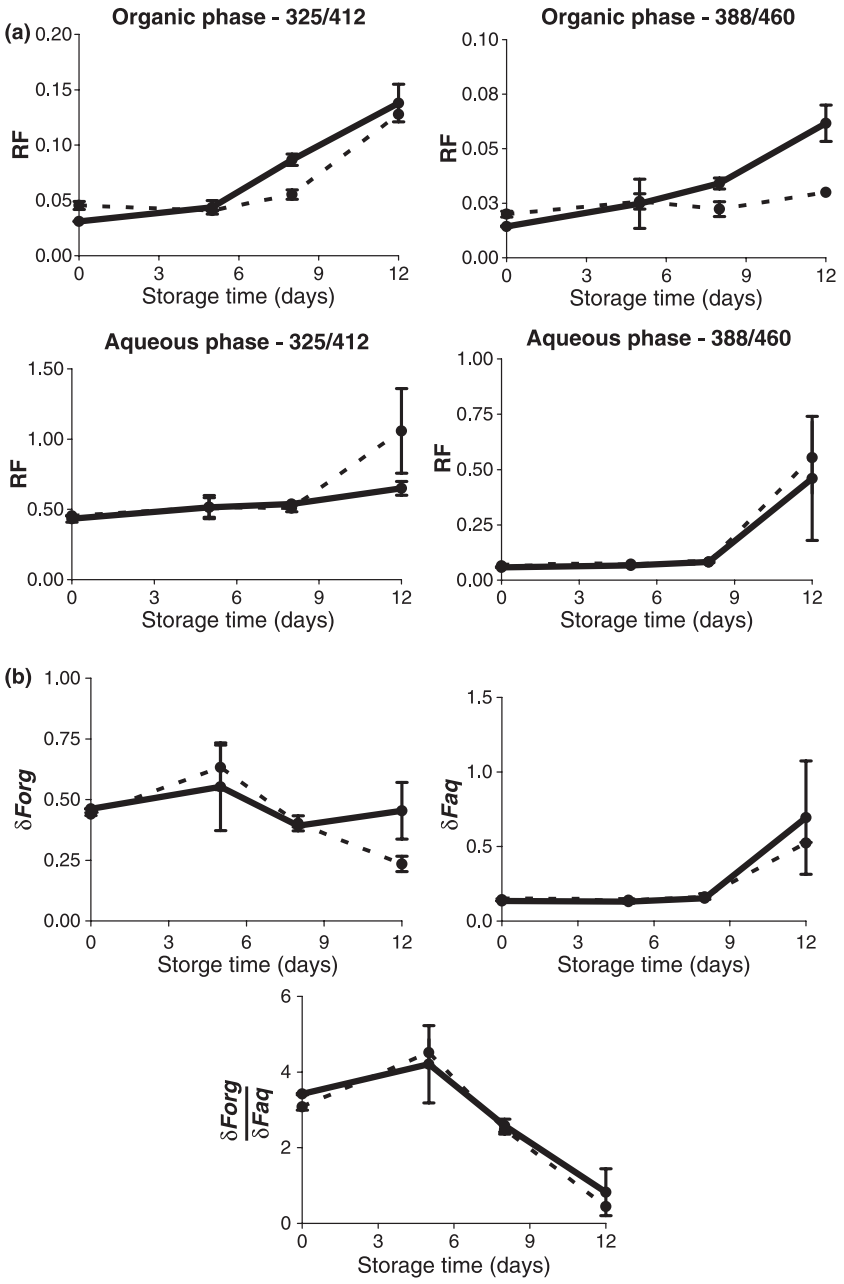

Figure 3 Time course of fluorescence compounds formation: (a) relative fluorescence (RF) in the excitation/emission maxima, (b) δ*F*or, δ*F*aq and δ*F*org/δ*F*aq as a function of chilled storage time of sea salmon muscle: UT —— and T200 – – – Results are expressed as the mean ± SD (n = 4).

6 days of storage for all the systems assayed. However, some differences between UT and rosemary extract-treated muscles appeared at longer storage times. Significant increases ($P < 0.05$) in myofibrillar protein fraction extractability were observed after 9 days of storage for T500 (Fig. 4) or 12 days for T200 (data not shown), probably because of protein degradation. These results suggest that protein insolubility in UT muscle during the first 6 days of storage is not related to the lipid oxidation process because protein extractability decreased in the presence and absence of antioxidant. Differences detected at longer storage times, when protein structure is degraded and disorganised, suggest some type of interaction between degraded protein and lipid oxidation products. However, such effects could not be observed by using other methodologies. SDS-PAGE, DSC and lysine content studies were performed with the aim to compare T200 and T500 with UT samples. SDS-PAGE and DSC results for rosemary extract–treated (200 and 500 ppm) muscles (data not

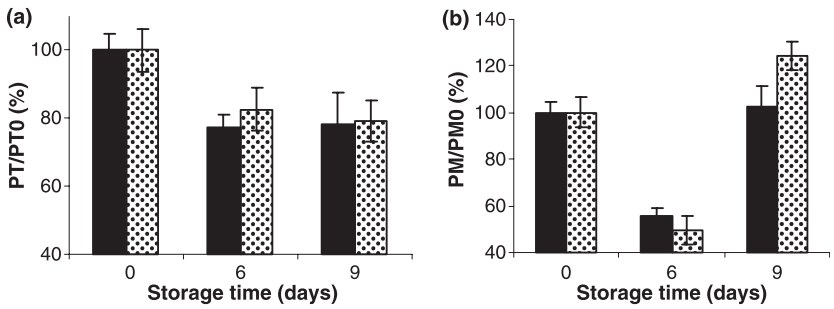

Figure 4 Protein extractability as a function of chilled storage time of sea salmon muscle: UT ■, and T500 ⊡. (a) Total extractable proteins respect to time 0 (PT/PT0 %); (b) myofibrillar extractable proteins respect to time 0 (PM/PM0 %). Results are expressed as the mean ± SD (n = 4).

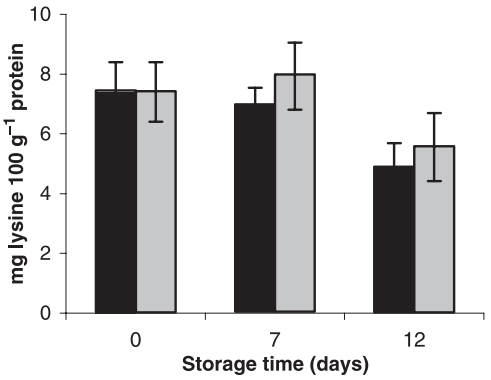

Figure 5 Available lysine content as a function of chilled storage time of sea salmon muscle: UT ■, and T200 ▨. Results are expressed as the mean ± SD (n = 2).

shown) did not differ significantly to those described for UT samples in a previous work (Tironi *et al*., 2007). Similarly, lysine content at different storage times did not significantly change as a function of the antioxidant application as is shown in Fig. 5 for T200 muscles, suggesting that antioxidant had not effect on protein modifications during chilled storage. These results are in agreement with the fact that determination of fluorescent compounds did not reveal interactions between lipid oxidation products and proteins, as was previously described.

It is important to remark that the fish specie studied in the present work has low lipid content. This, in addition with the application of a chilling process (also a relative short chilling time), could explain the low lipid oxidation development found, leading to low formation of interaction compound and short protein changes.

Effects of rosemary extract on colour changes

Changes in the colour of salmon flesh during chilled storage may be associated with the oxidation process. The L^*, a^* and b^* parameters were determined in UT, T200 and T500 muscles. We have found differences in muscle colour into the different experiments performed, some samples were whiter and others were redder. Figure 6a shows the results obtained in UT and T500 'white' muscle. None of the colour parameters differed significantly in the presence of antioxidant, indicating that the colour changes observed during chilled storage – increase in the value of a^* and decrease of b^* after 8 days – are not related to lipid oxidation. However, results differed in 'red' muscle (Fig. 6b). Note the difference in the initial value of a^* when compared with 'white muscle' (Fig. 6a). In this case, L^* differed significantly between UT and rosemary extract-treated samples after 4 days of storage. The red colour decreased in both UT and rosemary extract-treated samples as a function of storage time; a minor decrease was observed in treated muscles, as evidenced by the increase in a^* (Fig. 6b). These results indicate partial red colour preservation by the rosemary extract. The b^* parameter increased as a function of the storage time but was not significantly affected by rosemary extract. Total colour change, ΔE, was greatly affected by antioxidant. T500 muscle showed no change in this parameter throughout the chilled storage period (Fig. 6b). When T200 samples were analysed (data not shown) in comparison with UT, results obtained were comparables with those previously described for T500, this fact indicate that both rosemary extract concentrations assayed had a similar effect on colour preservation.

Rosemary extract produces only a partial preservation of red colour, because there are other causes for colour modification in addition to the oxidative processes investigated in this work. Possible causes for the decrease of a^* include the conversion of oxy- and deoxy-haemoglobin to met-haemoglobin, denaturation of myofibrillar proteins that produce a change upon interaction with haemoglobin, and surface dehydration. Similar results have been obtained by others (Wetterskog & Undeland, 2004) in cod muscle; a loss in red colour was detected despite total inhibition of the lipid oxidation process. In other way, interaction between oxidised lipids and proteins can produce changes in b^* (browning) (Aubourg, 1999). However, according to the present results, the increase detected in b^* could not be related to the appearance of lipid oxidation products and their interaction with proteins since it was registered in UT, T200 and T500 samples.

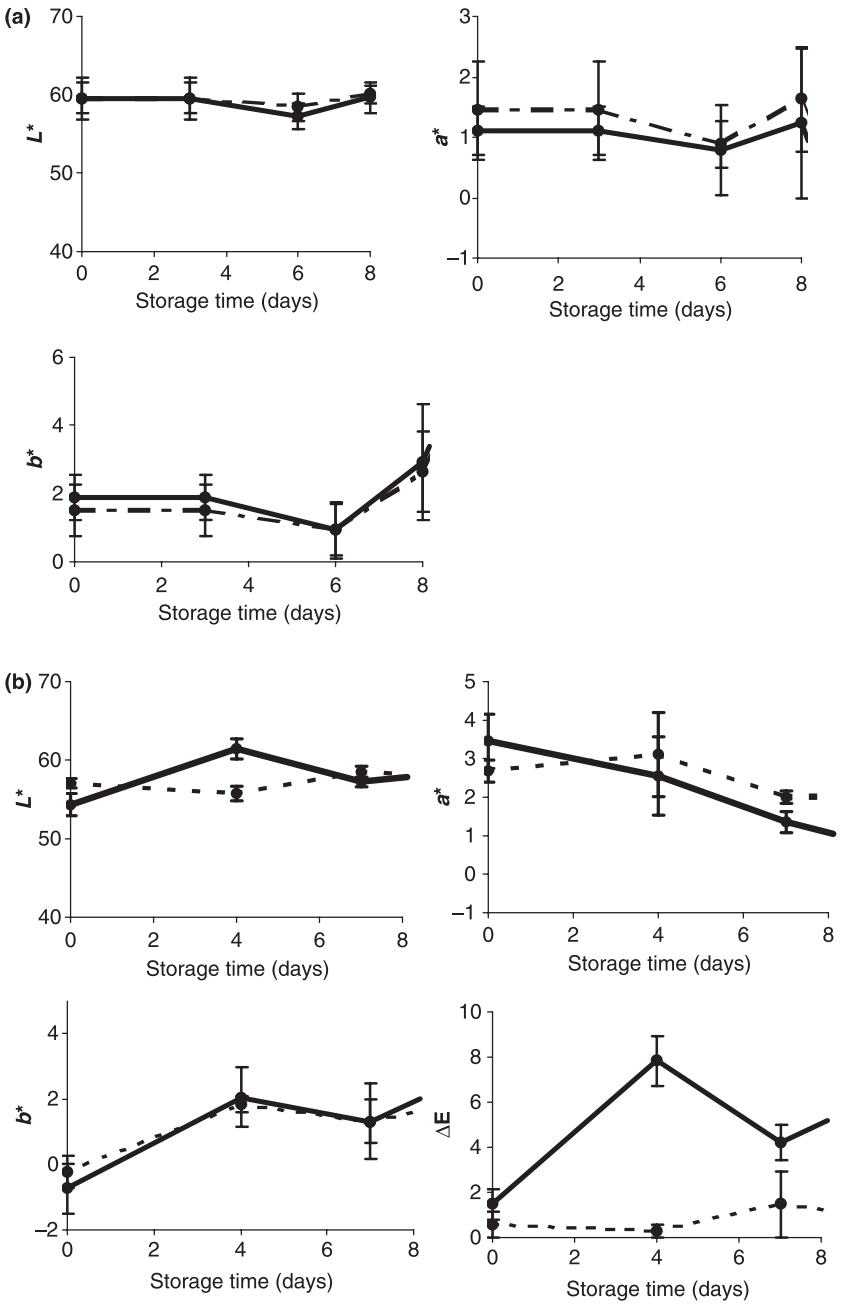

Figure 6 Colour parameters (a^*, b^* and L^*) as a function of the chilled storage time: (a) 'white', and (b) 'red' sea salmon muscle; UT —— and T500 – – –. Results are expressed as the mean ± SD (n = 10).

Conclusions

The present study assessed the antioxidant effects of applying rosemary extract to chilled sea salmon muscle. Both concentrations assayed (200 and 500 ppm) were very effective in preventing development of lipid oxidation products (TBARS) and the decrease in ω3-PUFAs. Rosemary extract had beneficial effects on colour preservation of muscle, partially preventing the loss of red colour. Changes in fluorescent compounds during long-term storage, mainly in the aqueous phase, cannot be attributed to interactions that involve lipid oxidation products. In contrast, changes in fluorescent compounds from the organic phase may be related to lipid oxidation, and likely reflect the interaction between lipid oxidation products and amine groups present in the phospholipid fraction. Fluorescence results obtained from the aqueous phase, as well as protein fraction

analysis, in the presence or absence of antioxidant, suggest that the lipid oxidation process is not the main source of the structural protein modifications that are detected during chilled storage of sea salmon. Together the results indicate that application of rosemary extract to foods containing minced sea salmon muscle may be advantageous.

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