Oxidative quality and color variation during refrigeration (4 °C) of rainbow trout fillets marinated with different natural antioxidants from oregano, quillaia and rosemary

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The study aimed at determining the antioxidant effect of natural extracts on the oxidative quality and color variation of rainbow trout fillets during storage at 4 °C. The fillets were marinated and samples from the belly flap area and Norwegian quality cuts were used for lipid oxidation determination while the dorsal region was used for color measurements. The fillets were marinated with the different treatments: 470 mg l^{-1} of oregano extract, 6.84 ml l^{-1} of quillaia extract, 7.2 ml l^{-1} of rosemary extract and 2 ml l^{-1} of a synthetic antioxidant. Maximum TBARS (Thiobarbituric acid reactive substances) values of belly flap and Norwegian quality cuts occurred at five and six days of storage, respectively. The susceptibility of treatments to lipid oxidation in decreasing order was: control>quillaia >oregano>rosemary>synthetic antioxidant. An increase in lightness (L*) and redness (a*) were observed for rosemary and quillaia extracts when compared to control samples. Marinates with natural antioxidants may be an alternative for extending shelf-life of trout fillets at least during the first six days of storage at 4 °C.

Key words: rainbow trout meat, lipid oxidation, colour, natural polyphenols

Introduction

After harvesting, oxidative rancidity (OR) is produced by enzymatic and non-enzymatic reactions which originates lipid-oxidation compounds altering physical-chemical properties of meat (Gomez and Montero 2007). The loss of quality and freshness in salmonids is sensory-associated to the release of unwanted odors and flavors, as well as the gradual decrease in intensity of color and brightness, texture, alteration of lipid distribution and gapping (Dawson et al. 2018).

Reddish color is a characteristic in the meat of salmonids that exerts a great influence on consumer´s acceptance and the final price of the product. The typical coloration in salmonids meat depends on carotenoid pigments content in the muscle. These pigments are labile to light, heat, and oxygen and may decrease during processing and storing (Dawson et al. 2018).

Applying synthetic antioxidants during the marinating process is a preservation technique widely used in meat products. However, the use of synthetic antioxidants (such as BHQ, TBHQ) has provoked strong controversies related to issues on food safety and toxicity (Decker 1998). Some plant species such as rosemary, quillaia, and oregano possess antioxidant properties due to their contents of bioactive substances such as phenolic compounds. Therefore, they could be used in seafood to avoid OR since their antioxidant action is similar to that of synthetic phenolic compounds (Gomez et al. 2007).

Foods with high content of polyunsaturated fatty acids, as trout meat, are susceptible to OR. Therefore, extension of the shelf life of the final product is a constant priority for the food industry (Medina et al. 2009). Thus, the main hypothesis of this study is that the addition of natural antioxidants during the marinating process keep and/or improve the quality of rainbow trout fillets. The objective of this study is to determine the effects of using natural antioxidants applied in the marinating process on the oxidative and sensory quality in rainbow trout fillets. In this study, oregano, rosemary and a synthetic antioxidant were used as known antioxidants. The quillaja extract was chosen because its antioxidant properties in trout meat remained unknown but the extract is readily available.

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Materials and methods

Samples and treatments

Twenty-five fillets of rainbow trout (*Oncorhynchus mykiss*), were obtained from a company located in the south of Chile (Puerto Montt). Each fillet weighed 750 g and corresponded to a type of cut called TRIM-C (loin-like fillets without skin, with belly flaps and with the same cutting orientation).

Four antioxidant marinade solutions (extracts of quillaia, rosemary, oregano and a synthetic antioxidant) in equivalent doses and a control treatment were used. The equivalent dose (ED) was the amount of natural antioxidant that equals in antioxidant capacity to 1000 ppm of the synthetic antioxidant measured with the ferric reducing antioxidant power (FRAP) method (Benzie and Strain 1996). For each treatment, five repetitions were performed absorbing 4% of the fillet weight in antioxidant marinade solution. Dose and composition of the antioxidant treatments of the natural extracts are shown in Table 1. Fillets were randomly assigned to each treatment for subsequent marinating by immersion with the different antioxidants. After this procedure, the regions of belly flap (ventral zone) and NQC (Norwegian cut) were delimited and cut into seven portions (Fig. 1), which were placed in polystyrene trays covered with a transparent plastic film and randomly assigned to the refrigeration treatment from 0 to 6 days at 4 °C. Then, samples were frozen at –80 °C until further analysis.

For color measurement, the cranial-dorsal area of each fillet was separated for each treatment. Samples were placed in polystyrene trays, covered with a transparent film and stored at 4 °C. Color measurements were made daily between 0 and 12 days of refrigeration.

Table 1. Antioxidant treatments and dosage used in the experiments

Antioxidants	Chemical composition	Doses	Reference
Rosemary extract	Carnosic acid, carnosol, rosmarinic acid,	7.2 ml l^{-1}	Yanishlieva et al. 2006
Quillaia extract	Polyphenols	6.84 ml l^{-1}	Fellenberg et al. 2010
Oregano extract	Carvacrol, thymoll, caffeic acid	470 mg l^{1}	Pokorný 2007
Synthetic antioxidant	BHT (10%), TBHQ (5.5%), BHA (2.5%) citric acid	2 ml l^{-1}	Cramer Ltda. Chile

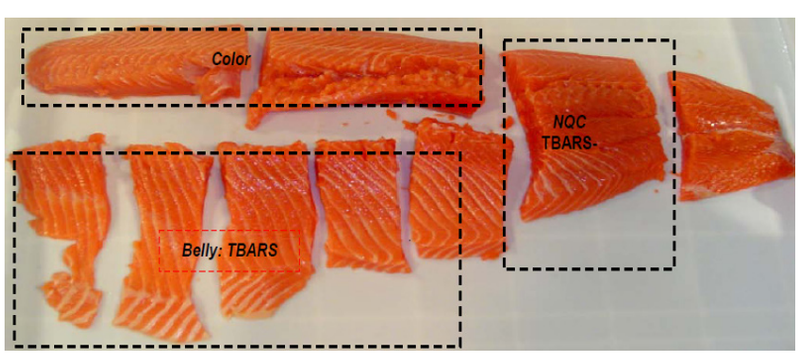

Fig. 1. Sampling zone for TBARS and color evaluation in trout fillets

Antioxidant capacity of plant extracts assessment

Solutions of different concentrations of plant extracts were prepared, with a final dilution factor of 1: 1000. Then, 30 µl of each solution were added to test tubes containing: 1000 µl of acetate buffer (300 mM, pH 3.6); 100 µl of $FeCl_3$ (20 mM) and 100 µl of 2,4,6-tripyridyl-S-triazine (10 mM) in HCl (40 mM) previously incubated for 4 min at 37 °C. For the blank, 30 µl of acetate buffer (300 mM, pH 3.6) were added to replace the different samples of the antioxidants. The mixture was incubated at 37 °C for 4 min, then its absorbance was measured at 593 nm on a Shimadzu® UV-1650 PC spectrophotometer. The antioxidant capacity of the extracts was determined using the

standard curve of the synthetic antioxidant, this data allowed to determine the equivalent dose of the extracts, which were incorporated into the fillet marinade.

Thiobarbituric acid reactive substances (TBARS) analysis

Samples of tissue (5 g each) were homogenized for 3 min in 12 ml of phosphate buffer $Na_2(H)PO_4$ (50 mM, pH 7.4). This homogenate was separated in two 25 ml flasks, where 50 µl of $FeCl_3$ (5.05 mM) were added to one of them to induce oxidation. An aliquot of 800 µl was taken from both bottles and mixed with 1200 µl of phosphate buffer to determine the basal state of the sample (basal lipid-oxidation). The rest of the solutions were incubated for 45 min at 37 °C with shaking. After the incubation, samples were cooled after which 800 µl aliquots were taken from both flasks. All aliquots taken at the basal state and after incubation were added to tubes containing 100 µl of TCA (60% w/v) and EDTA (2 mM) and centrifugated for 5 min at 12,000 x g at 25 °C.

In test tubes, 1 ml of TBA (0.67% w/v) in HCl (0.3 M) was mixed with 500 µl of supernatant. These tubes were incubated for 20 minutes in boiling water (95 °C). Then they were cooled and the absorbance was read at 532 nm in Shimadzu® spectrophotometer. Malondialdehyde (MDA) content was determined against a standard curve and results were expressed in µg of MDA g^{-1} of fresh tissue.

Color measurements were made daily during 12 days of refrigeration using a Minolta CR200 colorimeter (Minolta Co Ltd., Osaka, Japan), which presents an opening of 8 mm in diameter calibrated in a white reference plate. Measurements were made from three points of the fillet: front dorsal, middle dorsal and caudal dorsal areas, to obtain representative values of the appearance of color L*, a* and b*. In addition, Hue (H) and Chroma (C) chromatic parameters were calculated.

Statistical analysis

The design used for data analysis was a completely randomized model with repeated measurements of refrigeration over time (lipid-oxidation values and variation of color parameters).

$$Yijk = \mu + \alpha i + ßij + \gamma k + (\alpha\ \gamma)\ ik + \varepsilon ijk,$$

where:
Yijk = level of lipid-oxidation and color variation obtained for sample j subjected to treatment i in the cooling period
kµ = population mean
αi = Effect of the treatment
ißij = random effect of the sample (subject) j, assigned to the treatment i, this allows to test if there is a difference between treatments ignoring the cooling time effect
γk = cooling time effect k
(α γ) ik = interaction of the treatment i, in the cooling time k
εijk = is the error term that in the variances/covariance matrix represents two aspects: the covariance due to the fact that the measurements are made on the same sample, and the additional contribution to the covariance due to the proximity in the measurements

The model used allowed us to contrast the different antioxidants and the control group (without antioxidant). When the interaction of the antioxidant and the cooling time was significant (p <0.05), the comparison between antioxidants and the control group was made at each cooling time, when the interaction was not significant, this comparison was made in time average cooling. With the obtained results, the proposed model was evaluated, and the Tukey-Kramer test allowed comparison between the antioxidants and the control group. The data obtained were carried out with the statistical program SAS V.9.3.

Results

Lipid-oxidation

TBARS content was measured in rainbow trout marinated fillets (different antioxidants) preserved for six days in refrigeration. The measurements were made from the belly (Table 2) and NQC regions (Table 3).

Lipid-oxidation in the belly flap and NQC, showed significant interaction between treatments and refrigeration time. In general, TBARS values increased during the storage period under the different lipid-oxidation conditions.

Table 2. TBARS values (µg MDA g^{-1} of fresh tissue), obtained with equivalent dose and with 4% absorption of marinating in the belly flap region (average ± standard error of the mean)

Refrigeration time (d)	Antioxidants				
	Control	Quillay	Oregano	Romero	Synthetic
			Basal lipid-oxidation		
0	0.0755 ± 0.007	0.0719 ± 0.007	0.0827 ± 0.006	0.0611± 0.008	0.0680 ± 0.07
1	0.1222 ± 0.012	0.1186 ± 0.013	0.0970 ± 0.011	0.1114 ± 0.012	0.0755 ± 0.011
2	0.1617 ± 0.015	0.1437 ± 0.014	0.1150 ± 0.015	0.1042 ± 0.015	0.1078 ± 0.014
3	0.1941 ± 0.016^{a}	0.1905 ± 0.017^{a}	0.1402 ± 0.016^{ab}	0.1150 ± 0.015^{b}	0.1402 ± 0.015^{ab}
4	0.2767 ± 0.031^{a}	0.2480 ± 0.030^{ab}	0.2264 ± 0.029^{ab}	0.1473 ± 0.030^{b}	0.1330 ± 0.030^{b}
5	0.3055 ± 0.037^{a}	0.2552 ± 0.035^{ab}	0.2156 ± 0.036^{ab}	0.1468 ± 0.0032^{b}	0.1320 ± 0.036^{b}
6	0.1294 ± 0.040	0.2192 ± 0.038	0.1437 ± 0.41	0.1402 ± 0.043	0.0539 ± 0.042
			Lipid-oxidation at an incubation of 37 °C for 30 minutes		
0	0.1545 ± 0.006	0.1581 ± 0.008	0.1151 ± 0.007	0.0970 ± 0.007	0.1006 ± 0.008
1	0.3198 ± 0.011^{a}	0.2156 ± 0.012^{ab}	0.1545 ± 0.012^{ab}	0.1330 ± 0.013^{b}	0.1186 ± 0.012^{b}
2	0.5822 ± 0.014^{a}	0.3486 ± 0.012^{ab}	0.2120 ± 0.013^{b}	0.2192 ± 0.012^{b}	0.1150 ± 0.015^{b}
3	0.4708 ± 0.015^{a}	0.5462 ± 0.014^{a}	0.2480 ± 0.014^{b}	0.3162 ± 0.015^{ab}	0.1258 ± 0.015^{b}
4	0.6001 ± 0.030^{a}	0.5534 ± 0.032^{a}	0.4241 ± 0.032^{ab}	0.3701 ± 0.030^{ab}	0.1581 ± 0.030^{b}
5	0.8697 ± 0.035^{a}	0.7259 ± 0.034^{ab}	0.4456 ± 0.033^{bc}	0.3773 ± 0.33^{bc}	0.1366 ± 0.035^{c}
6	0.3306 ± 0.040	0.3773 ± 0.039	0.2839 ± 0.038	0.3162 ± 0.040	0.1437 ± 0.041
			Lipid-oxidation at an incubation of 37 °C for 30 minutes with Fe		
0	0.8733 ± 0.007^{a}	0.6037 ± 0.007^{b}	0.6289 ± 0.008^{ab}	0.2408 ± 0.007^{c}	0.1006 ± 0.007^{c}
1	1.4878 ± 0.011^{a}	1.4195 ± 0.013^{a}	0.6505 ± 0.012^{ab}	0.3773 ± 0.012^{b}	0.1689 ± 0.011^{b}
2	1.9226 ± 0.013^{a}	1.4626 ± 0.012^{ab}	0.7044 ± 0.012^{ab}	0.5786 ± 0.011^{ab}	0.1150 ± 0.012^{c}
3	1.7250 ± 0.015^{a}	1.4051 ± 0.014^{a}	0.7798 ± 0.014^{ab}	0.5780 ± 0.012^{b}	0.1186 ± 0.015^{b}
4	1.5489 ± 0.031^{a}	1.2686 ± 0.030^{ab}	1.0350 ± 0.032^{ab}	0.6576 ± 0.031^{bc}	0.1725 ± 0.030^{c}
5	1.7322 ± 0.034^{a}	1.5668 ± 0.033^{ab}	0.8589 ± 0.032^{bc}	0.4816 ± 0.32^{bc}	0.2444 ± 0.033^{c}
6	0.7331 ± 0.038	0.6612 ± 0.041	0.4744 ± 0.040	0.3450 ± 0.41	0.2623 ± 0.040

^{a,b,c} Different letters within each row denotes significant difference to the Tukey test $p < 0.05$). Each result is given as the mean value ± standard error of the mean, and corresponds to the chemical analyses in duplicate of 5 fillets of each treatment.

In basal conditions induced by temperature and induced by temperature plus Fe lipid-oxidation in belly region, the maximum TBARS values were recorded on the fifth day of refrigeration and the control treatment had the highest lipid-oxidation.

In the basal lipid-oxidation, since the third day, synthetic antioxidant and rosemary extract protected fillets (belly region) from oxidation compared with control treatment. TBARS content under inducted by temperature lipid-oxidation were higher in control and quillaia extract respect to the treatment with synthetic antioxidant. It should be noted that the treatment with synthetic antioxidant had low lipid-oxidation values, similar to the TBARS observed in the basal condition.

In lipid-oxidation induced by incubation with temperature plus Fe, since day zero, control treatment presented the highest TBARS content with respect to the extracts of quillaia ($p = 0.0496$), rosemary and synthetic antioxidant and it was maintained high until the fifth day of refrigeration. The treatment with synthetic antioxidant, presented low values of lipid-oxidation compared to the other treatments. It was lower from the beginning until the fifth day of storage. On day six, all treatments did not have any difference with regard to TBARS contents.

Table 3. TBARS values (µg MDA g^{-1} of tissue) obtained with equivalent dose and with 4% absorption of marinade measured in the NQC region (average ± standard error of the mean)

Refrigeration time (d)	Antioxidants				
	Control	Quillay	Oregano	Romero	Synthetic
		Basal lipid-oxidation			
0	0.0647 ± 0.003^{ab}	0.0719 ± 0.004^{a}	0.0722 ± 0.003^{a}	0.0575 ± 0.03^{b}	0.0649 ± 0.004^{ab}
1	0.1078 ± 0.011	0.1222 ± 0.010	0.1294 ± 0.012	0.0970 ± 0.011	0.0975 ± 0.010
2	0.2120 ± 0.025^{a}	0.1725 ± 0.024^{ab}	0.1330 ± 0.025^{ab}	0.1617 ± 0.024^{ab}	0.0755 ± 0.024^{b}
3	0.3019 ± 0.043^{a}	0.2552 ± 0.040^{ab}	0.2336 ± 0.042^{ab}	0.2048 ± 0.044^{ab}	0.1222 ± 0.043^{b}
4	0.4564 ± 0.066	0.3989 ± 0.065	0.2264 ± 0.065	0.2270 ± 0.066	0.1186 ± 0.065
5	0.4564 ± 0.083	0.3989 ±0.080	0.2444 ± 0.082	0.2911 ± 0.083	0.1473 ± 0.082
6	0.6828 ± 0.121^{a}	0.5247 ± 0.122^{ab}	0.4528 ± 0.121^{ab}	0.3126 ± 0.121^{ab}	0.1509 ± 0.120^{b}
		Lipid-oxidation at an incubation of 37 °C for 30 minutes			
0	0.1581 ± 0.077	0.1653 ± 0.080	0.3162 ± 0.078	0.1006 ± 0.081	0.0934 ± 0.077
1	0.2623 ± 0.058	0.3091 ± 0.065	0.2336 ± 0.060	0.1545 ± 0.062	0.1114 ± 0.060
2	0.3666 ± 0.131	0.4456 ± 0.141	0.3594 ± 0.138	0.1509 ± 0.133	0.1186 ± 0.131
3	0.4564 ± 0.064^{a}	0.4061 ± 0.060^{a}	0.4780 ± 0.064^{a}	0.2192 ± 0.067^{ab}	0.1006 ± 0.065^{b}
4	0.6792 ± 0.118^{a}	0.6001 ± 0.120^{ab}	0.4384 ± 0.116^{ab}	0.4205 ± 0.118^{ab}	0.1105 ± 0.117^{b}
5	1.0853 ± 0.187^{a}	0.9523 ± 0.179^{ab}	0.4395 ± 0.188^{ab}	0.4600 ± 0.178^{ab}	0.2300 ± 0.180^{b}
6	1.5848 ± 0.245^{a}	1.2542 ± 0.234^{ab}	0.8230 ± 0.243^{ab}	0.5139 ± 0.244^{b}	0.3666 ± 0.230^{b}
		Lipid-oxidation at an incubation of 37 °C for 30 minutes with Fe			
0	1.1069 ± 0.222^{a}	0.7978 ± 0.218^{abc}	1.0889 ± 0.217^{ab}	0.1294 ± 0.221^{c}	0.1545 ± 0.222^{bc}
1	1.2937 ± 0.217^{a}	0.9739 ± 0.217^{ab}	0.9559 ± 0.220^{ab}	0.1761 ± 0.220^{b}	0.1042 ± 0.217^{b}
2	1.7357 ± 0.266^{a}	1.1536 ± 0.270^{ab}	0.9236 ± 0.255^{ab}	0.2803 ± 0.271^{b}	0.1007 ± 0.265^{b}
3	1.4950 ± 0.183^{a}	1.4123 ± 0.180^{a}	1.1823 ± 0.0180^{ab}	0.5067 ± 0.0178^{bc}	0.1222 ± 0.183^{c}
4	1.5956 ± 0.181^{a}	1.5165 ± 0.184^{ab}	1.1572 ± 0.185^{ab}	0.7655 ± 0.180^{bc}	0.1473 ± 0.182^{c}
5	1.7250 ± 0.291	1.3620 ± 0.299	1.3045 ± 0.290	0.9775 ± 0.288	0.5211 ± 0.290^{b}
6	2.8642 ± 0.417^{a}	1.7789 ± 0.423^{ab}	2.1670 ± 0.420^{ab}	1.0062 ± 0.418^{b}	0.6001 ± 0.415^{b}

^{a,b,c} Different letters within each row denotes significant difference to the Tukey test ($p < 0.05$). Each result is given as the mean value ± standard error of the mean, and corresponds to the chemical analyses in duplicate of 5 fillets of each treatment.

Unlike results from the belly region, TBARS content in NQC cut gradually increased until the sixth day of refrigeration and control treatment had the highest values in all of the different lipid-oxidation conditions (Table 3). The initial TBARS content (day zero), in basal condition of lipid-oxidation, was lower in the treatment with rosemary extract compared to quillaia and oregano extracts.

By inducing lipid-oxidation with temperature, the TBARS content increased approximately twice the amounts observed in the basal condition. They were no difference until day 3. From that day, it was observed that the control group had higher values of lipid-oxidation with respect to the treatment with synthetic antioxidant. It was also observed that the control group had the highest level of lipid-oxidation in comparison to rosemary extract and synthetic antioxidant at day six of refrigeration.

Regarding lipid-oxidation with temperature plus Fe, it was observed that the control group, had a higher lipid-oxidation value compared to the treatment with synthetic antioxidant. At day six of refrigeration, the maximum lipid-oxidation values were recorded, with the control treatment being greater with respect to rosemary extract and synthetic antioxidant.

TBARS content in the NQC area of rainbow trout fillets under different lipid-oxidation conditions were increased until the sixth day of storage. On the other hand, lipid-oxidation levels decreased from higher to lower according to the following order of treatments: control, quillaia, oregano, rosemary and synthetic antioxidant, respectively.

Color measurements

The variation of the color parameters: luminosity (L*), red (a*), yellow (b*), Hue (tone) and Chroma (saturation) were recorded during the first twelve days of refrigeration (Figs. 1 a, b, c, d, e).

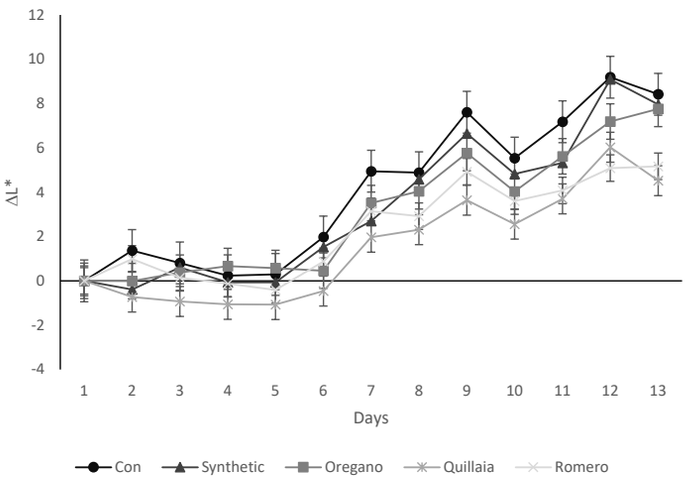

Fig. 1a. Variation of luminosity (L*) among 12 days of refrigeration at 4 °C

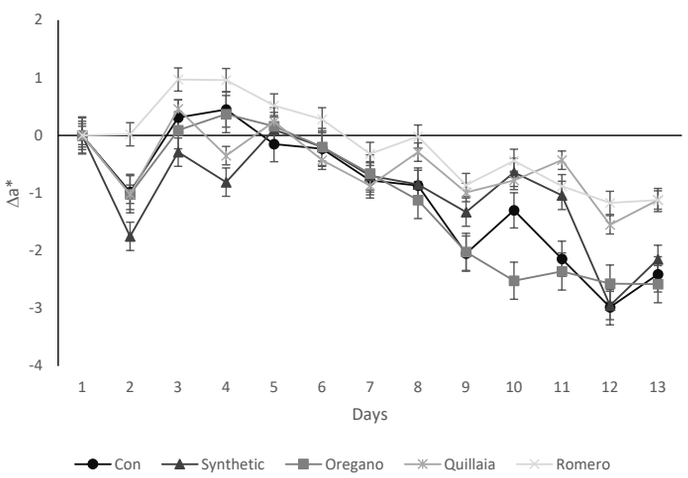

Fig. 1b. Variation of redness (a*) among 12 days of refrigeration at 4 °C

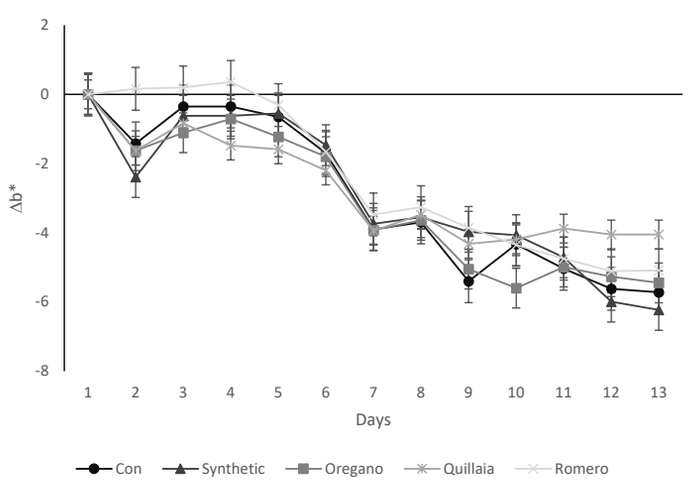

Fig. 1c. Variation of yellowness (b*) among 12 days of refrigeration at 4 °C

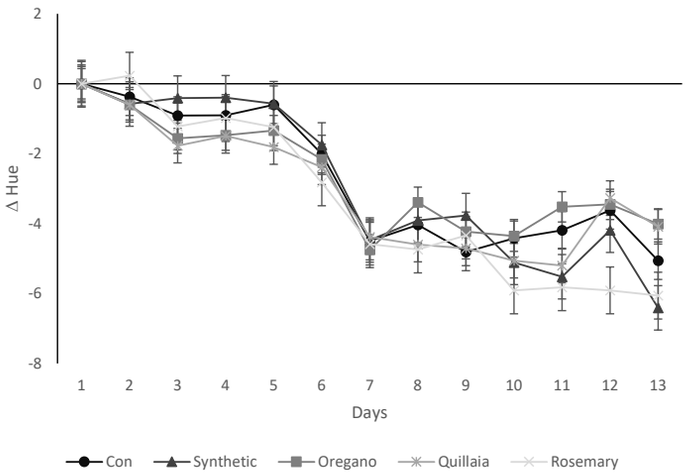

Fig. 1d. Variation of Hue (tone) parameter among 12 days of refrigeration at 4 °C

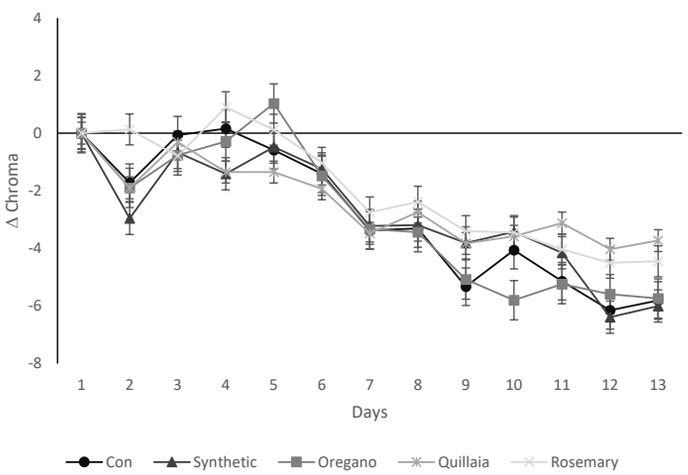

Fig. 1e. Variation of Chroma (color saturation) parameter among 12 days of refrigeration at 4 °C

In the present study, the determination of the variation of color (Δ) was made with respect to the average initial value of fillets, prior to marinating. The change in color did not show significant interaction between the treatments and the cooling time, so the comparison between treatments was made through the marginal means. When analyzing the color characterization, a significant difference was observed in ΔL*, Δa* and ΔChroma (Table 4).

Table 4. Values L, a *, b *, Hue, Chroma, in rainbow trout fillets (*Orcorhynchus mykiss*) marinated with different antioxidants in equivalent dose (4% absorption), and subsequent cooling between 0 and 12 days

	Antioxidant				
	Control	Synthetic	Oregano	Quillaia	Rosemary
ΔL* Luminosity					
Media	4.37^{a}	3.56^{ab}	3.33^{ab}	1.71^{c}	2.53^{bc}
Δa * (red)					
Media	-1.10^{ab}	-1.07^{ab}	-1.23^{a}	-0.63^{ab}	-0.17^{b}
Δ b * (yellow)					
Media	-3.17	-3.20	-3.37	-2.97	-2.60
Δ Hue					
Media	-2.95	-3.09	-2.90	-3.28	-3.62
Δ C* (Chroma)					
Media	-3.07^{ab}	-3.08^{ab}	-3.31^{a}	-2.61^{ab}	-2.01^{b}

^{a,b,c} Different letters within each row denotes significant difference to the Tukey test ($p < 0.05$). Each result is given as the mean value and corresponds to the chemical analyses in duplicate of 5 fillets of each treatment.

With regard to luminosity, control treatment presented the bigger color variation with respect to rosemary and quillaia extracts. Among treatments, the quillaia extract showed the lowest variation compared to oregano, synthetic antioxidant and control group (Fig. 1a).

The maximum average value of Δa* (red) found corresponds to the treatment with oregano extract, being lower in comparison to rosemary extract, which presented the lowest value between treatments. On the other hand, quillaia extract presented similar variations to rosemary extract, and was lower compared to the other treatments, not presenting a significant difference (Fig. 1b).

In the same way that Δa*, in comparison to the values obtained for ΔChroma, the treatment with oregano extract presented greater variation when compared to rosemary extract. In addition, treatment with quillaia extract (as well as rosemary extract) showed less variation of Chroma compared to treatments with oregano extract, synthetic antioxidant and control group, which did not represent a significant difference.

Discussion

In this study, lipid-oxidation in different conditions (basal, temperature and temperature plus Fe) increased progressively with regard the storage time. With a 4% absorption of antioxidant-marinated solution, the maximum values of lipid-oxidation were found at days 5 and 6 of cooled storage in the belly flap and NQC, respectively.

In basal lipid-oxidation, in both belly flap and NQC cut, maximum TBARS content was observed in the control group on the fifth and sixth day of refrigeration respectively, being lower in treatment with synthetic antioxidant. Furthermore, in the belly region, when the samples were incubated at 37 °C, the control group presented a higher value of TBARS compared to the synthetic antioxidant on the fifth day of refrigeration. These low levels of lipid-oxidation (TBARS), observed in the synthetic antioxidant, agreed with the data reported by Weilmeier and Regenstein (2004) when comparing the antioxidant action of different synthetic compounds, determined that the butyl hydroxytoluene (BHT) and citric acid applied by injection in horse mackerel fillets and lake trout, in a concentration of 0.5% w/w of marinade, maintains the permitted levels of TBARS until days five and ten of refrigeration, respectively.

In rainbow trout fillets under refrigeration conditions, little research has been done on the application of natural extracts as antioxidants in fillets, so little is known about the possible changes in fillets. In the present study, lipid-oxidation levels increased until the fifth and sixth day of refrigeration, then tended to be constant as reported by Tironi et al. (2007). The values of TBARS in basal lipid-oxidation condition of the rosemary extract and synthetic antioxidant treatments were lower compared to the control group in the belly area on the fifth day of refrigeration. This could be due to the fact that secondary lipid-oxidation products, such as malonaldehyde (MDA), would not have formed in the presence of rosemary extract (Tironi et al. 2009) because it contains in its chemical structure highly antioxidant compounds such as phenolic diterpenes (carnosic acid and carnosol) and other phenolic acids (rosmarinic acid, carvacrol, caffeic acid) (Al-Bandak and Oreopoulous 2007). These phenolic compounds have the ability to capture or inhibit the formation of free radicals by donating hydrogens, avoiding the formation of primary compounds such as radical peroxides or the degradation of hydroperoxide radicals (Kykkidou et al. 2009), diminishing lipid-oxidation.

The tendency to present low values of lipid-oxidation observed in the rosemary and oregano extracts agrees with that reported by Tironi et al. (2009), who established that the application of 200 ppm of rosemary extract in rainbow trout fillets maintains TBARS content low on the eighth day of refrigeration compared to the fillets without antioxidants, which increased on the fourth day of refrigeration. Regarding oregano extract, Frangos et al. (2010), determined that its application in rainbow trout fillets sealed in vacuum, increases the sensory shelf life up to nine days of refrigeration, however, when evaluating the levels of TBARS in the present study, to day six they decrease in the belly area, independent of antioxidant treatment.

Under similar conditions of basal lipid-oxidation, different tests have been conducted on different fish species, applying the combination of different packaging methodologies with extracts of oregano and rosemary. Rosemary extract in combination with ascorbic acid applied in Atlantic salmon fillets (*Salmo salar*) and packed in modified atmospheres under different lighting conditions, maintained low levels of oxidative rancidity (TBARS) and improved the quality of the meat during eight days of refrigeration (Giménez et al. 2005). On the other hand, the isolated application of rosemary compared to ascorbic acid, showed an antioxidant action and prolonged the shelf life in fillets of sea bream (*Sparus aurata*) (Giménez et al. 2004). Additionally, it has been reported that the application

of polyphenols such as carvacrol and thymol extracted from rosemary and oregano in carp fillets improves the shelf life to eight days of refrigeration at 5 °C (Mahmoud et al. 2004).

Previous work in poultry meat (Fellenberg et al. 2011), showed that quillaia extract is effective against lipid oxidation when it is applied by injection in marinade brine. In this study, the treatment with quillaia extract presented values close to the control group in the different lipid-oxidation conditions. Probably due to the fact that part of its polyphenolic content, such as quercetin and rutin (Kauffmann et al. 2004) are not evenly distributed through the sarcolemma, due to the formation of soluble and insoluble compounds with proteins (Tang et al. 2001). This protein-polyphenol interaction could have a masking effect on the antioxidant activity related to the capture of free radicals by polyphenols (Arts et al. 2002). Probably immersion is not a suitable way to distribute the antioxidant through the trout fillet. More research is required to determine if quillaia extract applied by injection in trout fillets has better performance as in poultry meat.

In lipid-oxidation induced by temperature both belly flap and NQC cut, marinated with natural antioxidants presented approximately twice the TBARS contents than basal lipid-oxidation. This could be due to a catalyzing process in the reduction oxide reactions according to the Arrhenius kinetics (Jacobson et al. 2008), which causes a considerable decrease in the induction lipid-oxidation. Furthermore, depending on the type of lipid substrate, the temperature increases the rate of formation and isomerization of the hydroperoxides formed from the rearrangement of peroxy radicals. These free radicals are formed from the beta cleavage of the pentadienyl radicals incubated between 50–60 °C. Therefore, the formation rate of volatile, polar and polymeric secondary compounds (Eldin et al. 2003) would increase and consequently, fillet shelf life would be reduced (Jacobson et al. 2008).

On other hand, it has been reported that the temperature affects the reactions of peroxy radical formation, isomerization and decomposition of hydroperoxides by forming reactive lipid-oxidation complexes (Vinagre et al. 2011). Eldin et al. (2003) and Vinagre et al. (2011) determined that the thermal treatment process produces catalytic changes at the structural level of the hemoproteins and inhibits the endogenous antioxidant enzymes. In this study, temperature remain constant with a relative high temperature. Also, we used an incubation temperature of 37 °C which was as challenging as adding Fe.

In salmonids, such as rainbow trout, muscle levels of hemoglobin are found in greater quantity than myoglobin, therefore, an increase in temperature could affect it structurally, which would constitute a prooxidant factor at the muscle level (Richards et al. 2005). Therefore, in the present study, the temperature incubation would decrease the levels of endogenous muscle antioxidants, such as astaxanthin and vitamin E, affecting lipid-oxidation levels (Giménez et al. 2005, Ortiz et al. 2008).

This may be because when the incubation temperature increases, the conditions become more prooxidant, and therefore the muscle spends its endogenous antioxidants.

As in the basal condition, the synthetic antioxidant treatment presented the lowest values of lipid-oxidation from the fourth day of refrigeration in belly flap and NQC cut of the fillet. The above agrees with Pazos et al. (2006) who reported that the application of the synthetic antioxidant propyl gallate (100 ppm) compared to the natural polyphenol hydroxytyrosol (50 ppm) in atlantic horse mackerel fillets post thermal incubation, maintains low values of formation of secondary oxidation products up to 21 days of refrigeration (0 °C).

Similar to our study, Tokur and Korkmaz (2007), reported that under conditions of oxidation catalyzed by iron (Fe^{2+}), observed that in both areas of the fillet, the lipid-oxidation levels of the different treatments increased in relation to the cooling time until day five (belly) and six (NQC) of refrigeration. The addition of ferrous ion, in synergy with thermal treatment and in the presence of hydrogen peroxide through the Fenton reaction could increase the formation of highly oxidizable compounds such as hydroxyradicals, which decompose form volatile compounds such as aldehydes and ketones (Eldin et al. 2003, Maestre et al. 2009).

The antioxidant protection of oregano extract, rosemary extract and synthetic antioxidant in comparison to the control treatment could be associated to its capacity to donate electrons and its content of phenolic compounds. The effective antioxidant capacity observed in both rosemary extract and oregano compared to control treatment could be due to the number of hydroxyl groups in the ortho position of phenolic compounds such as caffeic acid (Fellenberg 2013) and rosmarinic acid in both extracts and carnosic acid, carnosol in rosemary extract (Yanishlieva et al. 2006). Similarly, other polyphenols derived from hydroxycinnamic acid, such as ferulic acid and cumaric acid contained in oregano extract (Yanishlieva et al. 2006) and rosemary (Pokorný et al. 2007), presented similar

values of TBARS compared to propyl gallate to be incorporated by immersion in minced meat of dark muscle (high hemoglobin content) of atlantic jack mackerel. On the other hand, Pazos et al. (2006) and Maestre et al. (2009), determined that polyphenolic compounds present a directly significant correlation between the ability to donate electrons and their inhibitory action as an antioxidant under conditions of lipid-oxidation catalyzed by hemoglobin in Atlantic horse mackerel fillets.

With respect to the effective antioxidant action presented by the synthetic antioxidant, it could be due to the fact that it possesses a better capacity of absorption and penetration related to its hydrophilic nature with certain membrane transport proteins at the superficial level of the fillet in conditions of refrigeration (Ortiz et al. 2008). However, other studies, conducted by Medina et al. (2009) and Pazos et al. (2006) determined that a higher percentage of incorporation of synthetic polyphenol propyl gallate of lipophilic nature, at the level of the microsomal membrane in hake muscle produces lower lipid-oxidation (TBARS) when compared with the incorporation of natural hydrophilic polyphenols such as proanthocyanidins under conditions of oxidation with ferrous ion and in enzymatic and non-enzymatic processes. Knowing the molecular and electronic structure of polyphenols allows us to understand their antioxidant activity (Fellenberg 2013). In the same way, more research is required regarding polyphenols of the different plant extracts, to better understand and enhance their mechanism of antioxidant action.

On the other hand, in salmonids, meat color is an important quality parameter (Dawson et al. 2018). Nowadays, the color may be determinate through color parameters that has been standardized as: luminosity (L*), a* (red), b* (yellow), hue (Hue) and saturation (Chroma) (Dawson et al. 2018). In salmonids, research regarding color has been oriented mainly to define the optimal levels of carotenoids intake such as astaxanthin which is related to the parameter a* at muscular level (Schubring 2009). In this way, it has been established that the standardized values for salmonid meat of the parameters: a* vary from 5 to 26; for b* 7–31; and L* from 35 to 63 (Schubring 2009).

In the present study, ΔL* values were increased with respect to the cooling time, which agrees with that reported by Schubring (2009). Treatment with quillaia extract presented the best stability of ΔL*, Δa*, and ΔChroma, with respect to the other treatments. This could be due to the fact that part of their polyphenolic content, such as dihydroquercetin and rutin (Kauffmann et al. 2004) when found in glycosylated form, would be protecting the initial loss of color at the surface level of the fillets. In food emulsion systems, San Martín and Briones (1999), reported that quillaia phenolic glycosides prevent the gradual surface loss of color in suspended foods. Another explanation may be that quillaia extract did not move through the tissues and remained mainly at fillet surface protecting it. More research is necessary to understand this mechanism better.

Likewise, Robb et al. (2000) determined that the changes in L* values are related to the water holding capacity and storage temperature (Ozbay et al. 2006) due to the denaturation of the protein matrix of the meat, which would produce changes in the refraction of light from the surface of the fillets, causing non-uniformity in the pigmentation of salmonid meat (Ozbay et al. 2006). In frozen fillets of rainbow trout, it has been reported that lipid-oxidation leads to the deterioration of phospholipids at the level of cell membranes, which, when reacting with muscle proteins such as actinomyosin, produces a cross-linking, generating a decrease in protein solubility and gradual loss of astaxanthin and fillet translucency, being the main cause of color loss (Ozbay et al. 2006, Tironi et al. 2009).

In this study, it should be noted that the a* parameter showed a tendency to decrease as the cooling time advanced. As reported by Ozbay et al. (2006) lipid-oxidation and the rate of protein denaturation correlates with the increase in L* and the decrease in a*. They observed that color loss was mainly due to the loss of translucency. The lower value of Δa* obtained for the rosemary extract treatment agrees with that reported by Giménez et al. (2005), who determined that the value of parameter a* in atlantic salmon fillets tends to decrease over time, independent of storage conditions, and that the previous application of rosemary extract in the fillets, produces a protective effect of this parameter with respect to ascorbic acid. Antioxidant compounds, such as polyphenols contained in rosemary extract (Pokorný et al. 2007), would inhibit pigment loss such as astaxanthin through the regeneration and partial stabilization of carotenoid radicals caused by lipid-oxidation associated with pro-oxidant factors such as the presence of iron ions, temperature increase and storage light (Schubring et al. 2009). Previously, Akhtar et al. (1998) determined the protective effect of rosemary extract on color retention and lipid stability in rainbow trout meat stored at 4 °C. The decrease observed in a*, can be related to the whitening of the carotenoids as a consequence of its action as an antioxidant (Giménez et al. 2005). Similarly, Tironi et al. (2009) determined that the application of rosemary extract in Atlantic salmon muscle slightly decreased the values of a* during the first four days of storage in refrigeration and in freezing until three months of storage. As observed in the present study, the application of natural extracts produced a partial preservation of the red color.

Another possible cause in the gradual loss of the value of a*, includes the conversion of oxy and deoxy hemoglobin to meta hemoglobin (Schubring 2009).

In relation to ΔChroma, a downward trend was observed with respect to the cooling time, observing a greater stability during the first five days of refrigeration. However, significant differences were observed in the total average variation of the treatments, where rosemary extract was low if compares to oregano extract. The decrease of ΔChroma showed an increase in the opacity of the fillet as the cooling time advanced, possibly due to the loss of translucency due to muscle protein denaturation, which would affect the post-mortem color reading (Erikson and Misimi 2008). On the other hand, in previous trials (unpublished data), the value of Chroma and Hue constituted a reflection of the behavior of the parameters a* and b*, the decrease of this parameter being the reflection of the gradual loss of the astaxanthin pigment contained in the muscle of salmonids (Ortiz et al. 2008).

In summary, the data obtained shows a variation in the color parameters as the cooling time advanced. The brightness and red color increased and decreased respectively as the cooling time progressed, and the treatments with quillaia and rosemary extracts presented the best stability in the determination of the color.

Future studies should consider evaluating the antioxidant effect of the different treatments (used in this study) in other types of application to the fillet marinade such as glaze, spray and injection. Also, consider performing microbiological analyzes to evaluate if the different treatments have a bactericidal or bacteriostatic effect. Lastly, consider evaluating the protein oxidation associated with lipid oxidation through the determination of tertiary products of oxidative rancidity.

Conclusions

In this study, the antioxidant action in the different lipid-oxidation conditions vary effectively in the following way: Synthetic> Rosemary> Oregano> Quillaia> Control. Overall, the use of natural antioxidants in marinade such as oregano, rosemary and quillaia extracts improves oxidative stability at the level of lipid content and color in rainbow trout fillets by inhibiting the lipid-oxidation process and extending the shelf life to six days of refrigeration.

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