Research Article

Lipid and sensory quality of canned Atlantic salmon (*Salmo salar*): Effect of the use of different seaweed extracts as covering liquids

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The addition of natural compounds as additives in fish products is increasingly important to prevent or delay their deterioration. Nowadays, most of the additives used on seafood are synthetic, and their safety is being increasingly questioned. The aim of this research was to compare the effects of the addition of different seaweeds extracts on lipid and sensory quality parameters of canned Atlantic salmon (*Salmo salar*) muscle. For this purpose, four different seaweeds extracts were tested: cochayuyo, sea lettuce, ulte, and red luche as covering liquids against a standard, without seaweed extract. For each sampling day, three cans from each treatment were analyzed periodically, up to reach 140 days of storage at 40°C. The parameters that were measured are: fatty acids content (saturated, monounsaturated, and polyunsaturated), polyene index (PI), peroxide value (PV), *p*-anisidine value (pAV), astaxanthin content (AX), total tocopherols content, total volatile basic nitrogen (TVB-N), and sensory indicators of "characteristic flavor" and "rancid odor." All chemical parameters measured (fatty acids, PI, PV, pAV, AX, total tocopherols, and TVB-N) showed significant differences between all treatments and throughout storage time. Sensory parameters were not significantly different between canned salmons packed with different covering liquids, and they were always within acceptable limits.

Practical applications: The results obtained in this research show the possibility of the use of seaweeds as an alternative source of natural antioxidants in fatty fish canning. Next studies on the use of seaweeds to help in fish and seafood preservation should be focused on the use of mixtures of seaweeds as protective extracts. The results indicate that it is possible to obtain advantages in the preservation of canned salmon, based on the use of some seaweed extracts as covering liquid, which can help inhibit lipid peroxidation.

Keywords: Canned storage / Covering liquid / Lipid quality / Salmon / Seaweed

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Abbreviations: AX, astaxanthin; **pAV,** *p*-anisidine value; **PI,** polyene index; **PV,** peroxide value; **TVB-N,** total volatile basic nitrogen

1 Introduction

Canning of marine foods is a process that has been largely used in several countries in order to extend shelf life of fish and seafoods; however, the multistep nature of this process, and the use of pretreatments such chilling, freezing, cooking until sterilization, and canned storage can lead to the formation of several substances that may produce losses on quality and breakdown of beneficial nutrients in the final product [1].

At present, farming of salmonids has a crucial role in worldwide economy; and Chile is one of the major players in the market of these fish species [2, 3]. Canned salmon is one of the major fish products in countries like UK and USA. The salmon canning industry must have strategies to keep their products safe, healthy, convenient, and tasty during their shelf life time [4]. Difficulties in exporting of fresh and chilled salmon allow that a good quality canned salmon could become an attractive alternative to consider for export to distant markets, if it is handled in an efficient way.

Freshness is an important component of the quality of fish and seafoods: physical, microbial, biochemical, and sensory attributes are parameters that conform, and help to determine shelf life of these products [5]. Lipid peroxidation is an important factor in determining flavor and quality of muscle foods, and also allows deciding if their meat is suitable for eating [6]. A problem associated with lipid peroxidation is the appearance of rancid flavors and odors. This susceptibility is due to the deterioration of PUFA present in salmon lipids, particularly omega-3 [7].

Determinations of lipid peroxidation indices in muscle foods by chemical methods are based on the generation of primary, secondary, and tertiary products resulting from the reaction between unsaturated fatty acids present in this muscle with molecular oxygen, by means of free radical mechanisms [8]. In seafood industry, there is a great interest in developing effective methods to evaluate fish freshness by quantification of secondary volatile compounds from lipid peroxidation, which are the major contributors to the development of oxidized off-flavors and odors: they are good indicators that allow showing the rancidity degree of marine products [9].

Adipose tissue of the flesh of salmonids is known for their important content of omega-3 long chain-PUFA (ω3 LC-PUFAs): eicosapentaenoic acid (C20:5, EPA), and DHA (C22:6), which are effective in the prevention and treatment of various disorders on human health, such as cardiovascular disease, neurodegenerative diseases, cancer, inflammatory bowel disease, rheumatoid arthritis, and ischemia [10]. However, these fatty acids are very susceptible to lipid peroxidation due to the high number of double bonds contained in its molecules: to help prevent lipid peroxidation, synthetic and natural antioxidants are widely applied in fish factories; as the use of synthetic antioxidants in foods is being restricted due to its effects on human health – and so it is believed that in the future its use will be banned in some countries – the use natural antioxidants is gaining importance in many food industries [11, 12]. Also, the interest of consumers by eating foods with small amounts of artificial additives has led to an increase in the demand for natural compounds and spices that have antioxidant properties; therefore, in these days there is a strong trend to replace synthetics antioxidants with natural ones in meats and seafoods [13–17].

Seaweeds are becoming an important food ingredient in many coastal countries: they are used as source of bioactive nutritional compounds like dietary fibers, phospholipids, EFA, glycolipids, phytosterols, pigments, mannitol, iodine, tocols, and antioxidants, among others. Also, from algae are obtained substances, which are used as ingredients for the food industries, especially polysaccharides with hydrocolloid action, such as agar-agar, alginates, carrageenans, etc. Species such as *Durvillaea antarctica* (*cochayuyo*/*ulte*), *Pyropia columbina* (red *luche*), *Ulva lactuca* (sea lettuce), *Macrocystis piryfera* (*huiro*), and *Gracilaria chilensis* (*pelillo*) are of great importance for Chilean economy and gastronomy [18–20]. Numerous studies had shown that several kinds of seaweeds and their byproducts have minor compounds with antioxidant activity against lipid peroxidation [21–24]. *Cochayuyo*, *ulte*, sea lettuce, and red *luche* are very popular seaweeds in Chilean recipes for direct human consumption and this together with their high availability in Chilean coasts were the reasons why we chose them to perform the study.

The aim of this research was to evaluate the effect of the addition of different seaweed extracts on lipid quality, antioxidants, and selected sensory parameters of canned Atlantic salmon (*Salmo salar* Linnaeus), due to this effect to prolong the lipid quality of canned fish has not been reported until now.

2 Materials and methods

2.1 Raw material and processing of canned salmon

Atlantic salmon (*S. salar* Linnaeus) fish (ca. 2500 g weight, 50–65 cm length), were obtained from Puerto Montt (Región de los Lagos, Chile). The day after its capture, they were withdrawn, sacrificed by a sharp blow to the head, the gills cut and bled in a water-ice mixture, headed, gutted, and filleted. Then, fish were frozen at −40°C in a tunnel freezer, and packaged in individual low-density polyethylene bags, these including hermetic sealing, after that, they were transported in boxes with ice at our laboratory (Universidad de Chile, Santiago, Chile).

Samples of homogenized salmon muscle without skin (80 ± 0.1 g), were placed in epoxyphenolic enamelled cans (211×108). Then, they were precooked in a bath of boiling water for 20 min before adding 30 mL of the respective covering liquid. The filling media were added at 90°C. The cans were sealed in a manual sealer (Dixie Canner Co., Athens, GA, USA) and sterilized in a vertical retort (Küster, Berlin, Germany: 121.1°C, 30 min; $F_0 = 6$ min). Cans were placed at 40°C in a temperature controlled chamber, in order to accelerate the occurrence of lipid changes. Samples of canned salmon were evaluated for analysis on Days 0, 8, 60, 115, and 170 of storage at 40°C. From each treatment under study, three different cans were analyzed independently at each sampling time ($n = 3$). The standardized Norwegian Quality Cut (NQC) was used for analyses; this cut is defined as the region of fish from the end of the dorsal fin to the anus [25, 26].

According to the objectives of this research, five covering liquids were tested: a control, made with distilled water (CTR) and aqueous extracts of *cochayuyo* (CYO, frond of *D. antartica*), sea lettuce (SLC, *U. lactuca*), *ulte* (ULT, basal part of *D. antartica*), and red *luche* (RLC, *P. columbina*) in order to carry out a comparative study of the antioxidative performance of these media throughout the storage time on the canned salmon quality, by means of the evaluation of selected lipid, biochemical, and sensory parameters. All natural extracts were prepared with 500 g of each seaweed in 2 L of distilled water. Then, the mixtures were boiled for 3 h, filtered and packed in sealed glass bottles that were refrigerated at 4°C until its use.

2.2 Nutritional characterization of raw material

Moisture in samples were determined by drying to constant weight at 105 ± 2°C, according with 950.46B official method; ash was determined at 550°C by AOAC 920.153 method [27] and total protein ($N \times 6.25$) was determined by Kjeldahl method 928.08, alternative II [27]. Total carbohydrates were estimated by rounding up. In all cases, results were expressed as (g/100 g muscle).

2.3 Lipid composition analysis in canned salmon

The lipid fraction was extracted from the fish muscle by the Bligh and Dyer method [28]. Quantification results are expressed as (g lipid/100 g muscle).

Lipid extracts from the fish white muscle were converted into FAME by employing sodium methylate and analyzed by GC (Hewlett-Packard 5860 serie 2 chromatograph, Bellefonte, PA, USA) employing a fused silica capillary column BPX-70 (0.25 mm id $\times$ 50 m, 0.25 lm film, J&W Scientific, San Francisco, CA, USA), according to a modification of AOCS method Ce 1-62 [29]. A temperature gradient of 2°C/min between 150–240°C was used. A FID set at 240°C and H_2 as carrier gas were used. Peaks were identified by comparison of their retention times with standard FAME mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peaks were automatically solved with the Clarity-Chromatography SW (DataApex-2005) software for integration of the peaks areas. Polyene index (PI) was calculated as the following fatty acid ratio: PI = C20:5 + C22:6/C16:0 [30].

2.4 Lipid peroxidation assessment

The peroxide value (PV) was determined on the lipid extract by the Cd 8-53 iodometric method [29]. The results are expressed as mEq active oxygen/kg lipid.

The *p*-anisidine value (pAV) was determined in fish muscle according to the Cd 18-90 method, based on the reaction between α- and β-unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent [29]. Results are expressed as 100 times the absorbance measured at 350 nm in a 1 cm path length cuvette from a solution containing 10 g lipid/L reaction medium.

2.5 Analyses of antioxidants

Total tocopherols were determined in the lipid extracts by HPLC with fluorescence detection, following the standard method Ce 8-89 [29]. A LichroCART Superspher Si 60 column (25 cm $\times$ 4 mm id, particle size 5 μm; Merck, Darmstadt, Germany) was used. The mobile phase was propan-2-ol in *n*-hexane (0.5:99.5 v/v) at a flow rate of 1 mL/min. The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck, Darmstadt, Germany), a Rheodyne 7725i injector with 20 μL sample loop, a Merck-Hitachi F-1050 fluorescence detector equipped with Clarity-Chromatography SW (DataApex-2005) software for integration of the peaks areas. Peaks were detected at 290 and 330 nm, excitation and emission wavelengths, respectively. Tocopherols were identified using external standards (Merck). Results were expressed as (mg tocopherol/kg lipid muscle).

Astaxanthin (AX) content was measured according to Sheehan et al. [31] method. AX from salmon muscle was extracted with acetone. The combined extracts were dried under nitrogen flux and dissolved in the mobile phase, which consisted of 20% ethyl acetate and 80% methanol/water (9: 1) HPLC separation of the samples was carried out on a Nucleosil 5 C18 (25 cm $\times$ 4 cm id) reverse-phase column; detection was carried out at 470 nm. Results were expressed as (mg AX/kg salmon muscle).

2.6 Total volatile basic nitrogen determination

Total volatile basic nitrogen (TVB-N) was determined according to a trichloroacetic precipitation method [32] modified by Ortiz et al. [3], based on the precipitation of salmon muscle proteins with a solution 5 g/100 mL of trichloroacetic acid (CCl_3COOH; Merck) in water, followed by the separation of volatile bases of other nitrogen compounds present in the sample by steam distillation at alkaline pH-value, in a distillation unit (Büchi B-323 Labortechnik, Flawil, Switzerland) using magnesium oxide (MgO; Reutter, Santiago, Chile) as alkalizing agent. Then, TVB-N is collected in a solution 4 g/100 mL of orthoboric acid (H_3BO_3; Winkler, Santiago, Chile) in water, and quantified by means of titration with 0.01 mol/L hydrochloric acid (HCl; Merck). The results are expressed in (mg TVB-N/100 g of fish muscle.

2.7 Sensory analyses

"Characteristic flavor" and "rancid odor" parameters were evaluated by a sensory panel consisting of ten trained assessors (five females and five males) according to a modification of the method described by Green-Petersen et al. [33]. Panellists had been involved in sensory analysis of

different kinds of fish foods. At each sampling time, the fish muscle portions were presented to panellists in individual trays and were scored individually. Parameters were evaluated on a non-structured linear scale with numerical scores from 0 to 10, where score 0 represents the stage of no parameter at all, while score 10 corresponds to the stage where no increase in respective parameter is possible. Scores among panellists were averaged.

2.8 Statistical analyses

Data from the different measurements were subjected to two-way ANOVA, this one are focused to assess significant differences as a result of the diet provided and the frozen storage time; comparison of means was performed using a multiple range test (Tukey's Honestly Significant Differences, HSD). Statgraphics Plus 5.1® software (Statistical Graphics Corp, Rockville, MD, USA) was used to carry out these analyses. A significance level of $\alpha = 0.05$ was considered in all cases.

3 Results and discussion

3.1 Nutritional composition of raw material

Percent composition was determined in minced muscle of Atlantic salmon that was used as raw material for canning storage, and mean values of five ($n = 5$) independent determinations ± SDs are reported. Moisture content of salmon was 66.5% ± 0.1, lower than the values reported by others authors [34, 35] for coho salmon (*Oncorhynchus kisutch*) used as raw material for refrigerated and canned storage, respectively. Although moisture is a parameter easy to measure, is not considered a good index to determine the quality of salmon, due to the fluctuation observed in their content between individuals. Atlantic salmon used as raw material showed a little lower lipid content (12.6% ± 1.8) in relation to the range of values given by Valenzuela [36] for salmons cultivated in Chile. The differences in lipid composition may be due to factors such as sexual maturity of fish, size, sampling, environment, and even, post-mortem conditions [37]. Lipid content obtained for Atlantic salmon at the beginning of the study agree with values reported for coho salmon [38], but is much higher that the found by Rodríguez et al. [35] for coho salmon in the same stage of their research. Protein content (17.7% ± 2.2) was also lower than the values reported for the same specie [39] and for coho salmon. Ash content (1.0% ± 0.3) agrees with values reported for coho salmon [38]. Typically, lower moisture contents in fish are accompanied by an increase in lipid content, while protein and ash contents remained quite constant throughout the storage time [40]. It is noteworthy that proximate composition of muscle of farmed salmon species, especially the lipid content, is highly dependent on the diet provided to them [41]. Carbohydrate content, obtained by difference, was 2.2% ± 0.2.

3.2 Analyses of fatty acid composition

Table 1 shows the variation in the type of saturated fatty acids (SFAs), MUFAs, and PUFAs of all systems of canned salmon studied during storage at 40°C. The results show significant differences ($p \leq 0.05$) between samples and throughout storage time. For all cases, the content of SFAs, MUFAs, and PUFAs in the muscle of canned Atlantic salmon treated with seaweed extracts showed significant differences through the storage time studied, and between the five kinds of covering liquids considered in this research ($p \leq 0.05$). From these results, it may be deduced an inhibitory effect of antioxidants contents in the seaweed extracts on deterioration of MUFAs and PUFAs: a similar effect was observed in canned sardine when they used a previous slurry ice treatment [42].

SFA and MUFA contents in all samples of canned salmon from this study (with and without seaweed extracts) were always lower than those reported previously for canned salmons processed in the same factory at different seasons [43]. Canned salmon added with extracts from *ulte* and red *luche* displayed lower SFA contents, and higher contents of PUFAs through the storage time in comparison with control and the other two seaweed extracts used. A research suggests that the proportion of fatty acids and their variability in a canned product depends on intrinsic factors from the fish (sexual maturity, feed digestibility, etc.), post-harvest conditions, and processing techniques applied [44]. Moreover, PUFAs degradation due to the thermal processing may cause an increase in the concentration of other fatty acids [45], as occurs with MUFA contents of canned salmons when were used *cochayuyo* and sea lettuce extracts.

PI evolution in the salmon muscle is a measurement of the variation of LC-PUFAs during canned storage, relative to a saturated fatty acid representative of marine products such as salmon (16:0). Its index showed significant ($p \leq 0.05$) differences during the storage time and between the different covering liquids used (Table 2). Mean values for all treatments showed some decreasing tendency with time, with the lowest mean value measured at Day 170. The values of PI for canned salmon with *ulte* extract proves that this seaweed had a better performance for preserving the LC-PUFA during canned storage of salmon: samples added with *ulte* extract showed the highest PI values in comparison to all other extracts used.

3.3 Lipid peroxidation assessment

Primary lipid oxidation was measured by means of the PV evolution, while secondary oxidation was evaluated by the pAV during the 170 days of storage (Table 2).

The PV assessment showed significant differences ($p \leq 0.05$) for all kinds of samples between both, storage

Table 1. Fatty acids composition and polyene index in canned Atlantic salmon that was packed with different covering liquids[a)]

Storage time (days)	SFAs (g/100 g lipid)					Storage time (days)	MUFAs (g/100 g lipid)				
	CTR^{w}	CYO^{x,y}	SLC^{x}	ULT^{y}	RLC^{x}		CTR^{x}	CYO^{y}	SLC^{y}	ULT^{y}	RLC^{y}
8^{A,B}	27.32 ± 0.82	24.92 ± 0.10	25.65 ± 0.19	24.60 ± 0.19	24.79 ± 0.09	8^{A}	28.44 ± 0.44	28.83 ± 0.15	29.03 ± 0.05	29.05 ± 0.23	28.61 ± 0.02
60^{A}	26.33 ± 0.03	24.24 ± 0.02	25.38 ± 0.06	24.74 ± 0.08	25.20 ± 0.10	60^{B}	27.46 ± 0.31	28.23 ± 0.10	28.59 ± 0.01	27.90 ± 0.16	28.45 ± 0.09
115^{B}	27.15 ± 0.06	24.96 ± 1.23	25.93 ± 0.77	24.88 ± 0.01	26.72 ± 1.12	115^{C}	28.31 ± 0.08	28.85 ± 1.01	29.97 ± 0.30	30.06 ± 0.05	30.25 ± 1.13
170^{A,B}	27.22 ± 0.33	25.95 ± 0.06	25.52 ± 0.31	23.94 ± 0.17	25.43 ± 0.23	170^{A,C}	28.29 ± 0.10	29.79 ± 0.11	29.76 ± 0.09	28.50 ± 0.02	28.35 ± 0.26
Storage time (days)	PUFAs (g/100 g lipid)					Storage time (days)	Polyene index (−)				
	CTR^{w}	CYO^{w,x}	SLC^{x,y}	ULT^{z}	RLC^{y}		CTR^{x}	CYO^{x}	SLC^{x}	ULT^{y}	RLC^{x}
8^{A}	35.69 ± 0.09	36.91 ± 0.08	36.64 ± 0.15	37.50 ± 0.19	38.19 ± 0.13	8^{A}	1.42 ± 0.06	1.47 ± 0.01	1.40 ± 0.04	1.51 ± 0. 02	1.57 ± 0.03
60^{B}	35.33 ± 0.12	36.32 ± 0.13	36.59 ± 0.02	37.70 ± 0.25	36.46 ± 0.06	60^{A}	1.44 ± 0.03	1.47 ± 0.02	1.42 ± 0.01	1.49 ± 0.03	1.42 ± 0.04
115^{C}	36.20 ± 0.30	35.94 ± 0.08	36.47 ± 0.85	37.56 ± 0.14	34.00 ± 0.15	115^{B}	1.42 ± 0.02	1.42 ± 0.04	1.36 ± 0.07	1.58 ± 0.01	1.21 ± 0.07
170^{C}	34.82 ± 0.14	33.88 ± 0.13	34.73 ± 0.14	38.61 ± 0.01	36.60 ± 0.04	170^{B}	1.32 ± 0.01	1.31 ± 0.01	1.27 ± 0.02	1.60 ± 0.02	1.40 ± 0.02

[a)]For each parameter, mean values of five ($n = 5$) independent determinations ± SDs. Letters (w, x, y, and z) denote significant differences between covering liquid used ($p \leq 0.05$), and capital letters (A, B, C, and D) denote significant differences between storage time ($p \leq 0.05$).
SFAs, saturated fatty acids.
Covering liquids: CTR: control (distilled water), CYO: aqueous extract of *cochayuyo*, SLC: aqueous extract of sea lettuce, ULT: aqueous extract of *ulte*, RLC: aqueous extract of red *luche*.

Table 2. Development of primary and secondary lipid peroxidation in canned Atlantic salmon that was packed with different covering liquids[a)]

Storage time (days)	Peroxide value (meq O_2/kg lipid)					Storage time (days)	*p*-Anisidine value (−)				
	CTR^{w}	CYO^{x}	SLC^{y}	ULT^{w}	RLC^{z}		CTR^{x}	CYO^{y}	SLC^{z}	ULT^{z}	RLC^{y,z}
8^{A}	2.37 ± 0.05	1.17 ± 0.03	1.42 ± 0.22	2.17 ± 0.02	1.63 ± 0.11	8^{A}	3.38 ± 0.45	5.95 ± 0.10	4.86 ± 0.73	6.38 ± 0.27	5.06 ± 0.43
60^{B}	1.75 ± 0.03	2.68 ± 0.19	2.78 ± 0.04	1.40 ± 0.06	5.56 ± 0.09	60^{B}	4.16 ± 0.26	3.06 ± 0.10	4.09 ± 0.10	3.11 ± 0.27	3.22 ± 0.43
115^{C}	2.58 ± 0.08	2.07 ± 0.12	3.57 ± 0.14	1.57 ± 0.08	6.27 ± 0.42	115^{C}	7.21 ± 0.33	3.66 ± 0.06	4.81 ± 0.05	3.91 ± 0.14	3.39 ± 0.46
170^{D}	1.17 ± 0.04	3.36 ± 0.29	3.25 ± 0.15	1.72 ± 0.16	2.88 ± 0.16	170^{B}	7.06 ± 0.08	3.50 ± 0.03	3.53 ± 0.35	3.36 ± 0.16	2.58 ± 0.15

[a)]For each parameter, mean values of five ($n = 5$) independent determinations ± SDs. Letters (w, x, y, and z) denote significant differences between covering liquid used ($p \leq 0.05$), and capital letters (A, B, C, and D) denote significant differences between storage time ($p \leq 0.05$).
Covering liquids: CTR: control (distilled water), CYO: aqueous extract of *cochayuyo*, SLC: aqueous extract of sea lettuce, ULT: aqueous extract of *ulte*, RLC: aqueous extract of red *luche*.

time and the covering liquid used. A peroxide breakdown tendency was found higher for canned fish with red *luche*, sea lettuce and control after Day 115th of storage; some different breakdown rate could be concluded according to the covering liquid used. PV found were low in all cases, which can be explained by the acceleration of the generation of peroxides produced by the thermal treatment, as well as their rapid breakdown to secondary byproducts, or a possible reaction of the hydroperoxides with some constituents of salmon muscle [1]. The values found were also favored with the type of container used (tin), which exerts a barrier to oxygen input, and therefore, to the generation of hydroperoxides in canned salmon [46]. Nevertheless, only the *ulte* extract showed some inhibition in the hydroperoxides formation in canned salmon in comparison with the control.

The pAV evolution showed significant differences ($p \leq 0.05$) throughout the storage time for all kinds of samples. Control sample showed a clearly high increase of this value between 60th and 115th day, and for all samples contained seaweed extracts it was observed a significant decrease of pAV, this results could indicate that seaweed extracts have the ability of delay generation of secondary products of peroxidation, or breakdown them. When was used red *luche* extract as covering liquid, the most decrease of pAV was observed through the time, followed by *ulte* and *cochayuyo*. Comparison among all samples with seaweed extracts used as covering liquids, led to a higher pAVs ($p \leq 0.05$) for control samples. Other authors found pAV considerably higher (between 13.3 and 29.0) in canned coho salmon using different icing conditions as pretreatments [35]. It was postulated that a pAV above 20 reflects characteristics rancid off-flavors and odors in foods [47]; this value is not reached in any of the treatments studied here for canned salmon. It should be studied that a decrease tendency in secondary peroxidation compounds in the early stages of storage may be due to the changes caused by the sterilization process and the equilibration stage between solids and liquids, in which a stabilization process occurs between the raw material and covering medium [48]. This decrease also has been attributed to a dilution effect of secondary peroxidation products, which can be extracted with the covering liquid from the muscle of the fish, could be distributed throughout the muscle by the presence of the filling medium, or its compounds may be expelled from the muscle in the form of exudates [49]. Also, aldehydes can react with proteins or other biological compounds in systems oxidized during heat treatment, which can also lead to decreases in pAV [49].

3.4 Total tocopherols

In the course of this research, all systems studied showed a significant increase of tocopherols after the Day 8th of storage at 40°C ($p \leq 0.05$, Table 3). Canned salmon added with sea lettuce extract showed the highest value for tocopherols content at the beginning of the work (368 mg/kg), and at Day 170th with 729 mg/kg; showing differences with the control sample ($p \leq 0.05$). The increase in the amount of tocopherols

Table 3. Changes in antioxidant compounds formation in canned Atlantic salmon that was packed with different covering liquids[a)]

Storage time (days)	Total Tocopherols (mg/kg lipid)					Storage time (days)	Astaxanthin (mg/kg lipid)				
	CTR^{w}	CYO^{x}	SLC^{y}	ULT^{z}	RLC^{w}		CTR^{x}	CYO^{z}	SLC^{y}	ULT^{z}	$RLC^{y,z}$
8^{A}	349.65 ± 2.47	310.50 ± 0.28	368.45 ± 1.06	284.05 ± 7.28	294.25 ± 3.75	8^{A}	3.54 ± 0.58	2.21 ± 0.05	2.75 ± 0.04	2.83 ± 0.20	2.47 ± 0.20
60^{B}	457.10 ± 9.33	430.45 ± 8.56	505.70 ± 2.97	487.55 ± 0.49	501.95 ± 3.04	60^{B}	2.82 ± 0.03	1.70 ± 0.12	2.29 ± 0.08	1.80 ± 0.03	1.87 ± 0.43
115^{C}	453.47 ± 3.27	459.37 ± 8.59	490.18 ± 3.05	342.69 ± 1.55	484.73 ± 13.99	$115^{B,C}$	1.82 ± 0.34	1.39 ± 0.01	1.86 ± 0.29	1.58 ± 0.08	1.85 ± 0.03
170^{D}	554.31 ± 21.55	499.39 ± 30.26	728.69 ± 10.73	425.72 ± 29.01	514.47 ± 16.27	170^{C}	1.41 ± 0.08	1.48 ± 0.21	1.95 ± 0.11	1.14 ± 0.05	1.80 ± 0.07

[a)]For each parameter, mean values of five ($n = 5$) independent determinations ± SDs. Letters (w, x, y, and z) denote significant differences between covering liquid used ($p \leq 0.05$), and capital letters (A, B, C, and D) denote significant differences between storage time ($p \leq 0.05$).
Covering liquids: CTR: control (distilled water), CYO: aqueous extract of *cochayuyo*, SLC: aqueous extract of sea lettuce, ULT: aqueous extract of *ulte*, RLC: aqueous extract of red *luche*.

through storage time may occur mainly because tocopherols are supplied to farmed fish in diets during its growth period in the form of a tocopherol ester (α-tocopheryl acetate), of which only a fraction would be absorbed by lipids presents in the salmon flesh [50], while the other fraction of α-tocopheryl acetate remains bound esterified in the tissues. Thereby, the ester being in an aqueous medium would begin to hydrolyze, which causes a gradual releasing of tocopherol, and therefore, an increase of the quantity of free tocopherols in salmon flesh throughout storage time. In other research, authors found similar behavior for the content of α-tocopherol in the muscle of coho salmon during the first 8 months of frozen storage [51]. It was demonstrated that the majority of the tocopherols present in various species of fish are deposited in the liver, followed by gut and belly, and the remainder is deposited on the muscle [52]. Another probable reason why were not observed a decrease in the levels of tocopherols may be because the high temperatures help to decrease the solubility of oxygen in lipids, so the tocopherol autoxidation may occur more slowly and would be replaced by polymerization reactions [53].

3.5 Astaxanthin

The results of HPLC analyses indicated that the AX values determined during the canned storage (Table 3) showed significant differences between samples ($p \leq 0.05$), and ranged from 1.1 to 3.5 mg/kg in salmon muscle; these levels were lower than those determined previously [41], who found average concentrations of AX in salmonid muscle of 4.6 mg/kg. Furthermore, these authors observed that the pigmentation produced by the retention of carotenoids is influenced by genetic factors, sexual maturation (higher in immature fish), and their size (greater in largest fish). AX content in all samples of canned salmon decreased significantly during storage ($p \leq 0.05$), this behavior is similar to that found previously for frozen stored rainbow trout (*Oncorhynchus mykiss*) fed with two kinds of diets [54].

Research suggests that the higher level of retention of AX is reached when salmonids were fed with diets contained primarily synthetic AX: researchers were fed rainbow trout with diets supplemented with two vegetable sources of carotenoids (marigold flowers and red pepper) and synthetic AX, determined that the muscle of the fish retained more content of AX when they were fed with diets formulated with synthetic AX than with diets with the natural carotenoid [55]. Jensen et al. [56] found that the addition of α-T in the diet does not affect AX retention in the muscle of rainbow trout.

In order to evaluate the showed effect of seaweed extracts on AX content in canned salmon, it was calculated a value of pigment retention at the end of the study in relation to the content of AX in raw material: the most efficient retention of AX was achieved with red *luche* extract (73%), followed by the sea lettuce (71%) and *cochayuyo* (67%), leaving the last of *ulte*, which was achieved with a retention value identical to that reached by the control sample (40%). This agrees with that the most of the seaweed extracts exhibit good protection against depletion of AX, therefore in this case would not be used as an antioxidant, if not rather the constituents from the extracts accomplished this role. Values and behavior similar to those found in red *luche* exhibited canned salmon added with sea lettuce extract; however the descent stage lasted until Day 115. With respect to the observed decrease in the levels of AX after Day 60 in all cases, it can be inferred that, due to this compound is a potent antioxidant; it begins to be used in antioxidant functions, thus causing a loss of pigment, which can lead changes in color [57]. It is important to point out that the diminution of AX pigment in these concentrations (<6 μg of AX/g of fish muscle) has no great influence on changes in flavor of fish [31, 58].

3.6 Total volatile basic nitrogen

The results indicate that TVB-N increased steadily over time for the most of the cases, and showed statistically differences ($p \leq 0.05$) throughout the storage period (Fig. 1). Thermal

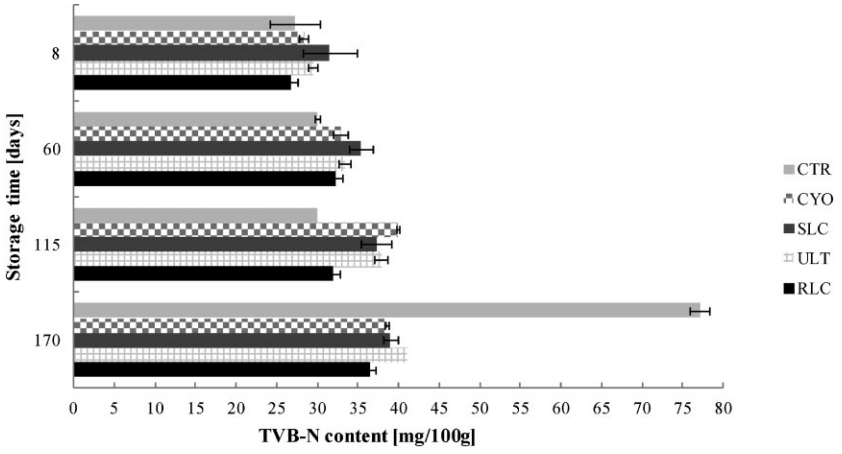

Figure 1. TVB-N content in canned Atlantic salmon that was previously packed with different covering liquids. *Mean values of five ($n = 5$) independent determinations. SDs are denoted by bars. Covering liquids: CTR: control (distilled water), CYO: aqueous extract of *cochayuyo*, SLC: aqueous extract of sea lettuce, ULT: aqueous extract of *ulte*, RLC: aqueous extract of red *luche*.

processes and the storage time cause an increase in TVB-N in marine products that is generated mainly by deterioration in trimethylamine oxide (a compound present in most fish and shellfish), causing deterioration in sensory and biochemical quality of canned fish due to the generation of amines of low molecular weight as alteration products [59, 60]. In the present study, all canned salmon showed values close to 30–35 mg TVB-N/100 g on the eighth day of storage at 40°C, which is established as the maximum limits for fresh products by Chilean legislation [61] and European Union for Atlantic salmon [62], respectively. The standard from National Fisheries Service (Chilean government entity) does not set limits for TVB-N in canned fish [63].

Results obtained for canned Atlantic salmon in this research were lower than those reported for canned golden kingclip (*Genypterus blacodes*) stored for 3 months at room temperature, finding values of 49.61 and 52.01 (mg TVB-N/100 g) when they was used water and brine as covering liquid, respectively [64]. Rodríguez et al. [35] reported values between 36.2 and 46.0 (mg TVB-N/100 g) in flesh of canned coho salmon after 3 months of storage at 15–18°C; similar values are reached in our study with all seaweeds extracts at 170th day of storage at 40°C. Canned Atlantic salmon with red *luche* as covering liquid showed the lowest values for TVB-N in muscle at the end of the study in comparison with the control samples. This may be due to a potential antimicrobial activity from some components contained in this seaweed.

3.7 Sensory evaluation

Sensory parameters of "characteristic flavor" and "rancid odor" were measured in flesh from all samples of canned salmon from this research (Table 4). None of these parameters showed significant differences between all samples of canned salmon ($p>0.05$), so the covering liquid (seaweed extract) that was used for their preparation mostly did not affect the generation of non-characteristic flavors and odors. In all cases, "characteristic flavor" scores were included in the acceptable domain at the end of the experiment (above 7.0); while "rancid odor" parameter was evaluated by the sensory panel with very low values, so that all kinds of canned salmon were scored as highly acceptable. A similar behavior for "rancid (oxidized) odor" was found by Rodríguez et al. [65] in samples of canned coho salmon pre-treated under different icing conditions.

Canned samples of Atlantic salmon with *cochayuyo* as covering liquid showed a higher score for "characteristic flavor," and together with *ulte*, showed the minor values for "rancid odor" development for all the canning period.

Table 4. Sensory quality at the end of the research in canned Atlantic salmon that was packed with different covering liquids[a)]

	Characteristic flavor	Rancid odor
CTR	8.22 ± 0.33	0.25 ± 0.05
CYO	9.37 ± 0.78	0.15 ± 0.02
SLC	7.30 ± 0.95	1.19 ± 0.24
ULT	7.99 ± 1.99	0.16 ± 0.02
RLC	6.92 ± 1.69	1.39 ± 0.12

[a)]For each parameter, mean values of ten ($n = 10$) panellists ± SDs were considered. No significant differences were found between different covering liquids used ($p>0.05$).
Covering liquids: CTR: control (distilled water), CYO: aqueous extract of *cochayuyo*, SLC: aqueous extract of sea lettuce, ULT: aqueous extract of *ulte*, RLC: aqueous extract of red *luche*.

4 Conclusions

In this study, we ventured into the use of seaweed extract as a unexplored way to improve the preservation of canned Atlantic salmon, a fatty fish. The present results have shown some advantages when employing seaweeds extracts (contain natural antioxidants) during the canned storage of salmon. After 170 days of canned storage at 40°C, all kinds of fish samples had acceptable oxidized odor and characteristic flavor scores. The use of seaweeds extracts as part of the covering liquid contributed to the decrease of secondary peroxidation (pAVs) in canned salmon, contrary to what was observed for the control sample. These results were accompanied by a higher PUFA and AX retention, and lower scores for oxidised odors. Concerning the sensory parameters evaluated, no effect could be shown as a result of employing different seaweeds extracts as covering liquids in canned Atlantic salmon.

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