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Rancidity development during the frozen storage of farmed coho salmon (*Oncorhynchus kisutch*): Effect of antioxidant composition supplied in the diet

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ABSTRACT

A commercial diet including synthetic antioxidants (BHT–ethoxyquin mixture) (diet I) was provided to coho salmon (*Oncorhynchus kisutch*) in parallel with two diets including natural antioxidants (tocopherol isomers–rich mixture, diet II; tocopherol isomers-rosemary extract mixture, diet III). A comparative study of the rancidity development in the corresponding frozen (−18 °C) products was undertaken. When compared to fish fed with diet I, individuals corresponding to diet II showed a greater ($p < 0.05$) retention of primary (conjugated dienes and peroxides content) and secondary (anisidine and thiobarbituric acid indices) lipid oxidation compounds that led to a lower interaction compound formation (fluorescence ratio ranges: 0.33–0.50 and 0.55–0.85, for diet II and diet I individuals, respectively); likewise, a higher polyene index (1.99–2.14 and 1.72–1.97, respectively) and lower oxidised taste scores (0.0–0.6 and 0.0–2.4, respectively) were obtained. No effect ($p > 0.05$) on lipid hydrolysis development (free fatty acid formation) could be found as a result of employing different diets.

1. Introduction

Freezing and frozen storage have largely been employed to retain fish quality before it is consumed or used in other technological processes. However, the presence in fish muscle of both a highly unsaturated lipid composition and a relevant prooxidant compound content can facilitate an important enzymatic and non-enzymatic rancidity development, this leading to sensory, physical and nutritional quality losses (Barroso, Careche, & Borderías, 1998; Erickson, 1997). To slow down such deteriorative pathways during frozen storage, previous treatments with synthetic antioxidants were successfully used, although their employment is actually not recommended because of safety concerns related to human health. In this sense, recent efforts have been focused on prior treatment with endogenous-type antioxidants (namely, tocopherol isomers) or with natural antioxidants present in plant extracts (namely, polyphenol compounds) (Kamal-Eldin & Appelqvist, 1996; Yanishlieva, Marinova, & Pokorný, 2006).

In recent years, the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability. This has prompted fish technologists and the fish trade to pay more attention to aquaculture as a source of fish and other seafood products. Because of its important role in the human health, great attention has been paid to the effect of diet provided on the fish food composition. Thus, great efforts have been made to enhance the $\omega-3$ fatty acid content in fish products by employing vegetable oil diets, including high levels of alpha-linolenic (C18:3 $\omega-3$) acid (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006; Visentainer, de Souza, Makoto, Hayashi, & Franco, 2005). Additionally, much research has been carried out on the effect of the diet provided on the physical and sensory properties of the processed fish, e.g. liquid holding capacity and texture (Rørå, Regost, & Lampe, 2003; Torstensen et al., 2005), colour changes (Choubert & Baccaunaud, 2006) and odour (Sérot, Regost, Prost, Robin, & Arzel, 2001).

Because of the important role of lipid oxidation development in processed fish, great attention has been paid to the endogenous antioxidant content of cultivated fish. Thus, fish farmers have included a wide range of allowed (both in the EC and the USA) synthetic antioxidants (namely, ethoxyquin, BHT and BHA) in order to enhance lipid stability in the corresponding processed food (Hertrampf & Piedad-Pascual, 2000; Southgate, 2003). However, recent efforts are focused on the replacement of synthetic antioxidants by natural ones, which may additionally provide nutritional and therapeutic effects (Frankel, 1995). Thus, diets including high contents of endogenous antioxidants have led to a partial inhibition of lipid oxidation development (Jittinandana, Kenney, Slider, Kamireddy, & Hakins, 2006; Stéphan, Guilaume, & Lamour, 1995). However, to our knowledge, comparison of the effects of diets including synthetic and natural antioxidants has not been achieved up to now.

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Among cultivated fish, coho salmon (*Oncorhynchus kisutch*), also called silver salmon, has received great attention because of its increasing production in countries such as Chile, Japan and Canada (FAO, 2007a) in parallel with important capture production in countries such as the USA, Russian Federation, Canada and Japan (FAO, 2007b). The present work focuses on the commercialisation of this species as a frozen product. In it, a commercial diet including both BHT (butyl-hydroxy-toluene) and ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) (diet I) was provided to salmon fish and compared to two other diets, one of them including a tocopherol isomer-rich mixture (diet II) and the other, a tocopherol isomers-rosemary extract mixture (diet III). A comparative study of diet effect on the fish product quality was achieved throughout frozen storage by means of rancidity development analysis.

2. Materials and methods

2.1. Experimental diets, raw material, processing and sampling

Coho salmon (*O. kisutch)* individuals used in this study were cultivated in three different tanks by EWOS Innovation Research (Colaco, Puerto Montt, Chile). Sandbed-filtered seawater (salinity range: 31.3–33.1 g kg^{-1}) was supplied to each tank over a temperature range of 11.2–12.8 °C. Feeding to satiety was carried out during the lighted period (photoperiod: 5.8–7.8 h) by employing a diet with the following general composition: protein (43.0%), fat (29.0%), moisture (7.0%), ash (6.5%), crude fibre (1.3%) and carbohydrates (13.2%). The distribution of fat composition into saturated, monounsaturated and polyunsaturated fatty acid groups was 32.5%, 27.0% and 40.2%, respectively.

Following the objectives of the work, each of the three tanks was fed with a different antioxidant composition, according to data shown in Table 1. Diet I included a relevant content of synthetic antioxidants in the meal (ethoxyquin) and in the oil (BHT); diet II provided a mixture of tocopherol isomers in both meal and oil; finally, diet III combined the presence of tocopherol isomers (in the meal) and a rosemary extract (in the oil).

Once individual salmons attained ca. 2500 g weight, 30 fish per tank were withdrawn, sacrificed by a sharp blow to the head, the gills cut and bled in a water–ice mixture, headed, gutted and kept in ice for 24 h until they arrived at our laboratory. The fish were then frozen at −40 °C in individual polyethylene bags, with hermetic sealing. After 3 days, the fish were stored at −18 °C. Frozen individuals were taken for analysis on months 0, 3, 6, 9, 12 and 18 of storage at −18 °C. From each tank under study, five different fish were independently analysed at each sampling time ($n = 5$).

2.2. Oxidised taste analysis

Oxidised taste analysis was conducted according to the quality descriptive analysis (QDA) method by a sensory panel consisting of ten experienced judges (five females and five males). Panellists were selected and trained according to International Standards (ISO, 1991) in use of sensory descriptors for thawed and cooked salmon of different quality conditions.

At each sampling time, fish samples were thawed and then cooked in polyethylene bags in a water bath. The fish muscle portions were presented to panellists in individual trays and were scored individually. The panel members shared samples tested. Oxidised taste was evaluated on a non-structured linear scale with numerical scores from 0 to 10. Score 0 represents the stage of no rancidity at all, while stage 10 corresponds to the stage where no increase in rancidity is possible; score 5.0 was considered the borderline of fish acceptability. Scores among panellists were averaged.

Table 1
Antioxidant composition (mg kg^{-1} muscle) included in the different diets provided to coho salmon*.

Antioxidant compound	Diet I	Diet II	Diet III
Total tocopherols	22.4	101	45
Ethoxyquin	19.3	2.9	2.3
BHT	3.0	ND	ND
Phenolic diterpenes	ND	ND	ND
Carnosic acid	ND	ND	18
Carnosol	ND	ND	13
Rosmarinic acid	ND	ND	ND

ND, not detected.
* Fish supplier's data.

2.3. Lipid composition analysis

The lipid fraction was extracted from the fish white muscle by the Bligh and Dyer (1959) method. Quantification results are expressed as g of lipid kg^{-1} muscle.

Lipid extracts from the fish white muscle were converted into fatty acid methyl esters (FAME) by using acetyl chloride and analysed by GC (Perkin-Elmer 8700 chromatograph), employing a fused silica capillary column SP-2330 (0.25 mm i.d. × 30 m, Supelco Inc., Bellefonte, PA, USA) (Aubourg, Medina, & Pérez-Martín, 1996). Carrier gas used was N_2 flowing with a linear velocity of 18 cm s^{-1}. A flame ionisation detector set at 250 °C was used. Peaks were identified by comparison of their retention times with standard FAME mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peaks were automatically integrated, 19:0 fatty acid being used as an internal standard for quantitative analysis. The polyene index (PI) was calculated as the following fatty acid ratio: PI = C20:5 + C22:6/C16:0.

2.4. Lipid damage analysis

Free fatty acid (FFA) content was determined on the lipid extract by the Lowry and Tinsley (1976) method which is based on complex formation with cupric acetate–pyridine, followed by spectrophotometric (715 nm) assessment. Results are expressed as g FFA kg^{-1} lipids.

Conjugated dienes (CD) formation was measured on the lipid extract according to the Kim and Labella (1987) method. The CD content results are expressed as absorption coefficients (AC), according to the formula: $AC = B \times V/w$, where B is the absorbance reading at 233 nm of an aliquot of the lipid extract, V denotes the aliquot volume (ml) and w is the mass (mg) of the lipid material included in the aliquot.

The peroxide value (PV) was determined on the lipid extract by the ferric thiocyanate method (Chapman & McKay, 1949). The results are expressed as meq active oxygen kg^{-1} lipids.

The anisidine value was determined in fish muscle according to the AOCS (1993) method, based on the reaction between α- and β-unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent. Anisidine value is 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^{-1} reaction medium.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloracetic acid extract of the fish muscle and thiobarbituric acid at high temperature (95–97 °C), the resulting chromophore being measured at 532 nm. Results are expressed as mg malondialdehyde kg^{-1} fish muscle.

2.5. Interaction compound formation

Formation of fluorescent compounds was determined with a Perkin Elmer LS 45 fluorimeter by measurements at 393/463 nm

and 327/415 nm as previously described (Aubourg, Sotelo, & Pérez-Martín, 1998). The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg ml^{-1} in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463}$ nm/$RF_{327/415}$ nm. The FR value was determined in the lipid extract of the fish muscle.

Browning development was measured from the lipid extract absorbances at 450 nm and 400 nm. Data are expressed as the 450 nm/400 nm absorbance ratio (browning ratio, BR).

2.6. Statistical analyses

Data from the different measurements were subjected to one-way analysis of variance; this served to assess significant differences as a result of the diet provided and the frozen storage time; comparison of means was performed using a least-squares difference (LSD) method. The SPSS 11.5 software for Windows (SPSS Inc., Chicago, Il, USA) was employed. Correlation analysis was achieved among the different parameters studied and with the storage time. A confidence interval at the 95% level ($p < 0.05$) was considered in all cases.

3. Results and discussion

3.1. Lipid hydrolysis assessment

FFA content increased ($p < 0.05$) in all kinds of fish samples throughout the frozen storage, indicating that hydrolytic enzyme activity continued under the storage temperature conditions (Fig. 1). Thus, good correlation values with storage time were obtained for individuals corresponding to the three feeding conditions ($r^2 = 0.92$–0.94, linear fitting). This linear FFA formation pattern is different from most reported experiments concerning fish frozen storage, where a logarithmic fitting is obtained between FFA formation and the storage time (Aubourg, Rodríguez, & Gallardo, 2005; Aubourg et al., 1998; Rodríguez et al., 2007). In such studies, a marked hydrolysis increase during the first period (ca. 0–3 months) was found, this being explained by a maximal lipase release from liposomes during this period, which then facilitates closer proximity between enzyme and substrate (Sikorski & Kolakowski, 2000).

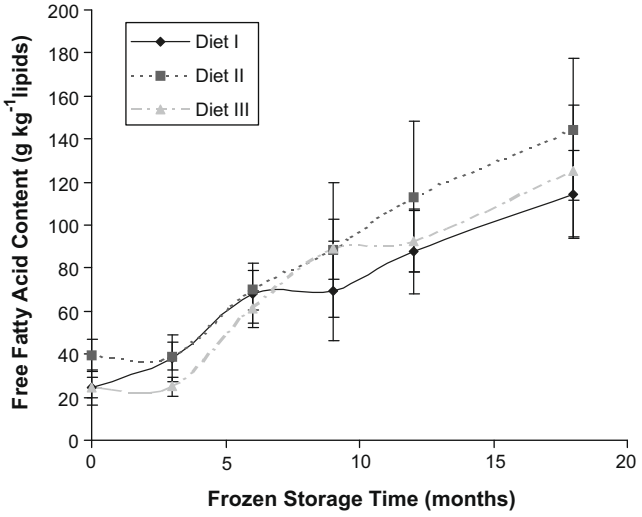

Fig. 1. Free fatty acid (FFA) formation in frozen coho salmon previously fed with different diets. Mean values of five ($n = 5$) independent determinations. Standard deviations are denoted by bars. Linear correlation values (r^2) between FFA content and frozen storage time are: 0.93 (diet I), 0.92 (diet II) and 0.94 (diet III).

Comparisons among individuals corresponding to different diets led to no significant differences ($p > 0.05$), although some higher mean values could be observed for individuals corresponding to diet II in the last period of the experiment (12–18 months). A definite effect of antioxidant composition of diets on lipid hydrolysis development in the frozen product is not clear.

According to previous research (Rodríguez et al., 2007), lipid content of the white muscle in the present study was in the 33.5–47.5 g kg^{-1} range. In spite of the known inverse ratio between lipid and FFA contents (Pearson, Love, & Shorland, 1977), a relatively high FFA content could be found in the present experiment when compared to previous reports on the same farmed species (Rodríguez et al., 2007) and on other fatty fish species (Aubourg et al., 1998, 2005) under the same frozen conditions. To explain such a difference, it should be taken into account that lipid hydrolysis development strongly depends on the hydrolytic enzyme content, this being highly influenced by different external and internal factors (Aubourg et al., 2005; Sikorski & Kolakowski, 2000).

The interaction of lipolysis and lipid oxidation is a particularly intriguing area of study as triglyceride hydrolysis has been shown to lead to increased oxidation, while phospholipid hydrolysis produces the opposite effect (Shewfelt, 1981; Sikorski & Kolakowski, 2000). The release of FFA from a triacylglycerol matrix may accelerate their interaction with oxidative catalysts and hence accelerate the rate of lipid oxidation and generation of off flavours (Sista, Erickson, & Shewfelt, 1997); this pro-oxidant effect has been explained as a catalytic effect of the carboxyl group on the formation of free radicals by the decomposition of hydroperoxides (Aubourg, 2001). In contrast, free fatty acid liberation from phospholipids would lead to a decreased interaction between oxidised and oxidisable fatty acids within the membrane matrix, thus inhibiting free radical propagation reactions (Shewfelt, 1981; Sista et al., 1997).

3.2. Biochemical lipid oxidation assessment

Frozen storage is known to be associated with fish lipid oxidation processes where different kinds of endogenous enzymes may be involved (Erickson, 1997). Freezing and thawing may cause lysis of mitochondria and lysosomes and alter the distribution of enzymes and factors affecting the rate of enzyme reactions in tissues, so that deteriorative damage in frozen fish could be accelerated. In the present experiment, different and complementary biochemical lipid oxidation indices were employed to evaluate the development of rancidity. Primary lipid oxidation was measured by means of the CD formation and the peroxide content evolution (Table 2), while secondary oxidation was evaluated by the AV and the thiobarituric acid-reactive substances (TBARS) formation (Table 3).

Throughout the frozen storage, the CD content showed a lower value ($p < 0.05$) for individuals previously fed with diet I than for their counterparts corresponding to diets II and III (Table 2). Being produced at a very early lipid oxidation stage, CD content can be considered the result of a formation/breakdown balance (Aubourg et al., 1998). In the present study, it is concluded that CD would breakdown more easily in fish previously fed with diet I than in their counterparts from natural diets. When comparing individual fishes from diets II and III, some lower values (months 6 and 18) were observed for those corresponding to diet II. For all kinds of samples, no differences ($p > 0.05$) could be assessed as a result of the frozen storage time in the 0–12 month period; then, an in-

Table 2
Development of primary lipid oxidation in frozen coho salmon previously fed with different diets*.

Frozen storage time (months)	Conjugated diene formation			Peroxide value (meq kg^{-1} lipids)		
	Diet I	Diet II	Diet III	Diet I	Diet II	Diet III
0	0.60 (0.06)	0.68 (0.03)	0.69 (0.06)	2.91 (1.01)	3.12 (0.81)	3.29 (0.82)
3	0.56 a (0.03)	0.64 b (0.03)	0.66 b (0.02)	3.29 (0.60)	3.05 (0.71)	3.33 (0.97)
6	0.53 a (0.08)	0.58 a (0.03)	0.74 b (0.05)	9.83 (0.99)	13.40 (3.88)	9.39 (3.08)
9	0.57 a (0.06)	0.68 ab (0.09)	0.74 b (0.07)	10.26 a (0.46)	13.59 b (1.37)	10.04 a (0.46)
12	0.60 (0.06)	0.66 (0.05)	0.63 (0.02)	7.69 a (0.62)	9.80 b (1.14)	9.38 b (0.68)
18	0.54 a (0.04)	0.80 b (0.07)	1.72 c (0.08)	4.83 a (1.25)	7.57 b (1.16)	4.35 a (0.60)

* For each parameter, mean values ($n = 5$) followed by different letters (a, b, c) denote significant differences ($p < 0.05$). Standard deviations are included in brackets.

Table 3
Development of secondary lipid oxidation in frozen coho salmon previously fed with different diets*.

Frozen storage time (months)	Anisidine value			Thiobarbituric acid index (mg malondialdehyde kg^{-1} fish muscle)		
	Diet I	Diet II	Diet III	Diet I	Diet II	Diet III
0	2.87 b (0.57)	3.32 b (0.52)	1.77 a (0.30)	0.08 a (0.03)	0.07 a (0.04)	0.14 b (0.01)
3	2.94 b (0.47)	2.66 ab (0.22)	2.35 a (0.05)	0.05 a (0.02)	0.05 a (0.03)	0.11 b (0.02)
6	3.61 ab (0.68)	5.17 b (0.95)	3.25 a (1.01)	0.05 a (0.01)	0.10 ab (0.07)	0.10 b (0.02)
9	4.71 a (0.57)	8.04 b (0.54)	7.18 b (0.59)	0.11 a (0.03)	0.29 b (0.11)	0.26 b (0.11)
12	7.03 a (0.76)	8.78 b (0.36)	8.22 b (0.12)	0.58 b (0.17)	0.63 b (0.18)	0.34 a (0.03)
18	8.03 a (0.99)	12.46 b (0.64)	8.92 a (0.29)	0.56 a (0.12)	0.79 b (0.10)	0.67 ab (0.11)

* For each parameter, mean values ($n = 5$) followed by different letters (a, b) denote significant differences ($p < 0.05$). Standard deviations are included in brackets. Linear correlation values (r^2) between anisidine value and frozen storage time are: 0.91 (diet I), 0.92 (diet II) and 0.89 (diet III). Linear correlation values (r^2) between thiobarbituric acid index and frozen storage time are: 0.74 (diet I), 0.90 (diet II) and 0.84 (diet III).

crease ($p < 0.05$) was observed for diets II and III. In all cases, bad correlation values were obtained for CD content and storage time.

The PV assessment showed a marked increase ($p < 0.05$) for all kinds of samples at month 6, reaching the highest mean value at month 9 (Table 2); then, a decreasing tendency could be observed in all kinds of samples. As in the case of CD formation, peroxide content can be considered to be the result of a formation/breakdown balance (Aubourg et al., 2005; Kim & Labella, 1987). Peroxide breakdown tendency was found to be higher ($p < 0.05$) for fish individuals from diet I, according to previous CD results; additionally, some lower values were obtained for individuals corresponding to diet III when compared to their counterparts from diet II (months 9 and 18). As a result, some different breakdown rate could apply for the diet previously provided.

The AV assessment showed an important increase ($p < 0.05$) throughout the frozen storage for all kinds of samples (Table 3), so that good correlation values were obtained in all cases with frozen time ($r^2 = 0.89$–0.92, linear fitting). This increase was markedly high at month 9 for individuals fed with diets II and III, and at month 12 for those from diet I. Finally, an important increase was produced at month 18 for individuals from diet II. Comparison among diets led to a higher ($p < 0.05$) value for those individuals corresponding to diet II. Comparison between the other two diets showed higher values for individuals from diet I at an early stage (0–3 months), but lower in a more advanced period (9–12 months).

The TBA-i did not provide differences ($p > 0.05$) with storage time during the 0–6 month period (Table 3). Then, an increasing tendency ($p < 0.05$) could be observed for all kinds of samples until the end of the experiment, so that fair correlation values with time could be found ($r^2 = 0.74$–0.90, linear fitting). This TBARS formation during the 9–18 month period was found to be higher ($p < 0.05$) for individuals previously fed with diet II than for their counterparts from diets I and III. Values attained at the end of the experiment can be considered similar to those reported for this farmed species (Rodríguez et al., 2007), but lower than for those wild fatty fish species stored under similar conditions (Aubourg et al., 1998, 2005).

As with primary oxidation formation, the content of molecules susceptible to measurement by AV and TBA-i is the result of a formation/breakdown balance (Kim & Labella, 1987). Thus, the present data show a higher ($p < 0.05$) secondary lipid oxidation compound retention in fish individuals corresponding to diet II than in their counterparts from diet I. According to the strong relationship reported between lipid oxidation and lipid hydrolysis, a good correlation was obtained between FFA content and AV ($r^2 = 0.88$, 0.94 and 0.90, for diets I, II and III, respectively) and fair for TBARS formation and FFA content ($r^2 = 0.73$–0.88 and 0.86, respectively).

3.3. Interaction compound formation study

Compound formation as a result of interaction between oxidised (primary and secondary) lipids and nucleophilic molecules (namely, protein-like) was assessed by fluorescence (FR) and browning (BR) development detection (Table 4).

The FR did not show differences throughout the frozen storage time for individuals from diets II and III, so that poor correlation values were obtained between such index and the storage time. However, an increasing tendency could be observed with frozen storage time ($r^2 = 0.83$, linear correlation) for individuals fed with diet I, according to a progressive formation of such interaction compounds. Comparison among diets led to a higher ($p < 0.05$) formation in the 6–18 month period for individuals previously fed with diet I. A lower interaction compounds formation occurs during the frozen storage for fish salmon previously fed with natural antioxidants.

As for the fluorescence detection, the BR showed an increasing tendency ($p < 0.05$) for individuals previously fed with diet I ($r^2 = 0.90$, linear fitting), while a clear tendency could not be found for their counterpart fishes from diets II and III. As shown in Table 4 for BR values, no significant differences could be observed as a result of the previous diet provided. However, and according to FR values, higher mean values could be obtained for individuals corresponding to diet I than for their counterparts previously fed with diets with relevant contents of natural antioxidants.

Table 4
Assessment of interaction compound formation in frozen coho salmon previously fed with different diets*.

Frozen storage time (months)	Fluorescence ratio (FR)			Browning ratio (BR)		
	Diet I	Diet II	Diet III	Diet I	Diet II	Diet III
0	0.55 (0.11)	0.39 (0.17)	0.41 (0.31)	8.97 (1.27)	8.14 (2.14)	8.07 (2.11)
3	0.55 (0.16)	0.50 (0.12)	0.44 (0.04)	9.88 (2.13)	8.11 (1.22)	8.85 (2.14)
6	0.69 b (0.06)	0.42 a (0.15)	0.43 a (0.11)	9.72 (2.11)	9.16 (2.07)	9.71 (2.11)
9	0.76 b (0.07)	0.35 a (0.13)	0.48 a (0.14)	9.79 (2.26)	9.99 (1.25)	9.86 (2.19)
12	0.81 b (0.15)	0.34 a (0.07)	0.45 a (0.12)	10.78 (2.26)	9.06 (1.24)	9.02 (2.07)
18	0.85 b (0.12)	0.33 a (0.16)	0.38 a (0.08)	11.73 (2.10)	9.05 (2.16)	9.00 (2.18)

* For each parameter, mean values ($n = 5$) followed by different letters (a, b) denote significant differences ($p < 0.05$). Standard deviations are included in brackets. Linear correlation values (r^2) between frozen storage time and FR and BR are 0.83 and 0.90, respectively, for individuals corresponding to diet I.

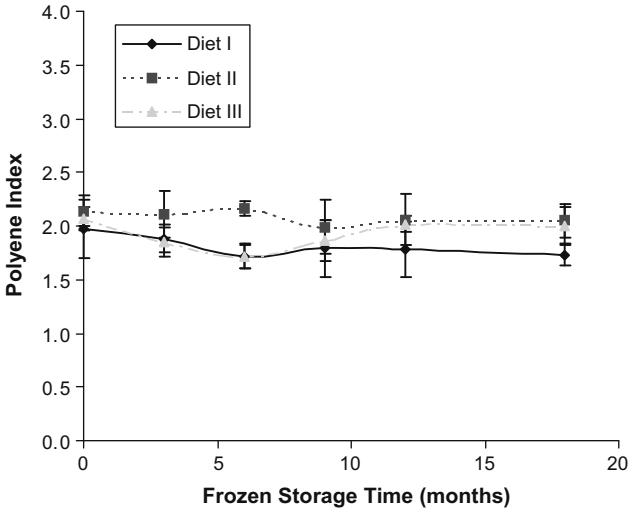

Fig. 2. Polyene index (PI) evolution in frozen coho salmon previously fed with different diets. Mean values of five ($n = 5$) independent determinations. Standard deviations are denoted by bars. Linear correlation value (r^2) between PI value and frozen storage time was – 0.84 for individuals corresponding to diet I.

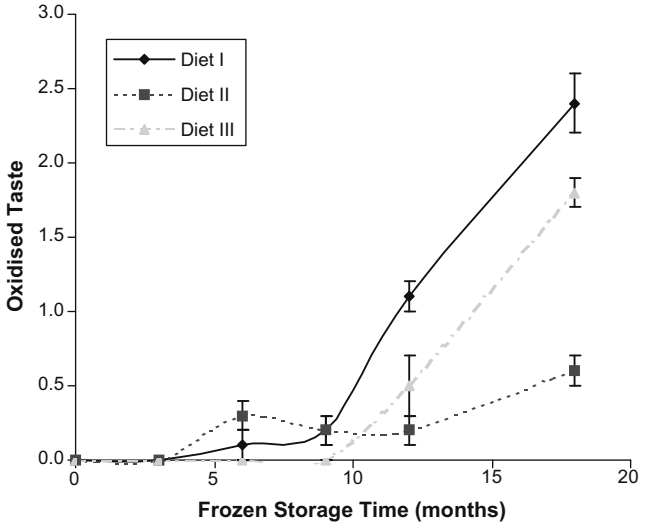

Fig. 3. Oxidised taste development in frozen coho salmon previously fed with different diets. Mean values of five ($n = 5$) independent determinations. Standard deviations are denoted by bars. Quadratic correlation values (r^2) between oxidised taste scores and frozen storage time are: 0.93 (diet I), 0.87 (diet II) and 0.92 (diet III).

According to previously mentioned results on primary and secondary lipid oxidation development (Tables 2 and 3), a higher interaction between nucleophilic compounds and lipid oxidation compounds was likely to occur in the present study, so that a higher fluorescence and browning (tertiary lipid oxidation compounds) development could be observed in fish previously fed with diet I. Such interaction compound formation has been shown to be responsible for important nutritional and sensory value losses during the frozen storage of fish species (Aubourg et al., 1998; Sikorski & Kolakowska, 1994).

3.4. Polyene index analysis

Polyunsaturated fatty acid breakdown was measured by following the PI of lipids in the white muscle (Fig. 2). This parameter showed no significant differences ($p > 0.05$) as a result of the frozen storage time for fishes corresponding to diets II and III, while a slight decreasing tendency ($r^2 = -0.84$, logarithmic fitting) could be observed for individuals from diet I. Fish individuals corresponding to diet II showed higher ($p < 0.05$) PI values than their counterparts from diet I; however, no significant differences ($p > 0.05$) could be assessed between fishes previously fed with both natural diets.

3.5. Sensory assessment of the oxidised taste

Oxidised taste was assessed in thawed and cooked fish. Progressive score increases with frozen storage time were observed in samples from all feeding conditions (Fig. 3), so that good correlation values (quadratic fitting) were observed in all cases ($r^2 = 0.93$, 0.87 and 0.92 for diets I, II and III, respectively). Very low scores (below 0.5) were given to all kinds of samples during the 0–9 month period; then, an increasing tendency period was observed for individuals from diets I and III. However, in all cases, taste scores were included in the acceptable domain, even at the end of the experiment (month 18). Comparison among diets led to the conclusion that individuals from diet I showed a higher rancid taste development for the 12–18 month period than their counterparts from diets I and III; additionally, a lower oxidised taste ($p < 0.05$) was developed at month 18 in individuals from diet II than in their counterparts from diet III.

Among the different biochemical lipid damage parameters studied in the present experiment, secondary lipid oxidation compounds are known to be the most closely related to the oxidised taste formation (White, 1994). However, correlation values of taste scores with AV ($r^2 = 0.67$–0.89) and TBA-i ($r^2 = 0.69$–0.79) were not found to be especially good. Better correlation values were obtained for the oxidised taste value with the FFA formation ($r^2 = 0.83$, 0.86 and 0.81 for diets I, II and III, respectively), according to previous research on frozen Atlantic salmon (Refsgaard, Brockhoff, & Jensen, 1998).

4. Conclusions

For the first time, to our knowledge, a comparative study on frozen fish quality was achieved, taking into account the effect of previous diets including synthetic and natural antioxidants.

Present results have shown an enhancement of lipid oxidation stability when employing a diet including natural antioxidants by replacement of synthetic ones during the commercialisation of frozen coho salmon. After 18 months of frozen storage at −18 °C, all kinds of fish samples provided acceptable oxidised taste scores. However, some different lipid oxidation development could be assessed for the different kinds of fish samples, according to the diet previously provided. Thus, replacement of synthetic antioxidants by a tocopherol isomers-rich mixture has shown a higher retention of primary and secondary lipid oxidation compounds, this leading to a lower formation of tertiary lipid oxidation compounds; such results were accompanied by a higher PI value and lower oxidised taste scores. Concerning lipid hydrolysis development, no effect could be found by employing different diets.

According to the wide range of benefits attributed to natural antioxidants, further studies focussing on this kind of diet replacement are to be continued. In addition to quality enhancement of the corresponding processed product, the fish development and growing improvement and the commercialisation of human foods including functional components are likely to be encountered.

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