Free Polyunsaturated Fatty Acids Cause Taste Deterioration of Salmon during Frozen Storage

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Increased intensity of train oil taste, bitterness, and metal taste are the most pronounced sensory changes during frozen storage of salmon (Refsgaard, H. H. F.; Brockhoff, P. B.; Jensen, B. Sensory and Chemical Changes in Farmed Atlantic Salmon (*Salmo salar)* during Frozen Storage. *J. Agric. Food Chem*. **1998a**, *46*, 3473−3479). Addition of each of the unsaturated fatty acids: palmitoleic acid (16:1, $n - 7$), linoleic acid (C18:2, $n - 6$), eicosapentaenoic acid (EPA; C20:5, $n - 3$) and docosahexaenoic acid (DHA; C22:6, $n - 3$) to fresh minced salmon changed the sensory perception and increased the intensity of train oil taste, bitterness, and metal taste. The added level of each fatty acid (∼1 mg/g salmon meat) was equivalent to the concentration of the fatty acids determined in salmon stored as fillet at −10 °C for 6 months. The effect of addition of the fatty acids on the intensity of train oil taste, bitterness and metal taste was in the order: DHA > palmitoleic acid > linoleic acid > EPA. Formation of free fatty acids was inhibited by cooking the salmon meat before storage. Furthermore, no changes in phospholipid level were observed during frozen storage. The results suggest that enzymatic hydrolysis of neutral lipids plays a major role in the sensory deterioration of salmon during frozen storage.

Keywords: *Free fatty acids; flavor; hydrolysis; salmon*

INTRODUCTION

The most pronounced sensory changes during frozen storage of salmon are increased intensity of train oil taste, bitterness and metal taste (Refsgaard et al., 1998a). It was not established which products that caused the sensory deterioration, but the peroxide value and the content of free fatty acids were the instrumental data describing the sensory data best. Relationships between hydrolysis and lipid oxidation are a matter of debate. It is generally accepted that free fatty acids are oxidized more readily than esterified fatty acids (Labuza, 1971), but in tissue free fatty acids are reported both to enhance lipid oxidation (Han and Liston, 1987) and to inhibit it (Mazeaud and Bilinski, 1976). A linkage between phospholipid hydrolysis and lipid peroxidation during frozen storage is reported for lean fish muscle (Han and Liston, 1987), but no conclusive data have previously been published for salmon. Triglyceride hydrolysis is suggested to lead to increased oxidation while phospholipid hydrolysis produces the opposite effect (Shewfelt, 1981).

Formation of volatile lipid oxidation products during frozen storage is shown in salmon (Milo and Grosch, 1996; Refsgaard et al., 1998a) and trout (Milo and Grosch, 1993). We demonstrated that formation of volatile oxidation products is not the cause of the most pronounced sensory changes found during frozen storage of salmon (Refsgaard et al., 1998a) and suggested that compounds of low volatility contributed to the increased intensity of train oil taste, bitterness and metal taste. Hydrolysis, resulting in free fatty acids of low volatility, has previously been suggested to be more important than lipid oxidation for deterioration of trout during frozen storage (Ingemansson et al., 1995). We have shown a substantial increase in free fatty acid content during frozen storage of salmon and a correlation between the sensory perception and the level of free fatty acids (Refsgaard et al., 1998a). Here we demonstrate that polyunsaturated fatty acids added to fresh salmon mince in levels equivalent to the levels formed during frozen storage contribute to a similar sensory perception as after frozen storage. We also present evidence that enzymatic hydrolysis of neutral lipids has a key role in the sensory deterioration of salmon during frozen storage.

MATERIALS AND METHODS

Materials. *Salmon*. Farmed salmon (*Salmo salar*) of 4−4.5 kg were from Sekkingstad A/S (Skogsvåg, Norway). The fish had been fed with Vextra Omega, a commercial diet from Ewos Aqua A/S (Bergen, Norway). Details of feed composition and average flesh content of lipid, tocopherols, astaxanthin and fatty acid composition have been described previously (Refsgaard et al., 1998b). The salmon were kept for 3 days on ice during transport and were stored at −10 °C or −20 °C as fillets for the sensory analysis and raw or cooked as cutlets for the chemical analyses. Both the fillets and cutlets were wrapped in aluminum foil and polyethylene bags during the storage.

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10.1021/jf000021c CCC: $19.00
Published on Web 07/29/2000

Cutlets of 60 g to be stored as cooked were heated for 20 min at 100 °C in a hot-air oven before freezing.

Fatty Acids. Palmitic acid (16:0), palmitoleic acid (16:1, $n-7$), oleic acid (18:1, $n-9$), linoleic acid (18:2, $n-6$), eicosenoic acid (20:1, $n-9$), eicosapentaenoic acid (EPA; C20:5, $n-3$), docosahexaenoic acid (DHA; C22:6, $n-3$) and other fatty acid standards were from Nu-Chek-Prep (Elysian, MN). Possible traces of volatile oxidation products, which could contribute with flavor, were removed from each fatty acid by purging with 340 mL/min argon for 30 min at room temperature. The purified fatty acids were analyzed for the presence of volatiles by purging 10 mg fatty acid for 10 min with 340 mL/min nitrogen at 45 °C and collecting the volatiles on a Tenax-trap. No volatiles were found by GC−MS after thermal desorption of the trap.

Descriptive Sensory Analysis. Seven odor and eight taste attributes were selected for the cooked minced salmon. Salmon samples of 60 g were placed in porcelain bowls covered with aluminum foil and heated for 20 min at 100 °C in a hot-air oven and immediately chopped for 1 min in a kitchen chopper. Purified fatty acids (1−1.5 mg/g salmon) were added singly during the chopping. For each sensory attribute the score sheets carried unstructured scales of 15 cm anchored 1 cm from the ends with terms which limit the attributes. The panel consisted of 10 trained persons, six females and four males between the ages of twenty five and sixty, who were selected by use of screening tests (ISO, 1991). The assessors were trained using both fresh salmon and salmon stored at −10 °C for 7 months. The panelists evaluated four samples twice at each session, but were not informed that they only were served four different samples nor that fatty acids were added to some of the samples.

Statistical Analysis. A mixed model analysis of variance with the three factors, assessor, session, and sample, was applied to test for significant differences between the samples for each attribute. Effects due to the assessors (main and interaction effects) were considered random. The session effect was considered a block effect, i.e., in the analysis the main effects was corrected for. For the attributes with significant sample differences the fresh samples with added fatty acids and the stored salmon samples were compared with the fresh samples using Dunnett's t-tests. The SAS procedure MIXED was used (Littell et al., 1996). In addition, a canonical variates analysis (CVA) was performed to study the sample differences from a multivariate perspective. For this analysis the Assessor*Sample interaction term was used as error term, corresponding as closely as possible to the univariate mixed models applied for each attribute. For this analysis the SAS procedure GLM was used.

Dynamic Headspace Sampling and GC−MS. Immediately after addition of the purified fatty acids the volatiles in the minced samples were collected on Tenax-traps as described by Refsgaard et al. (1999), to test if volatile oxidation products were formed during the 1 min chopping**.** Volatiles from the same salmon without addition of fatty acids were also collected**.** The volatiles were desorbed by use of an automatic thermal desorber (ATD400, Perkin-Elmer) and identified and quantified by GC-MS, using a HP 5890 IIA gas chromatograph equipped with a DB 1701 column (0. 25 mm × 30 m × 1.0 μm; J&W Scientific, CA) coupled to a HP 5972A mass selective detector. The helium flow was 1.3 mL min^{-1} and the temperature program was: 65 °C for 1 min, raised by 4 °C min^{-1} to 90 °C and further raised by 20 °C min^{-1} to 240 °C, and held for 4 min. Ionization energy of the mass spectrometer was set at 70 eV in the EI mode and the detector operated with a mass range between 30 and 250 with a scan rate at 3.4 scan s^{-1}. For quantification calibration curves from the headspace sampling of five concentration levels of each volatile compound were used (Refsgaard et al., 1999).

Bligh-and-Dyer Extraction. Salmon samples were minced and two portions of 10 g of mince were extracted with methanol−chloroform (Bligh and Dyer, 1959).

Separation of Lipid Classes. The lipid in the Bligh-and-Dyer extracts was separated in three classes: neutral lipids, free fatty acids and polar lipids by use of 3 cm^3 aminopropyl Sep-Pak Vac solid-phase extraction cartridges (Waters, Marlborough, MA) as described by Kaluzny et al. (1985).

Fatty Acid Composition. *Preparation of Methyl Esters*. Fatty acids of the lipids in the Bligh-and-Dyer extracts were trans-esterified to methyl esters using a base-catalyzed transesterification followed by a boron-trifluoride-catalyzed esterification according to the AOCS method Ce 1b-89 (AOCS, 1994). The methyl esters were dissolved in *n*-heptane to a concentration of about 20 mg mL^{-1}.

Gas Chromatographic Analysis. A HP 5890 gas chromatograph (Hewlett-Packard, Avondale, PA), equipped with a flame ionization detector was used. The column was an Omegawax 320 fused silica capillary column (0. 32 mm × 30 m × 0.25 μm; Supelco, Bellefonte, PA). The injection volume was 0.2 μL, injected in the split mode with a split ratio of 1:25. The injection and detection temperatures were 250 °C and 240 °C, respectively. The initial oven temperature program was 160 °C, immediately raised by 3 °C min^{-1} to 200 °C, held for 1 min, further raised by 3 °C min^{-1} to 220 °C, and held for 12 min. The helium carrier gas flow was 21 cm s^{-1}.

Free Fatty Acids (FFA). FFA-contents were determined by acidometric titration of the Bligh-and-Dyer extracts after adding ethanol and using phenolphthalein as an indicator. The FFA-content was calculated as oleic acid (AOCS Official Method Ca 5a-40) (AOCS, 1994).

Peroxide Values. Peroxides values were determined by iodometric titration of the Bligh-and-Dyer extracts after addition of acetic acid and using a reaction time of 5 min after addition of potassium iodide; otherwise the procedure was identical to the AOCS Official Method Cd8−53 (AOCS, 1994). Peroxide values were calculated as milliequivalents per kilogram of lipid.

RESULTS

Increased levels of free fatty acids during frozen storage are reported in several fish species (Shewfelt, 1981). The free fatty acid level increased from 0.6 to 10% of the total lipids in salmon stored as fillet at −10 °C for 7 months (Refsgaard et al., 1998a). The fatty acids: Palmitic acid (16:0), palmitoleic acid (16:1, $n-7$), oleic acid (18:1, $n-9$), linoleic acid (18:2, $n-6$), eicosenoic acid (20:1, $n-9$), eicosapentaenoic acid (EPA; C20:5, $n-3$) and docosahexaenoic acid (DHA; C22:6, $n-3$) were the major fatty acids and contributed each with 5 to 15% of the total fatty acids in fresh salmon (Refsgaard et al., 1998b). As expected these fatty acids were also the major compounds of the free fatty acid fraction after storage (Table 1). Taste assessment by descriptive sensory analysis of each of these six fatty acids in water showed that palmitoleic acid, linoleic acid, EPA and DHA had high intensity of train oil, bitter and metal taste (not shown).

Perception of Salmon with Added Free Fatty Acids. The level of EPA and DHA in raw salmon stored at −10 °C for 6 months was approximately 1 mg/g salmon and about 0.5 mg/g for palmitoleic acid and linoleic acid (Table 1). Each of these four fatty acids were added, in turn, at the 1 mg/g level and for DHA also at 1.5 mg/g, to fresh, cooked minced salmon and their effect was evaluated by descriptive sensory analysis. The addition of the fatty acids significantly increased the intensity of train oil taste, bitterness and metal taste (Figure 1). Addition of DHA, especially at the concentration of 1.5 mg/g salmon, changed the sensory perception of fresh salmon to a perception equal to that observed after 7 months storage at −10 °C. Addition of free fatty acids did not effect the odor.

In the canonical variates analysis only a single canonical component was significant. This means that the sample differences for all the attributes can be

Table 1. Fatty Acid Composition of the Neutral Lipid (NL), the Phospholipid (PL), and Free Fatty Acid (FFA) Fractions in Salmon (mg Fatty Acid/g Salmon)[a,b]

	fresh salmon			−10 °C, raw			−10 °C, cooked	−20 °C, raw	−20 °C, cooked
fatty acid	NL	PL	FFA	NL	PL	FFA	FFA	FFA	FFA
14:0	6.1	0.09	0.05	6.5	0.02	0.5	0.07	0.2	0.06
15:0	0.4	nd	nd	0.4	nd	0.04	0.01	0.01	0.01
16:0	15	0.4	0.2	16	0.5	1.8	0.3	0.5	0.3
16:1 ($n-7$)	7.5	0.04	0.06	8.0	0.03	0.5	0.08	0.2	0.07
16:2	0.6	nd	nd	0.6	nd	0.05	0.01	0.01	nd
17:0	0.3	nd	nd	0.3	nd	0.03	0.02	0.01	0.01
18:0	3.1	0.03	0.05	3.4	0.05	0.3	0.05	0.08	0.05
18:1 ($n-9$)	15	0.08	0.1	16	0.1	0.9	0.14	0.3	0.1
18:1 ($n-7$)	3.5	0.01	0.03	3.7	0.04	0.3	0.05	0.1	0.04
18:2 ($n-6$)	4.7	0.03	0.06	5.0	0.06	0.4	0.07	0.1	0.04
18:3 ($n-3$)	1.3	0.01	0.02	1.4	0.02	0.1	0.02	0.02	0.02
18:4 ($n-3$)	2.5	0.01	0.02	2.8	0.04	0.2	0.03	0.05	0.02
20:1 ($n-9$)	9.7	0.01	0.08	10	0.03	0.6	0.09	0.2	0.09
20:2 ($n-6$)	0.5	nd	nd	0.5	nd	0.04	0.01	0.02	0.01
20:4 ($n-6$)	0.6	nd	nd	2.2	0.04	0.06	0.01	0.02	0.01
20:3 ($n-3$)	0.2	nd	nd	0.2	nd	0.02	nd	nd	nd
20:4 ($n-3$)	2.0	0.03	0.02	2.2	0.04	0.2	0.03	0.05	0.02
20:5 ($n-3$)	9.4	0.3	0.1	10	0.3	1.1	0.2	0.3	0.2
22:1 ($n-11$)	9.8	0.01	0.1	11	0.02	0.6	0.1	0.2	0.1
22:1 ($n-9$)	1.2	nd	0.02	1.3	nd	0.09	0.02	0.03	0.1
22:5 ($n-3$)	3.9	0.9	0.03	4.1	0.1	0.3	0.05	0.08	0.04
22:6 ($n-3$)	11	1.2	0.1	13	1.5	1.2	0.3	0.3	0.2
24:1 ($n-9$)	0.8	nd	0.02	0.9	0.02	0.08	0.02	0.03	0.02
total	109	3.1	1.1	120	2.9	9.4	1.7	2.8	1.5

[a] The salmon were fresh or stored for 6 months as cutlets ($n = 2 \times 2$), and the standard deviation from the mean was 3−10% within the same salmon and 10−30% on the given mean values for two salmon. [b] nd: none detected.

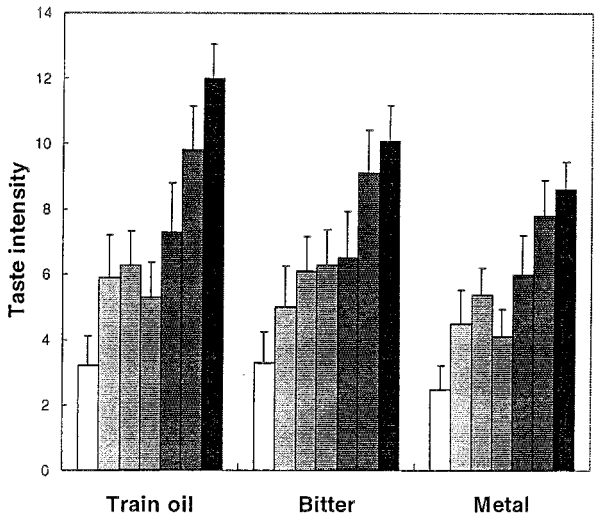

Figure 1. Taste scores for fresh and stored salmon and fresh salmon with added fatty acids. The bars show the mean values for each of the following samples: (1) fresh salmon, (2) fresh salmon with 1 mg/g palmitoleic acid, (3) fresh salmon with 1 mg/g linoleic acid, (4) fresh salmon with 1 mg/g eicosapentaenoic acid (EPA), (5) fresh salmon with 1 mg/g docosahexaenoic acid (DHA), (6) fresh salmon with 1.5 mg/g DHA, (7) salmon stored for 7 months at −10 °C. Standard deviation from the mean is shown.

expressed through a single linear combination of the fifteen attributes or that the positions of the samples in the fifteen-dimensional attribute space was essentially on a line. Differences between samples were expressed by the canonical scores (Table 2) and addition of fatty acids to fresh salmon changed the sensory perception against the stored samples. DHA had the largest effect followed by palmitoleic acid, linoleic acid, and EPA.

Only small amounts of oxidation products from the fatty acids were formed after addition of fatty acids and chopping of the salmon meat. The increase of hexanal, heptanal and octanal was in the range: 5−100 μg/kg salmon, 1−15 μg/kg salmon and 5−30 μg/kg salmon,

Table 2. Canonical Scores from a Canonical Variates Analysis on the Sensory Data[a,b]

sample	score	standard error
fresh salmon	0.86	0.16
+16:1 ($n-7$)	0.22	0.22
+18:2 ($n-6$)	0.27	0.16
+20:5 ($n-3$)	0.39	0.18
+22:6 ($n-3$)	−0.20	0.27
+22:6 ($n-3$)[c]	−0.91	0.25
stored salmon	−1.64	0.20

[a] The fatty acids were added singly to fresh salmon at the 1 mg/g salmon level. [b] The canonical variates analysis was performed as described in the Methods section. [c] 1.5 mg/g salmon.

respectively. No other oxidation products could be detected and there was no systematic relationship between the level of oxidation products and the degree of unsaturation of the fatty acid added. The odor threshold of hexanal and heptanal in a meat model system is 5870 μg/L and 230 μg/L, respectively (Brewer and Vega, 1995) and the flavor threshold of octanal in oil is 900 μg/L (Lea and Swoboda, 1958). It is therefore not likely that these three lipid oxidation products caused the altered perception of fresh salmon with fatty acids added.

Chemical Changes During Frozen Storage of Salmon. The formation of free fatty acids during frozen storage depended on the storage temperature and heat treatment before storage (Figure 2). Only in the raw samples stored at −10 °C a pronounced increase of free fatty acids was found, suggesting that lipolytic enzymes were heat-inactivated by cooking. The level of free fatty acids was also higher in the raw compared to the cooked samples when stored at −20 °C. Furthermore, for the cooked samples, at both storage temperatures, almost no change in the free fatty acid level was found during storage time.

Table 1 shows the composition of the free fatty acid fraction in salmon after 6 months of storage. The level of formation of free fatty acids depended on the storage temperature (Figure 2), but the composition of the free

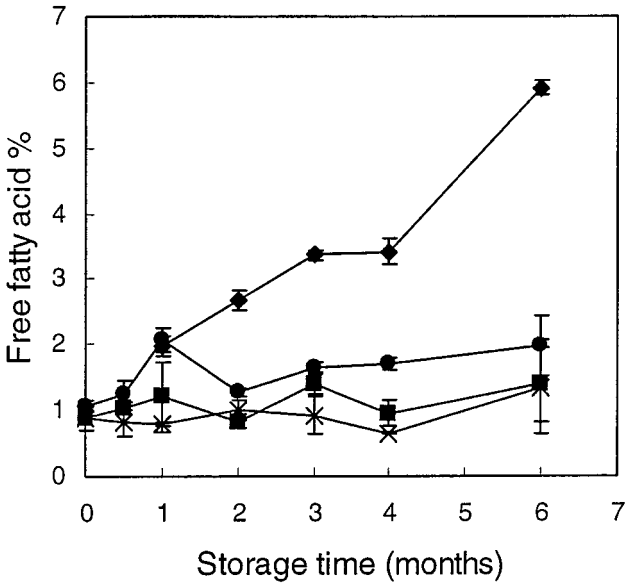

Figure 2. Changes in free fatty acid level during frozen storage of salmon: ◆, raw salmon stored at −10 °C; ■, cooked salmon stored at −10 °C; ●, raw salmon stored at −20 °C; ×, cooked salmon stored at −20 °C. Standard deviation from the mean is shown.

Table 3. Lipid Classes, Neutral Lipids (NL), Free Fatty Acids (FFA), and Phospholipids (PL), Given as mg/g Salmon[a,b]

treatment	NL	FFA	PL
raw, fresh	109 ± 3	1.1 ± 0.1	3.1 ± 0.6
cooked, fresh	100 ± 29	1.1 ± 0.1	2.7 ± 0.2
raw, −10 °C for 6 months	120 ± 20	9.4 ± 2.1	2.9 ± 0.3
cooked, −10 °C for 6 months	113 ± 2	1.7 ± 0.1	3.3 ± 0.1
raw, −20 °C for 6 months	124 ± 10	2.8 ± 0.6	2.8 ± 0.4
cooked, −20 °C for 6 months	112 ± 2	1.5 ± 0.2	3.8 ± 1.1

[a] Analysis on two salmon stored as cutlets ($n = 2 \times 2$). [b] ±: standard deviation from the mean.

fatty acid fraction did not change by cooking or due to the storage temperature. The fatty acid composition of the free fatty acid fraction was similar to the composition of the neutral lipids (Table 1).

The amount of neutral lipids, phospholipids, and free fatty acids are given in Table 3. The level of phospholipids did not change during storage which indicates that the formation of free fatty acids during the frozen storage was not due to hydrolysis of the phospholipids. Additionally, a higher level of free fatty acids was determined after storage for 6 months of raw salmon at −10 °C than the level of phospholipid detected in the salmon. These results suggest that the formation of free fatty acids was due to enzymatic hydrolysis of the neutral lipids. The mean values of the neutral lipid fractions had a high standard derivation (Table 3) which can be explained by large differences in the lipid content between the two salmon due to biological variation and distribution differences of the lipid (Refsgaard et al., 1998b).

Formation of peroxides was, like the formation of free fatty acids, dependent on the storage temperature and heat treatment before storage (Figure 3). Again, the most pronounced increase in peroxides was in raw salmon stored at −10 °C. This could be due to free fatty acids being oxidized more readily than fatty acids bound in the triacylglycerol structure. It is of interest that the cooking of the meat did not cause additional formation of peroxides. Cooking would destruct membrane structures, facilitate release of heme iron and thereby one could expect faster oxidation processes.

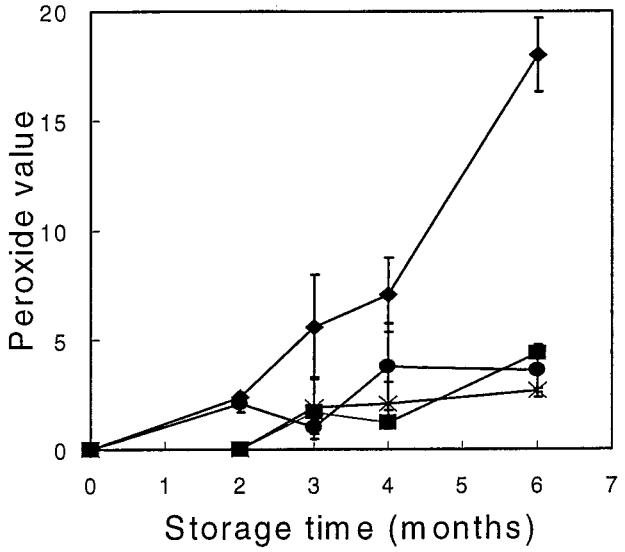

Figure 3. Changes in level of lipid peroxides (mequiv/kg) during frozen storage of salmon: ◆, raw salmon stored at −10 °C; ■, cooked salmon stored at −10 °C; ●, raw salmon stored at −20 °C; ×, cooked salmon stored at −20 °C. Standard deviation from the mean is shown.

DISCUSSION

Addition of long-chain polyunsaturated fatty acids deteriorated the flavor of fresh salmon by increasing the intensity of train oil, bitterness and metal taste (Figure 1). An increased intensity of these taste attributes is the most pronounced sensory change during frozen storage of salmon (Refsgaard et al., 1998a). The even-numbered fatty acids from butyric (C4:0) to lauric (C12:0) accounted for the fatty acid contribution to rancid flavor when added to fresh milk (Scanlan et al., 1965). They also tested C16:0, C18:0 and C18:1 but these fatty acids did only contribute little, if any, to the rancid flavor. Taste of long-chain fatty acids has not been demonstrated before, but indirect effects of free fatty acids on textural changes by promotion of protein denaturation and on flavor deterioration by enhancing lipid oxidation have been suggested (Shewfelt, 1981). Linoleic acid hydroperoxides, the primary oxidation products of linoleic acid, are transformed into mono-, di- and trihydroxy acids and some of these derivatives are reported to have a bitter taste (Belitz and Grosch, 1987). In the present experiment, there was only few minutes between the addition of the fatty acid and the sensory analysis. It is therefore not likely that formation of hydroxy acids was the cause of the changed perception of the fresh salmon with added free fatty acids.

Formation of free fatty acids during frozen storage has previously been suggested to be more important than lipid oxidation for deterioration of trout (Ingemansson et al., 1995). Here we demonstrate that polyunsaturated fatty acids added to fresh salmon mince in levels equivalent to the levels formed during frozen storage contribute to the same sensory perception as that observed after frozen storage. We also show evidence that enzymatic hydrolysis of neutral lipids has a key role in sensory deterioration of salmon during frozen storage.

The release of lipases from lysosomes in trout during frozen storage decrease at lower temperatures (Geromel

and Montgomery, 1980), which is in agreement with the higher level of free fatty acids formed in salmon stored at −10 °C compared to −20 °C (Figure 2). In mackerel hydrolytic activity was retarded by cooking or by lowering the storage temperature (Hwang and Regenstein, 1996), like seen here for salmon (Figure 2). The almost complete inhibition of the formation of free fatty acids in the cooked samples made us conclude that enzymatic activity is the major cause of hydrolysis during frozen storage of salmon.

Lipolysis in raw tuna muscle occurs preferentially in the *sn*-1 and *sn*-3 acyl positions of triacylglycerols, with a consequent release of saturated and monounsaturated fatty acids (Medina et al., 1994). The composition of the free fatty acid fraction of salmon was similar to the composition of the neutral lipid (Table 1) and no pattern for the enzymatic hydrolysis was seen, but the level of palmitic acid and EPA was higher in the free fatty acid fraction and the levels of oleic acid, eicosenoic acid and cetoleic acid (C22:1, $n - 11$) lower. The level of phospholipids did not change during storage (Table 3) which indicate that hydrolysis of neutral lipids dominates in salmon.

Relationships between hydrolysis and lipid oxidation during frozen storage of fish are a matter of debate. A linkage between phospholipid hydrolysis and lipid oxidation during frozen storage has been reported in low fat fish muscle (Han and Liston, 1987). Shewfelt (1981) suggests that triglyceride hydrolysis leads to increased oxidation while phospholipid hydrolysis produces the opposite effect. In mackerel the rate of oxidation was not influenced by lipolysis (Hwang and Regenstein, 1996; Brake and Fennema, 1999). Our results in Figures 2 and 3 suggest a relation between hydrolysis and formation of lipid peroxides in salmon. From Figure 3 can be seen that the cooking process did affect the formation of peroxides when the salmon were stored at −10 °C, but not at −20 °C. This can be explained by inactivation of enzymes involved in the initiation of oxidation processes or as discussed above, by the fact that free fatty acids are more easily oxidized than fatty acids in the triglyceride structure. The decrease in the ratio of C22:6 to C16:0 has been used as an index of oxidation (Khayat and Schwall, 1983). We found a decrease in this ratio for the fatty acids and phospholipids in stored salmon, but not for the neutral lipids, indicating that only the free fatty acids and phospholipids were oxidized. The lowest ratios were for salmon stored raw at −10 °C. German et al. (1992) studied 12-lipoxygenase and 15-lipoxygenase in carp and trout at 0 °C, their half-life was <3 and 10 h, respectively. The 15-lipoxygenase had very little activity in trout. These results suggest that lipoxygenase activity is not a major factor in the oxidation and support the hypothesis that in frozen salmon hydrolysis of neutral lipids is the initial process of deterioration followed by oxidative reactions of the free fatty acids.

ABBREVIATIONS

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid(s); GC−MS, gas chromatography−mass spectrometry

ACKNOWLEDGMENT

The authors are grateful to Inge Holmberg and Pernille Jørgensen for technical assistance. Jonas Nordahl and the panelists are thanked for participation in the sensory assessments.

LITERATURE CITED

AOCS. *The Official Methods and Recommended Practices of the American Oil Chemists' Society*; The American Oil Chemists' Society: Champaign, IL, 1994.

Belitz, H.-D.; Grosch, W. *Food Chemistry*; Springer-Verlag: New York, 1987.

Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.

Brake, N. C.; Fennema, O. R. Lipolysis and lipid oxidation in frozen minced mackerel as related to Tg′, molecular diffusion, and presence of gelatin. *J. Food Sci.* **1999**, *64*, 25−32.

Brewer, M. S.; Vega, J. D. Detectable odor thresholds of selected lipid oxidation compounds in a meat model system. *J. Food Sci.* **1995**, *60*, 592−595.

German, J. B.; Zhang, H.; Berger, R. Role of lipoxygenase in lipid oxidation in foods. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; American Chemical Society: Washington, DC, 1992; pp 74−92.

Geromel, E. J.; Montgomery, M. W. Lipase release from lysosomes of rainbow trout (*Salmo gairdneri*) muscle subjected to low temperatures. *J. Food Sci.* **1980**, *45*, 412−419.

Han, T.-J.; Liston, J. Lipid peroxidation and phospholipid hydrolysis in fish muscle microsomes and frozen fish. *J. Food Sci.* **1987**, *52*, 294−296, 299.

Hwang, K. T.; Regenstein, J. M. Lipid hydrolysis and oxidation of mackerel (*Scomber scombrus*) mince. *J. Aquat. Food Prod. Technol.* **1996**, *5*, 17−27.

Ingemansson, T.; Kaufmann, P.; Ekstrand, B. Multivariate evaluation of lipid hydrolysis and oxidation data from light and dark muscle of frozen stored rainbow trout (*Oncorhynchus mykiss*). *J. Agric. Food Chem.* **1995**, *43*, 3, 2046−2052.

ISO 3972. *Sensory analysis − Methodology − Method of Investigating Sensitivity of Taste*; International Standardization Organization: Geneva, Switzerland, 1991.

Kaluzny, M. A.; Duncan, L. A.; Merritt, M. V.; Epps, D. E. Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J. Lipid Res.* **1985**, *26*, 135−140.

Khayat, A.; Schwall, D. Lipid Oxidation in Seafood. *Food Technol.* **1983**, 130−140.

Labuza, T. P. Kinetics of lipid oxidation in foods. *CRC Crit. Rev. Food Techn.* **1971**, *2*, 355−405.

Lea, C. H.; Swoboda, P. A. T. The flavour of aliphatic aldehydes. *Chem. Ind. (London)* **1958**, *1958*, 1289−1290.

Littell, R. C.; Milliken, G. A.; Stroup, W. W.; Wolfinger, R. D. *SAS System for Mixed Models*; SAS Institute Inc.: Cary, NC, 1996.

Mazeaud, F.; Bilinski, E. Free fatty acids and the onset of rancidity in rainbow trout (*Salmo gairdneri*) flesh. Effect of phospholipase A. *J. Fish. Res. Board Canada* **1976**, *33*, 1297−1302.

Medina, I.; Sacchi, R.; Aubourg, S. ^{13}C nuclear magnetic resonance monitoring of free fatty acid release after fish thermal processing. *J. Am. Oil Chem. Soc.* **1994**, *71*, 479−482.

Milo, C.; Grosch, W. Changes in the Odorants of Boiled Trout (*Salmo fario*) as Affected by the Storage of the Raw Material. *J. Agric. Food Chem.* **1993**, *41*, 2076−2081.

Milo, C.; Grosch, W. Changes in the odorants of boiled salmon and cod as affected by the storage of the raw material. *J. Agric. Food Chem.* **1996**, *44*, 4, 2366−2371.

Refsgaard, H. H. F.; Brockhoff, P. B.; Jensen, B. Sensory and Chemical Changes in Farmed Atlantic Salmon (*Salmo salar)* during Frozen Storage. *J. Agric. Food Chem.* **1998a**, *46*, 3473−3479.

Refsgaard, H. H. F.; Brockhoff, P. B.; Jensen, B. Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem.* **1998b**, *46*, 808−812.

Refsgaard, H. H. F.; Haahr, A.-M.; Jensen, B. Isolation and Quantification of Volatiles in Fish by Dynamic Headspace Sampling and Mass Spectrometry. *J. Agric. Food Chem*. **1999**, *47*, 1114−1118.

Scanlan, R. A.; Sather, L. A.; Day, E. A. Contribution of free fatty acids to the flavor of rancid milk. *J. Dairy Sci*. **1965**, *58*, 1582−1584.

Shewfelt, R. L. Fish muscle lipolysis − a review. *J. Food Biochem*. **1981**, *5*, 79−100.

Received for review December 30, 1999. Revised manuscript received May 26, 2000. Accepted June 1, 2000. This work was supported by The Danish Research and Development Program for Food Technology through the LMC Center for Advanced Food Studies.

JF000021C