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ORIGINAL PAPER

Development of Lipid Changes Related to Quality Loss During the Frozen Storage of Farmed Coho Salmon (*Oncorhynchus kisutch*)

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Abstract Lipid changes related to quality loss were evaluated during frozen storage of coho salmon for up to 15 months. Biochemical indices concerning lipid hydrolysis (free fatty acids, FFA) and oxidation (peroxide value, PV; thiobarbituric acid index, TBA-i; fluorescent compounds, FR; polyene index, PI) were determined and compared to sensory (odor and taste) and endogenous antioxidant (tocopherol isomers and astaxanthin) assessments. As a result of the frozen storage, lipid hydrolysis was shown to develop according to the increase in FFA content ($p < 0.05$). However, most biochemical lipid oxidation indices (PV, TBA-i and FR) led to a low degree of rancidity development ($p < 0.05$) when compared to other fatty fish species under similar frozen storage conditions. The PI value decreased ($p < 0.05$) at month 10 but then remained unchanged until the end of the experiment. Rancid odor and taste development were shown to be low throughout the experiment, according to the biochemical indices mentioned above. However, a progressive decrease ($p < 0.05$) in the original fresh odor and taste of salmon fish flesh occurred with increasing frozen storage time, such that fish samples had the poorest scores by month 15. Endogenous antioxidants were remarkably stable throughout the experiment and which might contribute to the oxidative stability of frozen farmed coho salmon lipids.

Keywords Coho salmon · Aquaculture · Frozen storage · Rancidity · Endogenous antioxidant

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Introduction

Marine foods have recently attracted more attention from consumers as sources of nutritional components that have positive benefits in human health and nutrition [1]. However, 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 [2].

The importance of aquaculture production of fish is best represented by the increasing production of coho salmon (*Oncorhynchus kisutch*) or silver salmon. Increasing production of this salmon in countries such as Chile, Japan and Canada has been reported [3]. This source is an important supplement to capture production in countries such as USA, Russian Federation, Canada and Japan [4]. Most research on this fish species has been carried out on genetic aspects and farming conditions during aquaculture production [5, 6]. However, previous composition or quality research is scarce. The composition studies related to fatty acid distribution [7, 8] and lipid changes related to quality loss during chilled storage [9].

The unusual rapid rate of deterioration of seafood quality has been recognized for many years. The role of spoilage may be characteristic of individual species or related to the mode of preservation thus a large range of metabolites related to quality loss have been proposed [10].

Freezing and frozen storage of fish have largely been employed to retain sensory quality and nutrients [11]. However, marine species with both highly unsaturated lipid composition and prooxidant molecules suffer from enzymatic and non-enzymatic rancidity and quality loss [12, 13]. Under frozen conditions, lipid oxidation compounds have been shown to facilitate protein denaturation [14] and nutrient losses [15].

The objective of this work was to evaluate biochemical and sensory changes in coho salmon produced via aquaculture during frozen storage.

Experimental Procedures

Raw material, Processing and Sampling

Farmed coho salmon (*Oncorhynchus kisutch)* samples used in this study were cultivated in a single tank by EWOS Innovation Research (Colaco, Puerto Montt, Chile). Sand-bed-filtered seawater (salinity range: 3.13–3.31 g/100 g) was supplied to the 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 commercial diet (Tables 1, 2) from EWOS Innovation Research.

The fish samples (weight range: 3.0–3.4 kg) were 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. After 3 days, the fish were placed at –20 °C. In addition to starting analyses with fresh fish, frozen individuals were taken for analysis on months 0, 4, 6, 8, 10, 12 and 15 of storage at –20 °C. Five different fish were analyzed separately at each sampling time ($n = 5$). According to lipid distribution variation in fish tissues [16], biochemical and sensory assessments were carried out on the dorsal muscle from the anterior part of the fish body.

Chemicals used in the present work (solvents, reagents) were reagent grade (Sigma Chemical Co., St Louis, MO, USA).

Table 1 Composition of the diet employed

Feed component	Content
General composition (g/100 g)	
Protein	40.0
Fat	28.4
Moisture	7.5
Ash	6.0
Crude fiber	1.6
Carbohydrates	16.5
Antioxidants (mg/kg)	
Ethoxyquin	22.0
Astaxanthin	70.0
BHT	3.0
Alpha-tocopherol	280.0
Gamma-tocopherol	22.4
Ascorbic acid	150.0

Fish supplier's data

Table 2 Fatty acid composition of the total lipids included in the diet employed

Fatty Acid	g/100 g FAME[a]	g/100 g diet
C 12:0	0.12	0.03
C 14:0	6.81	1.77
C 16:0	20.20	5.23
C 18:0	4.77	1.24
C 20:0	0.32	0.08
C 22:0	0.23	0.06
C 24:0	0.09	0.02
Total saturated	32.54	8.43
C 14:1	0.45	0.12
C 16:1	6.28	1.63
C 18:1	16.89	4.38
C 20:1 n9	2.38	0.62
C 22:1 n9	0.33	0.09
C 24:1	0.64	0.17
Total monounsaturated	26.96	6.99
C 18:2 n6	6.52	1.69
C 18:3 n6	0.73	0.19
C 18:3 n3	1.78	0.46
C 20:2 n6	0.29	0.07
C 20:3 n6	0.00	0.00
C 20:3 n3	0.00	0.00
C 20:4 n6	0.95	0.25
C 20:5 n3	12.94	3.35
C 22:5 n3	2.12	0.55
C 22:6 n3	14.83	3.84
Total polyunsaturated	40.16	10.40

Fish supplier's data

[a] *FAME* fatty acid methyl esters

Water and Lipid Contents

Water content was determined by the difference between the weight of fresh homogenized muscle (1–2 g) and the weight recorded after 24 h at 105 °C. Results are expressed as g water/100 g fish muscle. Lipids were extracted by the Bligh and Dyer method [17]. Quantification results are expressed as g lipid/100 g fish muscle.

Lipid Damage Analysis

Free fatty acid (FFA) content was determined by the Lowry and Tinsley [18] method based on complex formation with cupric acetate-pyridine followed by spectrophotometric (715 nm) assessment. Results are expressed as g FFA/100 g lipids. The peroxide value (PV), expressed as meq oxygen/kg lipid, was determined by the ferric thiocyanate method [19]. The thiobarbituric acid index (TBA-i) was determined according to Vyncke [20]. Results are expressed as mg malondialdehyde/kg fish muscle.

The formation of fluorescent compounds was determined with a Perkin Elmer LS 3B fluorimeter by measurements at 393/463 nm and 327/415 nm, as previously described [21]. 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 sulfate solution (1 μg/ml 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 aqueous phase resulting from the lipid extraction [17].

Lipid extracts were converted into fatty acid methyl esters by employing acetyl chloride and then analyzed by gas chromatography according to a previous procedure [22]. The polyene index (PI) was calculated as the following fatty acid ratio: C 20:5 + C 22:6/C 16:0.

Endogenous Antioxidant Assessment

Tocopherol isomers were analyzed according to the method of Cabrini et al. [23]. Both alpha and gamma isomers were detected in the subject salmon samples, and their content was expressed as mg/kg fish muscle.

Astaxanthin content was measured according to Sheehan et al. [24]. Absence of 9Z- and 13Z-isomers was confirmed; only E-isomers were detected in the present salmon samples. Results are expressed as mg all-E-astaxanthin (AX)/kg fish muscle.

Sensory Analyses

A quantitative descriptive analysis (QDA) was used to assess the expected rancid odor and taste development, as well as the loss of the original fresh odor and taste of salmon flesh. For it, ten panelists were selected. Prior to the study, panelists were trained by employing individual salmon of different degrees of quality [25].

At each sampling time, five fishes ($n = 5$) were cooked in polyethylene bags in a water bath and then submitted independently to the sensory panel. Rancid odor and taste development was evaluated using a 10 cm unstructured lineal scale from 0 (stage of no rancidity) to 10 (stage where no increase in rancidity is possible). Original fresh salmon odor and taste were also evaluated using a 10 cm unstructured lineal scale from 0 (stage where the original odor and taste are no more noticeable) to 10 (stage of full original odor and taste of salmon flesh). In all cases, score 5.0 was considered the borderline of fish acceptability. Scores among panelists were averaged.

Statistical Analyses

Data from the different biochemical and sensory analyses were subjected to one-way analysis of variance ($p < 0.05$) and to correlation analysis; comparison of means was performed using a least-squares difference (LSD) method [26]. Factor analysis (principal components) was carried out with all quality parameters; a Varimax normalized rotation was employed for factor rotation [26].

Results and Discussion

Water and Lipid Contents

The water content of salmon muscle ranged between 69.5 and 71.5% and extractable lipid matter fell within the range 3.5–5.5%. Variations in contents of both constituent (water and lipids) may be explained as a result of individual fish variation, and not arising from the frozen storage time. Water content was less than in the case of leaner fish species, according to an inverse ratio between water and lipid matter [9].

Lipid Hydrolysis

FFA increased ($p < 0.05$) during the storage indicating that lipid hydrolysis continued even at low temperatures (Table 3). This increase was found markedly higher during the first stage of the experiment (months 0–4), which agrees with previous research [21, 27]. This behavior has been explained as a result of a maximal lipase release from liposomes during the first month [28], which then facilitates closer proximity between enzyme and substrate. After that time, hydrolysis would develop more slowly because component migration is less likely to occur during frozen storage [21, 27]. From the present results, a fair logarithmic fitting was obtained between the FFA formation and the storage time ($r^2 = 0.81$).

While the formation of FFA itself does not lead to nutritional losses, its assessment is deemed important when considering the development of rancidity. Thus, a prooxidant effect of FFA on lipid matter has been proposed and explained on the basis of a catalytic effect of the

Table 3 Assessment of biochemical indices related to lipid damage during frozen storage of coho salmon

Frozen storage time (months)	FFA	PV	TBA-i	FR	PI
0	1.04 a (0.58)	2.31 a (1.25)	0.10 a (0.05)	0.31 a (0.07)	1.31 bc (0.05)
4	5.03 b (1.75)	3.58 a(0.90)	0.09 a (0.02)	0.28 a (0.08)	1.37 c (0.09)
6	5.03 b (1.60)	4.26 a (1.58)	0.16 a (0.09)	0.32 a (0.04)	1.29 bc (0.16)
8	6.77 bc (3.39)	5.20 ab (2.79)	0.20 a (0.13)	0.37 a (0.13)	1.37 c (0.19)
10	6.66 bc (2.16)	5.45 ab (2.90)	0.29 a (0.15)	0.71 b (0.24)	1.06 a (0.11)
12	7.46 bc (2.42)	4.60 ab (2.12)	0.23 a (0.15)	0.89 b (0.24)	1.15 ab (0.12)
15	8.11 c (2.14)	8.59 b (2.06)	0.54 b (0.27)	1.39 c (0.55)	1.03 a (0.08)

Abbreviations: *FFA* free fatty acids, *PV* peroxide value, *TBA-i* thiobarbituric acid index, *FR* fluorescence ratio, *PI* polyene index

In each column, mean values ($n = 5$) followed by different letters are significantly ($p < 0.05$) different. Standard deviations are indicated in brackets. Starting fresh fish values: 0.59 ± 0.32 (*FFA*), 1.43 ± 0.37 (*PV*), 0.05 ± 0.06 (*TBA-i*), 0.23 ± 0.07 (*FR*) and 1.29 ± 0.13 (*PI*)

carboxyl group on the formation of free radicals by the decomposition of hydroperoxides [29]. In addition, FFA have shown to interact with proteins leading to texture deterioration [14].

Lipid Oxidation

Different and complementary biochemical lipid oxidation indices were employed to evaluate the development of rancidity in the present experiment. Only a moderate peroxide formation was detected during the frozen storage period (Table 3), except for the end of the experiment when the highest mean value was obtained. However, values obtained throughout the experiment were relatively low when compared to fish species including a similar fat content and stored under the same frozen conditions [27, 30]. A marked development of primary oxidation cannot be verified in the present experiment.

Similar results were obtained for the secondary lipid oxidation assessment. Thus, a very low thiobarbituric acid reactive substance formation could be inferred in the 0–12 month period (Table 3), that was followed by a marked increase at the end of the storage. However, even the value obtained at month 15 (0.54 ± 0.27) can be considered relatively low if we compare it with results obtained on fish species with a similar fat content and kept frozen under similar conditions [21, 27, 31].

Complex formation as a result of interaction between oxidized lipids and nucleophilic molecules (mainly proteins) was measured by fluorescence (Table 3). Fluorescence detection provided very low mean values during the 0–8 month period. Then, a marked increase was obtained at month 10, followed by a sharp increase at month 15, which agrees with the increases in PV and TBA-i at the end of the storage. FR values obtained were lower than those observed for fish species (sardine, mackerel) including a similar fat content and kept frozen under similar conditions [21, 27].

As in the case of the FFA assessment, a fair correlation with the storage time was obtained ($r^2 = 0.89$, quadratic fitting) for the FR value. However, both indices (FFA and FR) showed a different behavior throughout the experiment in the sense that the first (FFA) showed the highest increase in the first stage (months 0–4) of the experiment (logarithmic fitting), while the FR increased markedly in the last stage (months 10–15) of the study (quadratic fitting).

Damage to polyunsaturated fatty acids during frozen storage was measured by the PI value, which was not significantly different during the 0–8 storage period (Table 3). Then, a decrease was observed at month 10, which also corresponds to PUFA damage produced by lipid oxidation. After this time, no more differences throughout the remaining storage time were observed. An inverse relationship could be inferred from the PI decrease and the FR increase throughout the present study. As a good correlation value was obtained between both indices ($r^2 = -0.88$).

Sensory Analysis

As expected, a progressive sensory quality loss was observed as a result of the frozen storage time. A significant ($p < 0.05$) rancid odor and taste development was observed in salmon fish (Fig. 1). Both parameters (odor and taste) led to very good correlation values with the storage time ($r^2 = 0.95$ and $r^2 = 0.96$, respectively; both quadratic fittings). However, even at month 15, rancidity scores were found to be very low for both sensory parameters, showing a good agreement with the conclusions obtained from the lipid oxidation biochemical indices (Table 3). Fair correlation values for both rancid odor and taste assessments were obtained with the FR value ($r^2 = 0.82$ and $r^2 = 0.88$, respectively).

Rancid odor development has been shown to be the limiting factor of acceptability for most wild fatty fish species under the same freezing conditions, where 7 months shelf-life were obtained [27]. However, according to the

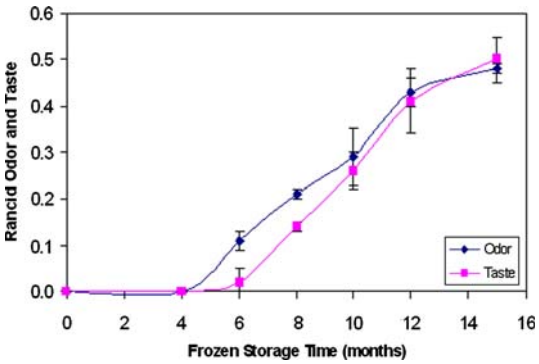

Fig. 1 Rancid odor and taste detection during coho salmon frozen storage. *Bars* denote standard deviations of the mean ($n = 5$). Starting fresh fish values: 0.0 for both parameters

present results, rancid odor development was not found to be markedly relevant as it is during the frozen storage of other farmed salmonoid species such as rainbow trout [32] and Atlantic salmon [33].

The loss of the original fresh odor and taste of salmon fish flesh was also evaluated (Fig. 2). In this case, a marked ($p < 0.05$) quality loss was obtained with storage time. The odor value was less than 5.0 even at the end of the experiment indicating poor quality. Good correlation values were obtained for the odor and taste assessments with the storage time ($r^2 = -0.87$ and $r^2 = -0.95$, quadratic and linear fittings, respectively). When compared to biochemical indices, the best correlation values of original freshness (odor and taste) loss were obtained with the FR value ($r^2 = -0.78$ and $r^2 = -0.70$, respectively).

Multivariate Analysis

In order to segregate the different parameters (biochemical and sensory indices) into different factors, principal component analysis (PCA) was carried out. Thus, 78.97% of the variability of the variables under study could be explained with two factors. Following a Varimax rotation (Table 4), it was found that Factor 1 alone accounted for 64.04% of the variability. Factor 1 had relatively high loadings (0.85–0.93) for the four sensory parameters, and fair loadings for the biochemical indices (0.62–0.83). Factor 2 accounted for only 14.94% of the variability.

Results obtained by PCA indicated that sensory determination was more accurate than biochemical assessment to evaluate the degradation in quality of coho salmon during frozen storage. This conclusion agrees with the above mentioned results where sensory parameters afforded better correlation values with time than the biochemical indices.

Endogenous Antioxidant Assessment

Tocopherol isomer assessment is expressed in Fig. 3. The content of such compounds provided some differences throughout the experiment that could be explained as a result of differences from fish to fish and not as a result of the frozen storage. Both isomers (alpha and gamma) showed similar results to those obtained when studying other cultivated fish species such as rainbow trout [34] and Atlantic salmon [35]. In the present experiment, both tocopherol isomers did not decrease significantly ($p > 0.05$) with the storage time, although previous research indicates important losses in both isomers during the frozen storage of other farmed species such as blue and red tilapia [36] and channel catfish [37].

Different tocopherols (α-, β- ,γ- and δ-tocopherol) have been identified in plants and all have been found in most seaweeds and unicellular algae. However, α-tocopherol has been reported to be the only tocopherol, which accumulates in higher marine animals from natural diets that presumably originally included all four tocopherols [38, 39]. Deposition of different tocopherol molecules (primarily

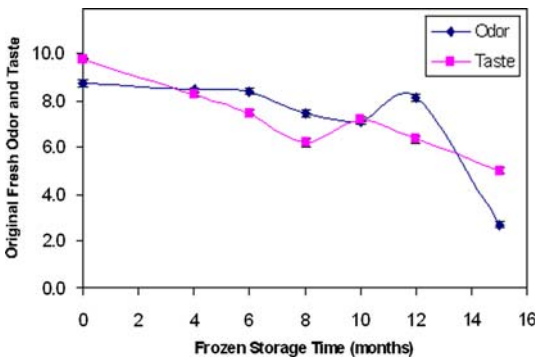

Fig. 2 Detection of original fresh odor and taste during coho salmon frozen storage. *Bars* denote standard deviations of the mean ($n = 5$). Starting fresh fish values: 10.0 for both parameters

Table 4 Factor loadings from principal component analysis of biochemical and sensory parameters measured in frozen coho salmon

Parameter	Factor 1	Factor 2
Free fatty acids	0.702	–0.525
Peroxide value	0.633	–0.643
Thiobarbituric acid index	0.755	–0.230
Fluorescence ratio	0.824	0.354
Polyene index	–0.622	–0.627
Rancid odor	0.926	0.158
Rancid taste	0.931	0.209
Original fresh odor	–0.852	–0.032
Original fresh taste	–0.884	0.114

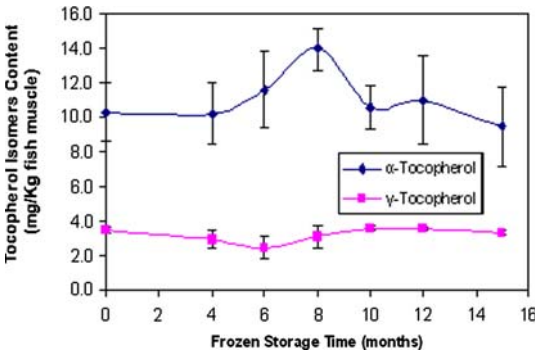

Fig. 3 Tocopherol isomers present during coho salmon frozen storage. *Bars* denote standard deviations of the mean ($n = 5$). Starting fresh fish values: 11.0 ± 0.5 and 3.3 ± 0.4 for alpha and gamma isomers, respectively

α, γ) in farmed fish did occur and could be strongly influenced by the feed provided [40, 41].

Recent demands for new sources of feed constituents have been addressed by oils from terrestrial plants, so that the presence of a large range of bioactive substances such as the different tocopherol isomers would guarantee a positive effect on the fish health and growth [42]. However, a lack of the long chain polyunsaturated fatty acid content (ω-3 series, particularly) has been recognized as a major disadvantage. Therefore, finishing diet strategies for fish have been developed, where diets based on plant oil are fed during the growing phase and then replaced by a fish oil diet in the final months prior to slaughter [42].

Similar conclusions can be obtained from the astaxanthin (AX) content analysis (Fig. 4). Again, content differences during storage can be explained as a result of fish to fish variation, and not from the frozen treatment. Values range (8.0–10.0) was similar to the one obtained previously for cultivated coho salmon [9], but higher than the one determined for wild coho salmon [43]. Compared to other cultivated fish species, the actual starting fish value (8.6 ± 1.4) was higher than that reported in previous research on Atlantic salmon [35, 44] and rainbow trout [32, 34].

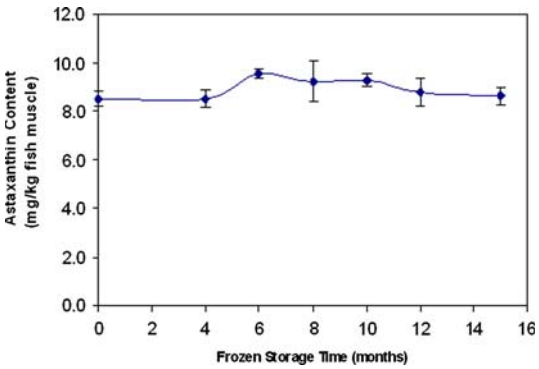

Fig. 4 Astaxanthin present during coho salmon frozen storage. *Bars* denote standard deviations of the mean ($n = 5$). Starting fresh fish value: 8.6 ± 1.4

Previous research has shown that AX can deteriorate either due to non-enzymatic degradation, e.g., by light, heat, oxygen, or to enzymatic degradation, e.g., lipoxygenase, peroxidase [45]. In the present case, a decreasing tendency in AX content was not observed with frozen storage time, according to a previous research carried out on a chilled storage experiment on coho salmon [9]. In contrast, previous studies have shown an AX content decrease during the frozen storage of Atlantic salmon [32] and rainbow trout [34] and as a result of post-harvesting of Atlantic salmon [35].

Marine lipids are constituted of highly unsaturated fatty acids that are known to be very prone to oxidation, leading to an important effect on fish quality loss [12]. However, according to biochemical lipid damage indices closely related to rancidity development (PV, TBA-i and FR), such a damage pathway was found to be slow in frozen farmed coho salmon, and values attained were low when compared to other fatty fish species kept under the same storage conditions. This result was corroborated by the rancid odor and taste development, which was still very low even after 15 months of frozen storage. However, an important quality loss was observed by the sensory panel according to the loss of the fresh odor and taste that were initially present in the salmon flesh; such scores led to non-acceptable fish at the end of the experiment. Accordingly, a quality loss pathway other than rancidity development may account for the sensory changes in the present experiment.

Carotenoids and tocopherols are known as endogenous antioxidants that can act as scavengers of free radicals, so that protection against the very early stages of lipid oxidation would be favored [34]. In addition, AX is well known as the main pigment responsible for the pink color of salmonoid fish species, so that its retention during processing should be very important to guarantee consumer acceptance and retain the commercial value of the product. Since relatively high tocopherol isomers and AX contents in salmon muscle were maintained throughout the present experiment, their presence is likely most responsible for the lipid stability observed in this study.

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References

1. Simopoulos A (1997) Nutritional aspects of fish. In: Luten J, Börrensen T, Oehlenschläger J (eds) Seafood from producer to consumer, integrated approach to quality. Elsevier, London, pp 589–607
2. Stickney R (1990) A global overview of aquaculture production. Food Rev Intern 6:299–315
3. FAO (2006) Aquaculture production Yearbook 2004 vol 98/2. Food and agriculture organization of the United Nations, Rome, pp 72
4. FAO (2006) Capture Production, Yearbook 2004, vol 98/1. Food and agriculture organization of the United Nations, Rome, pp 109
5. Estay F, Díaz N, Neira R, García X (1997) Reproductive performance of cultured female coho salmon in Chile. Prog Fish Cult 59:36–40
6. Winkler F, Bartley D, Díaz N (1999) Genetic differences among year classes in a hatchery population of coho salmon (*Oncorhynchus kisutch*) in Chile. Aquaculture 173:425–433
7. Gruger E, Nelson R, Stansby M (1964) Fatty acid composition of oils from 21 species of marine fish, freshwater fish and shellfish. J Am Oil Chem Soc 41:662–667
8. Braddock R, Dugan L (1969) Fatty acids of lake Michigan coho salmon. J Food Sci 37:426–429
9. Aubourg S, Vinagre J, Rodríguez A, Losada V, Larraín MA, Quitral V, Gómez J, Maier L, Wittig E (2005) Rancidity development during the chilled storage of farmed coho salmon (*Oncorhynchus kisutch*). Eur J Lipid Sci Technol 107:411–417
10. Gill TA (1990) Objective analysis of seafood quality. Food Rev Intern 6:681–714
11. Erickson M (1997) Lipid oxidation: flavor and nutritional quality deterioration in frozen foods. In: Erickson M, Hung YC (eds) Quality in frozen food. Chapman and Hall, New York, pp 141–173
12. Harris P, Tall J (1994) Substrate specificity of mackerel flesh lipopolygenase. J Food Sci 59:504–506, 516
13. Richards M, Hultin H (2002) Contributions of blood and blood components to lipid oxidation in fish muscle. J Agric Food Chem 50:555–564
14. Mackie I (1993) The effects of freezing on flesh proteins. Food Rev Intern 9:575–610
15. Castrillón A, Álvarez-Pontes E, García M, Navarro P (1996) Influence of frozen storage and defrosting on the chemical and nutritional quality of sardine (*Clupea pilchardus*). J Sci Food Agric 70:29–34
16. Zhou S, Ackman RG, Morrison C (1996) Adipocytes and lipid distribution in the muscle tissues of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 53:326–332
17. Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
18. Lowry R, Tinsley I (1976). Rapid colorimetric determination of free fatty acids. J Am Oil Chem Soc 53:470–472
19. Chapman R, McKay J (1949) The estimation of peroxides in fats and oils by the ferric thiocyanate method. J Am Oil Chem Soc 26:360–363
20. Vyncke W (1970) Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. Fette Seifen Anstrichm 72:1084–1087
21. Aubourg S, Sotelo C, Pérez-Martín R (1998) Assessment of quality changes in frozen sardine (*Sardina pilchardus*) by fluorescence detection. J Am Oil Chem Soc 75:575–580
22. Lepage G, Roy C (1986) Direct transesterification of all classes of lipids in a one step reaction. J Lipid Res 27:114–120
23. Cabrini L, Landi L, Stefanelli C, Barzanti V, Sechi M (1992) Extraction of lipid and lipophilic antioxidants from fish tissues: a comparison among different methods. Comp Biochem Physiol B Biochem Mol Biol 101:383–386
24. Sheehan E, O'Connor T, Sheehy P, Buckley D, Fitzgerald R (1998) Stability of astaxanthin and canthaxanthin in raw and smoked Atlantic salmon (*Salmo salar*) during frozen storage. Food Chem 63:313–317
25. Sveinsdottir K, Martinsdottir E, Hyldig G, Jørgensen G, Kristbergsson K (2002) Application of quality index method (QIM) scheme in shelf-life study of farmed Atlantic salmon (*Salmo salar*). J Food Sci 67:1570–1579
26. Statsoft (1994) Statistica for Macintosh. statsof, USA
27. Aubourg S, Rodríguez A, Gallardo J (2005) Rancidity development during frozen storage of mackerel (*Scomber scombrus*): effect of catching season and commercial presentation. Eur J Lipid Sci Technol 107:316–323
28. Sikorski Z, Kolakowski E (2000) Endogenous enzyme activity and seafood quality: influence of chilling, freezing, and other environmental factors. In: Haard N, Simpson B (eds) Seafood enzymes. Utilization and influence on postharvest seafood quality. Marcel Dekker, New York, pp 451–487
29. Yoshida H, Kondo I, Kajimoto G (1992) Participation of free fatty acids in the oxidation of purified soybean oil during microwave heating. J Am Oil Chem Soc 69:1136–1140
30. Verma J, Srikar L, Sudhakara N, Sarma J (1995) Effects of frozen storage on lipid freshness parameters and some functional properties of oil sardine (*Sardinella longiceps*) mince. Food Res Intern 28:87–90
31. Kurade S, Baranowski J (1987) Prediction of shelf life of frozen minced fish in terms of oxidative rancidity as measured by TBARS number. J Food Sci 52:300–302, 311
32. Christophersen A, Bertelsen G, Andersen H, Knuthsen P, Skibsted L (1992) Storage life of frozen salmonids. Effect of light and packaging conditions on carotenoid oxidation and lipid oxidation. Z Lebensm Unters Forsch 194:115–119
33. Refsgaard H, Brockhoff P, Jensen B (1998) Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar*) during frozen storage. J Agric Food Chem 46:3473–3479
34. Jensen C, Birk E, Jokumsen A, Skibsted L, Bertelsen G (1998) Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. Z Lebensm Unters Forsch 207:189–196
35. Gordon Bell J, McEvoy J, Webster J, McGhee F, Millar R, Sargent J (1998) Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). J Agric Food Chem 46:119–127
36. Erickson M, Thed S (1994) Comparison of chemical measurements to differentiate oxidative stability of frozen minced tilapia fish muscle. Int J Food Sci Technol 29:585–591
37. Brannan R, Erickson M (1996) Quantification of antioxidants in channel catfish during frozen storage. J Agric Food Chem 44:1361–1366
38. Syväoja EL, Salminen K, Piironen V, Varo P, Erojoki O, Koivistoinen P (1985) Tocopherols and tocotrienols in Finnish foods: Fish and fish products. J Am Oil Chem Soc 62:1245–1248
39. Sigurgisladóttir S, Ackman RG, O'Keefe SF (2003) Selective deposition of alpha-tocopherol in lipids of blue mussels (*Mytilus edulis*). J Food Lipids 1:97–109
40. Sigurgisladóttir S, Parrish C, Ackman RG, Lall S (1994) Tocopherol deposition in the muscle of Atlantic salmon (*Salmo salar)*. J Food Sci 59:256–259
41. Parazo M, Lall S, Castell J, Ackman RG (1998) Distribution of α- and γ-tocopherols in Atlantic salmon (*Salmo salar*) tissues. Lipids 33:697–704
42. Pickova J (2006) Plant oils as fish oil replacements in fish feeds. Lipid Technol 18:173–176

43. Iwamoto R, Myers J, Hershberger W (1990) Heritability and genetic correlations for flesh coloration in pen-reared coho salmon. Aquaculture 86:181–190
44. Bjerkeng B, Refstie S, Fjalestad K, Storebakken T, Rødbotten M, Roem A (1997) Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. Aquaculture 157:297–309
45. Krinsky N (1989) Antioxidant functions of carotenoids. Free Radic Biol Med 7:617–635