Sensory and Chemical Changes in Farmed Atlantic Salmon (*Salmo salar*) during Frozen Storage

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Farmed Atlantic salmon (*Salmo salar*) were stored as fillets at −10 and −20 °C and whole at −30 °C. The most pronounced sensory changes were first recognized by the assessors, when the salmon samples were in the oral cavity, and were significant increases in train oil taste, metal taste, and bitter taste in the fillets. This was shown by mixed model analysis of variance and canonical variates analysis. Volatile lipid peroxidation products such as aldehydes and ketones were identified and quantified in the salmon. For most of the peroxidation products the concentration increased during storage. The content of lipid hydroperoxides and free fatty acids also increased during storage, and the changes were fastest in salmon stored at −10 °C. A decrease in highly unsaturated fatty acids was observed in salmon stored at −10 and −20 °C. Peroxide values and the content of free fatty acids were shown by a partial least-squares analysis to be the best of the instrumental data in describing the sensory changes.

Keywords: *Salmon; taste; volatiles; canonical variates analysis; lipid peroxidation; lipid hydroperoxides; free fatty acids*

INTRODUCTION

The high levels of polyunsaturated lipids make fish highly susceptible to deterioration by oxidation processes. Rancidity in fatty fish such as salmon is not well-characterized and has been described as fish oil taste (Andersen and Steinsholt, 1992; Sylvia et al., 1995) and as fatty and train-oily odors (Milo and Grosch, 1996). The rancid off-flavor in salmon is caused by formation of volatile oxidation products such as aldehydes and ketones (Milo and Grosch, 1996). Some of these volatile compounds have very intense odors and flavors and are, even in small concentrations, able to affect the sensory quality. The rancid off-flavor of salmon is mainly caused by an increase in (*E*,*Z*)-2,6-nonadienal with a cucumber odor, (*Z*)-3-hexenal with a green odor, and (*Z*,*Z*)-3,6-nonadienal with a fatty odor (Milo and Grosch, 1996). These three aldehydes are formed from n-3 unsaturated fatty acids by oxidative processes (Grosch, 1987).

To describe and quantify sensory changes in salmon during storage conditions relevant for retail, we have developed and used descriptive sensory analysis. In addition we have followed the development of volatiles with the objective of determining the cause of some of the sensory changes in salmon. As shown by Bell et al. (1998) and in our own investigations (Refsgaard et al., 1998), the lipid content and concentration of lipid constituents such as tocopherols and astaxanthin are highly variable also in farmed salmon. The biological variation coefficients of these components were about 25% (Refsgaard et al., 1998), and this would probably cause a very high variation in storage stability and sensory quality. Therefore, in addition to sensory evaluation of salmon fillets and determination of content of volatiles, we have measured lipid content, fatty acid composition, and concentration of tocopherols and astaxanthin in each salmon before and after storage.

MATERIALS AND METHODS

Salmon. Farmed salmon (*Salmo salar*), 56 fish 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., 1998). The salmon were kept for 3 days on ice during transport and were stored as fillets at −10 or −20 °C wrapped in aluminum foil and polyethylene bags. Salmon used as references were stored whole at −30 °C after having been blast-frozen at −45 °C, glazed, and wrapped in aluminum foil and polyethylene bags. After storage, the fillets were thawed in water at 0 °C for 16 h. Each fillet was cut transversally into seven cutlets, ≈3 cm wide (cooked samples), and six cutlets, ≈1 cm wide (raw samples). From each fillet, the third cutlet counting from the head end was used for headspace sampling of volatiles, whereas the other cutlets were sensorily assessed.

Chemicals. DL-α-, -γ-, and -δ-tocopherol were from Merck (Darmstadt, Germany). *all-trans*-Astaxanthin and *all-trans*-canthaxanthin packed under nitrogen in sealed ampules were obtained from Hoffmann-La Roche (Basel, Switzerland). Fatty acid standards were from Nu-Chek-Prep (Elysian, MN). Refined and deodorized fish oil was a gift from the pilot plant at the Technical University of Denmark, courtesy of Anne-Mette Haahr. (*E*)-2-Hexenal (99%), heptanal (95%), (*E*,*E*)-2,4-heptadienal (90%), nonanal (95%), octanal (98%), and (*E*)-2-octenal (94%) were from Aldrich Chemie (Steinheim, Germany). Hexanal (98%) was from Merck, and decanal (99%) was from Sigma (St. Louis, MO).

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S0021-8561(98)00309-4 CCC: $15.00
Published on Web 08/22/1998

Descriptive Sensory Analysis. Ten sensory attributes were selected for raw and twenty-five for cooked salmon. For cooking, salmon samples of 60 g were placed in porcelain bowls covered with aluminum foil and heated for 16 min at 100 °C in a hot-air oven. 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, seven females and three males between the ages of 25 and 60, who were selected by use of screening tests (ISO 3972, 1991). The assessors were trained using both fresh salmon and salmon stored for half a year at −20 °C. At each assessment, salmon stored at −30 °C was presented to the panelists and the intensities of the sensory attributes of this open reference were placed on the score sheets. The panelists evaluated four randomized cooked samples, including a hidden reference and four raw samples also including a hidden reference after the open raw reference sample. The panelists were neither informed that one of the random samples was a hidden reference nor that the experiment was a storage experiment.

Additionally two panelists assessed the color of raw samples using a Roche color card for salmon.

Headspace Sampling of Volatiles. The cooked and raw samples were cut in pieces and frozen in liquid nitrogen and powderized. To salmon powder (20 g) was added 25 mL of water. The aqueous suspensions of salmon powder were purged with 340 mL/min nitrogen at 45 °C for 30 min, and volatiles were collected on Tenax-GR (Chrompack, Bergen op Zoom, The Netherlands) traps (225 mg in Perkin-Elmer (Buckinghamshire, U.K.) 1/4 in. steel tubes). Water was blown off the traps using 50 mL min^{-1} nitrogen at ambient temperature for 15 min. All collections were made in triplicate.

Headspace Sampling of Standards. For quantification purposes C_6−C_{14} aldehydes and some ketones dissolved in fish oil in five sets of concentrations were added to samples of 20 g of powderized fresh salmon with a low concentration of volatiles. The standards were collected as described above.

GC-MS. Volatiles were desorbed from the Tenax traps by use of an automatic thermal desorber system ATD400 from Perkin-Elmer. A Hewlett-Packard (Palo Alto, CA) 5890 IIA gas chromatograph equipped with a HP 5972 A mass selective detector was used for identification and quantification. A DB 1701 column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, Folsom, CA) with a flow of 1.3 mL of helium min^{-1} and the following temperature program was used: 25 °C for 1 min, 25−175 °C at 4 °C min^{-1}, 175−240 °C at 20 °C min^{-1}, and hold at 240 °C for 7 min. The mass selective detector used ionization at 70 eV and 50 μA emission. Scans were performed in the range from 30 to 350 amu at a rate of 2.2 scans s^{-1}.

Quantification. Results from the collections of standards described above were used to prepare a calibration curve for each standard compound by use of HP ChemStation software.

GC-Olfactometry. The volatiles were thermally desorbed from Tenax traps and separated on a DB 1701 column (30 m × 0.32 mm × 1.0 μm; J&W Scientific) using the temperature program described above. The odors were perceived at a sniffing port (olfactory detector outlet, OD-1, from SGE, Ringwood, Australia).

Sample Preparation. Before storage about 100 g of muscle was taken from each right-side fillet. The sample was taken from near the head end (at the pectoral fin) as a transversal cut from back to belly and including the dark muscle. After storage, about 100 g of muscle was taken in the same manner from each left-side fillet. The skin was removed, and the muscle samples were cut into pieces, frozen in liquid nitrogen, vacuum-packed in Riloten 40/50 bags (Otto Nielsen Emballage, Lyngby, Denmark), and stored for not more than 1 month at −40 °C before analysis. All samples were still frozen when minced, and two portions of 10 g of mince from each fish were extracted with methanol−chloroform (Bligh and Dyer, 1959). Oil percent, concentration of astaxanthin, canthaxanthin, and tocopherols, peroxide values, and the content of free fatty acids were determined in duplicate. Single determinations were used for the fatty acid composition.

Oil content was determined gravimetrically after evaporation of solvent from the Bligh-and-Dyer extracts. Evaporation was done overnight at room temperature followed by drying at 105 °C for 1 h.

Astaxanthin Content. The solvent from 4.00 g of Bligh-and-Dyer extract was evaporated under nitrogen at a maximum temperature of 30 °C, and the residue was redissolved in 2.00 mL of *n*-heptane. Canthaxanthin and astaxanthin isomers were separated by isocratic HPLC using a LKB (Bromma, Sweden) 2150 HPLC pump, a LKB 2157 autosampler, and a LKB 2151 variable wavelength monitor. The separation was carried out using a LiChrosorb Si60 column (100 mm × 3 mm × 5 μm; Chrompack) with a Chromsep silica (s2) (10 mm × 2 mm) guard column, eluting with 1.2 mL min^{-1} *n*-heptane:acetone (86:14 (v/v)) and detection at 470 nm.

Tocopherol Content. The solvent from 4.00 g of Bligh-and-Dyer extract was evaporated under nitrogen at a maximum temperature of 30 °C, and the residue was redissolved in 2.00 mL of *n*-heptane. Samples were analyzed by isocratic HPLC using a LKB 2150 HPLC pump, a LKB 2157 autosampler, and a Shimadzu (Kyoto, Japan) fluorescence HPLC monitor. Separation was carried out on a Spherisorb s5w column (250 mm × 4.6 mm; Phase Separations Ltd., Deeside, U.K.), using a flow of 1.0 mL min^{-1} *n*-heptane:2-propanol (100:0.4 (v/v)) and fluorescence detection (excitation at 290 nm; emission at 330 nm) (Official Method Ce 8−89 (AOCS, 1994)).

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 trans-esterification 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. An 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 1:50. The injection and detection temperatures were 250 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}.

Peroxide Values. Peroxide values (PVs) 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). PVs were calculated as milliequivalents per kilogram of lipid.

Free Fatty Acids. Free fatty acid (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 (Official Method Ca 5a-40 (AOCS, 1994)).

Statistical Analysis. For the sensory attributes, analysis of variance was performed to test for the significance of any kind of treatment or time effects. For these analyses 420 observations (=10 assessors × 7 time points × 3 treatments × 2 salmon) were used, except for missing values. A mixed model was used, in which the time and temperature were considered fixed effects. The main effect of assessor together with the three assessor interaction terms was considered random. The salmon-to-salmon biological variation (two salmon within each time−temperature combination) was also considered as a random effect. This means that the effects of time and temperature and their interactions were tested versus a combination of the assessor−time−temperature interaction effects and the biological salmon variation. This is done by the SAS procedure MIXED (Littell et al., 1996).

To study the multivariate sensory structure, we used canonical analysis (see, e.g., Mardia et al. (1979)). Like principal component analysis (PCA), canonical variates analysis (CVA) finds a low-dimensional space spanning the major variation between certain means of the data. One of the

Table 1. Intensity of Sensory Attributes[a] in Salmon Stored at −10 °C and Results from the Analysis of Variance of the Sensory Data

	intensity score after storage for given no. of weeks								results from ANOVA[c]		
attribute	0[b]	2	7	11	15	17	26	34	time	temp	time × temp
					Raw Salmon						
cucumber odor	10.3	8.7	7.5	7.4	7.7	7.8	4.9	7.4		**	
earthy odor	3.6	1.2	3.0	2.6	2.8	3.3	1.8	3.3		***	
train oil odor	0	1.0	0.9	1.5	1.5	0.6	4.7	3.0		*	
linseed oil odor	0	0.7	0.2	0.2	0.2	0.2	1.9	0.9		**	
color	6.8	7.3	6.1	5.9	6.9	6.9	7.8	5.1	*		
					Cooked Salmon						
boiled potato odor	10.0	9.0	8.5	8.7	9.2	8.5	9.3	6.5	*		
train oil odor	0	0.5	1.2	1.0	0.4	0.6	1.1	2.3		*	
color	12.0	10.5	9.3	9.5	10.7	9.9	11.1	8.4	*	*	
earthy taste	10.5	8.6	8.3	8.2	7.4	6.9	8.5	4.8	***	***	
fish oil taste	7.0	7.1	5.2	6.2	6.4	6.0	5.4	5.5		***	
train oil taste	0	0.7	3.3	2.4	1.9	3.3	2.8	4.7	**	***	*
metal taste	2.7	2.6	3.8	3.6	2.8	4.0	3.3	5.4	**	**	**
bitter taste	0	0.3	1.8	1.7	0.4	1.1	2.2	3.1	*	*	*
firmness	9.3	7.9	10.7	10.2	9.8	11.0	9.8	10.0		**	
juiciness	11.0	9.0	5.0	6.5	4.8	4.3	6.2	6.9	***		
fibrousness	0.4	1.7	3.6	3.9	3.6	3.1	2.6	3.7		**	

[a] Sensory attributes for which significant effects were found. [b] Intensities for the references. [c] Key to asterisks: *, **, *** represent significant effects at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

strengths of CVA is that it takes the error structure into account as opposed to PCA performed on mean sensory scores. CVA can be viewed as a PCA, where the variables are weighted according to their error ("noise") level and their within treatment interrelations. In combination with a mixed model approach, we can thus take the assessor variability as well as the biological variation into account in the multivariate analysis. These two variations define two different ways of measuring the error of an average sensory score. An approximate confidence region was computed for the canonical score plot using the average number of observations for each combination of storage time and temperature (Mardia et al., 1979). We present the results of a CVA where the salmon effect (within time and temperature) was used as the multivariate error term. The effects of investigation were the combined time, temperature, and time−temperature effects, corresponding to a PCA on mean scores for each time−temperature combination. This means that variables with a large mean-square will be down weighted in the analysis. The CVA was performed in SAS procedure GLM using the MANOVA statement with the E-option to define the multivariate error term.

For the aldehydes and ketones we also performed analysis of variance on the raw and the cooked salmon data separately. For the analyses of the chemical data 56 observations (=7 time points, 2 salmon stored at 2 temperatures, 4 salmon at the control temperature (−30 °C)) were used, except for missing values, since the average was taken of the replicate measurements on the same salmon.

Correlations between the sensory data and the chemical data were investigated by partial least-squares (PLS) regression. The Unscrambler software package version 3.54 (CAMO A/S, Trondheim, Norway) was used for the analysis with full systematic cross-validation as the validation method.

RESULTS

Sensory Changes during Frozen Storage of Salmon. Of the 35 sensory attributes used to describe the sensory impression of salmon, 16 showed significant temperature, time, or temperature−time interaction effects (Table 1). The odor of fresh raw salmon was characterized as cucumber-like with weak sweet, sourish, and fish oil notes. For the fresh and cooked salmon the same descriptors were used, but a boiled potato odor was the most pronounced attribute instead of cucumber.

The taste of fresh cooked salmon was characterized by a high intensity of earthy, sweet, sourish, and fish oil notes. The most pronounced sensory changes of salmon during frozen storage were first recognized by the assessors when the salmon samples were in the oral cavity. For train oil, metal, and bitter taste significant time−temperature effects were determined (Table 1). The intensities of train oil, metal, and bitter taste increased during storage at −10 and −20 °C (Figure 1). The intensity of earthy and fish oil flavor decreased in salmon stored at the high temperatures.

Both for cooked and raw samples significant color changes were found during the storage time (Table 1). The salmon color intensity decreased during frozen storage, and this change was not dependent on the storage temperature. In addition fillets stored at −10 °C for 34 weeks had a lower score on the Roche color map. An average score of 4 was given for all other samples, and fillets stored at −10 °C for 34 weeks obtained a score of 2.

Significant changes of the texture were also determined for salmon stored at −10 and −20 °C. The texture of a fresh cooked salmon was described as flaky, firm, and juicy. Storage at −10 and −20 °C changed the texture to more firm, less juicy, and more fibrous.

The CVA revealed that with a *P*-value of 0.499 we can accept that two canonical components are sufficient to describe the systematic multivariate time−temperature sensory structure. The first CVA component was the linear combination of all the sensory attributes that discriminates the best among samples in the sense of having the largest *F*-value for sample differences. The second CVA component expressed the second largest *F*-value. The test for only one component had a *P*-value of 0.0843, and we have chosen to retain the second component. The first two components accounted for 72.6% of the variation. The canonical coefficients (in SAS:"raw canonical coefficients") for the first canonical component were most extreme for earthy odor for raw salmon (+1.58), metal taste (−1.41), fish oil taste (−1.13), and fibrous texture (−1.13) for cooked salmon. The remaining coefficients were all numerically below 1. These are the weights with which each attribute enter the "best" linear combination of sensory attributes when emphasis is to discriminate treatment effects.

In Figure 2 the best canonical scores and "loadings" are plotted for the first two components. By "loadings" we mean the correlations, computed across time−

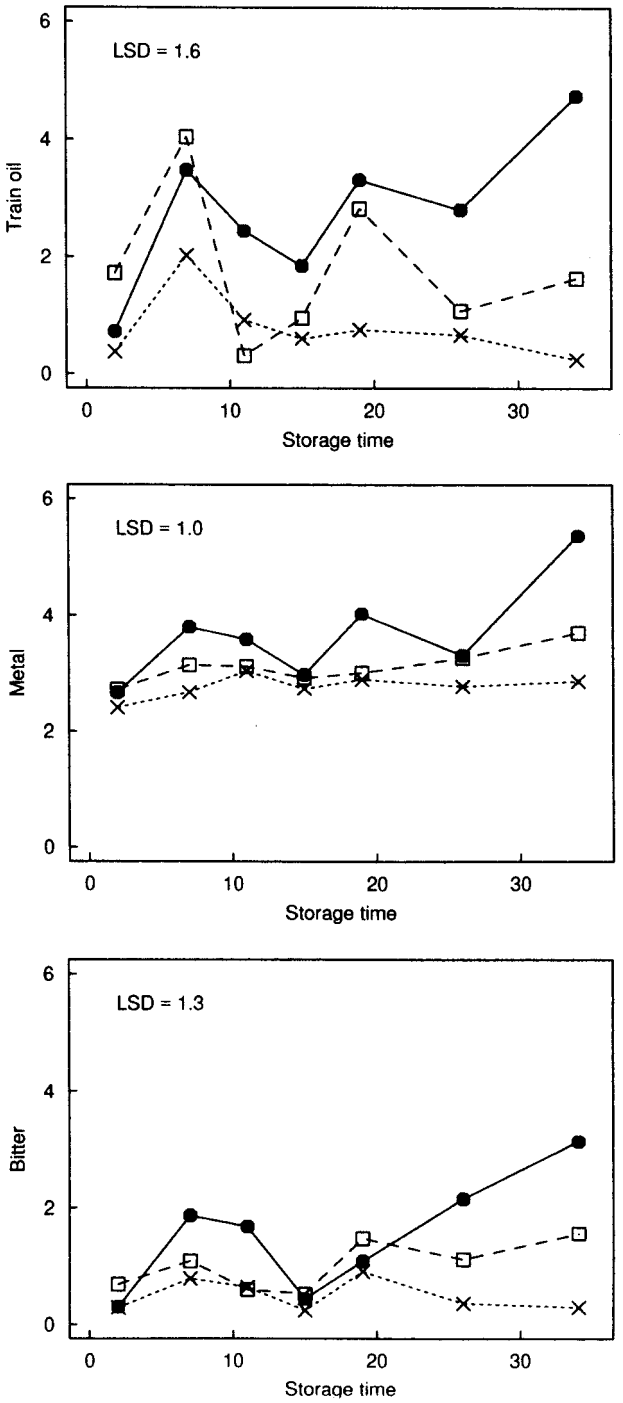

Figure 1. Changes in train oil taste, metal taste, and bitter taste of salmon during frozen storage. Score of taste intensity of salmon after weeks of storage at −10 °C (− ● −), −20 °C (- -□- -), and −30 °C (···×···). The values are the average of the panelists' assessments on an unstructured scale of 15 cm of two samples which have been treated identically. The LSD values are approximate 95% least significant difference values based on the mixed model analysis of variance and the average number of observations in each time−temperature combination.

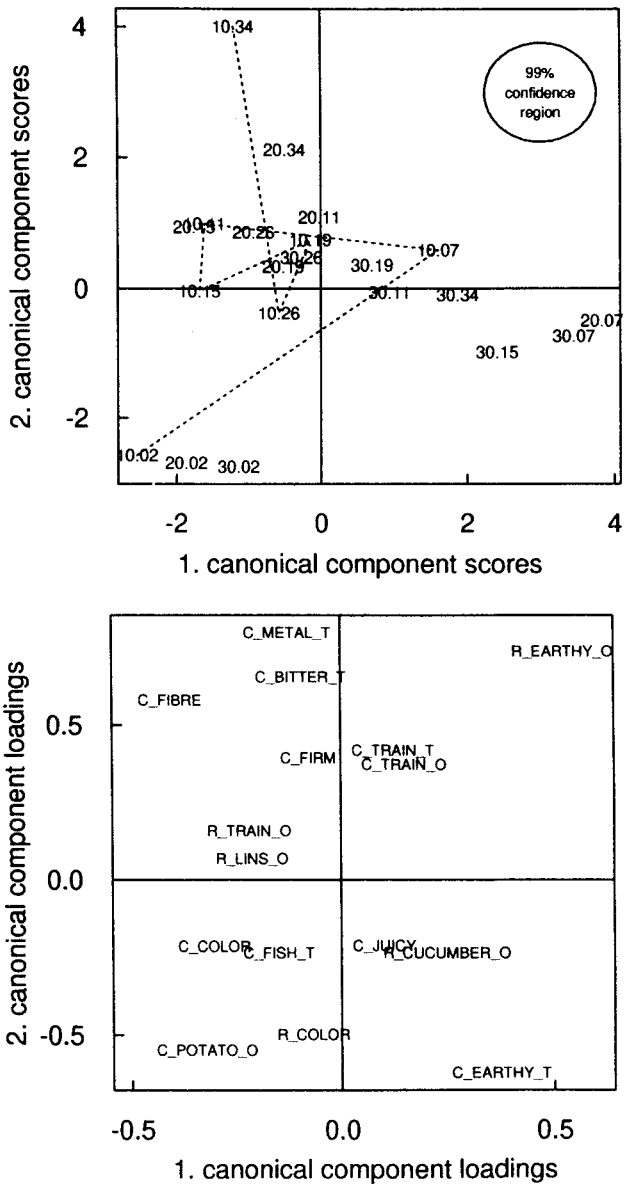

Figure 2. Canonical scores and loadings from a canonical variates analysis on time−temperature effects with the biological salmon variation (within time and temperature) used as error term. The 99% confidence region expresses the estimation error of the points in the score plot. The dotted line shows the time development of salmon stored at −10 °C. In the score plot the labels aa.bb indicate storage at −aa °C for bb weeks.

temperature combinations, between each attribute and the two canonical components. Salmon stored frozen for 2 weeks had a high intensity of potato odor and fish oil taste and was low in train oil odor and taste for the cooked samples and low in earthy odor for the raw salmon (Figure 2). As can be seen from the score plot, the sensory changes were most pronounced and fastest for salmon stored at −10 °C. After 7 weeks of storage at −10 °C, the figure shows train oil odor and taste had high intensities. Metal and bitter taste and fibrousness increased during frozen storage, and the earthy taste decreased.

Chemical Changes during Frozen Storage of Salmon. Of the 15 identified and quantified aldehydes and ketones, only 7 could be measured in the majority of the salmon. For hexanal, heptanal, *(E)*-2-hexenal, *(E,E)*-2,4-heptadienal, and nonanal were found significant time effects due to increasing concentrations during storage, both in raw and cooked samples independent of storage temperature (Table 2). For hexanal and *(E,E)*-2,4-heptadienal were found significantly higher concentrations in salmon stored at −10 and −20 °C compared to the reference treatment. A train oil or a linseed oil odor could not be detected in stored samples by GC-sniffing. But more odorants were registered in

Table 2. Concentrations (μg/kg) of Volatiles in Raw Salmon Stored at −10 °C and Results from Analysis of Variance of the Concentrations of Volatiles in 56 Salmon

		concn after given no. of weeks				results from ANOVA[b]	
volatile	RI[a]	2	19	26	34	time	temp
hexanal	885	14	12	24	24	***, **[c]	*
(E)-2-hexenal	967	1.5	1.4	3.8	5.1	**, **[c]	*
heptanal	989	2.0	4.7	4.4	6.8	*, *[c]	
octanal	1093	5.0	3.7	5.7	5.4		
(E,E)-2,4-heptadienal	1152	5.5	6.4	17	15	**, ***[c]	
(E)-2-octenal	1183	2.0	2.0	3.0	3.2		
nonanal	1197	13	7.4	11	12	**, ***[c]	

[a] RI: retention index on DB 1701 column. [b] Key to asterisks: *, **, *** represent significant effects at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [c] Samples which were cooked before being analyzed.

Table 3. Content of Lipid Constituents in Salmon Stored at −10 °C[a] and Results from Analysis of Variance of the Ratio in Content before and after Storage for the Chemical Data

	lipid content for given no. of weeks in storage								results from ANOVA on ratios[c]		
compd/param	0[b]	2	7	11	15	17	26	34	time	temp	time × temp
astaxanthin (mg/kg)	5.5	5.5	4.1	6.2	6.8	4.9	5.4	4.7		*[d]	
α-tocopherol (mg/kg)	39.0	36.3	39.0	34.0	37.8	31.6	34.2	29.6		***[d]	
C20:5(n-3) (%)	6.0	6.0	6.1	6.0	6.2	5.6	5.8	5.6		**	**
C22:5(n-3) (%)	2.8	2.6	2.8	2.8	2.8	2.8	2.7	2.8	*	*	*
C22:6(n-3) (%)	11.0	11.3	11.6	11.6	10.8	11.3	10.5	11.2	*		**
peroxide value (mequiv/kg)	0.0	1.9	1.6	2.7	3.6	5.7	9.4	10.3	**	***	***
free fatty acids (%)	1.0	1.9	3.9	5.0	3.8	6.1	6.0	8.7	***	***	***

[a] Mean values for 2 salmon measured after storage. [b] Mean values for 145 fresh salmon (Refsgaard et al., 1998). [c] The ratio between the level of a compound/parameter after storage and the level determined in the same individual before storage. Key to asterisks: *, **, *** represent significant effects at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. [d] Results from analysis of variance on the concentrations after storage.

Table 4. Results from Partial Least-Squares Analysis of the Sensory and the Chemical Data[a]

X	Y	*C*	val corr	cal corr	variable with largest correlation
ln GC	earthy odor (raw)	2	0.457	0.800	nonanal, 0.763
	train oil odor (raw)	2	0.358	0.638	heptanal, − 0.324
	metal taste (cooked)	2	0.359	0.598	hexanal, −0.304
fatty acid levels	cucumber odor (raw)	1	0.305	0.503	20:1(n-9), −0.516
	train oil odor (raw)	1	0.462	0.781	24:1(n-9), 0.577
	linseed oil odor (raw)	1	0.222	0.548	22:1(n-9 and n-11), 0.506
fatty acid ratios[b]	potato odor (cooked)	2	0.422	0.847	20:1(n-9), 0.623
	bitter taste (cooked)	2	0.411	0.627	20:1(n-9), 0.521

[a] C, number of PLS components, chosen as the first minimum of the residual cross-validation diagram; val corr, correlation between the observed and the predicted values in the cross-validation; cal corr observed correlation between PLS component computed on all observations and the Y value; ln GC, logarithm to concentrations of seven peroxidation products determined by GC/MS. [b] The ratio in content of the 25 fatty acids measured before and after storage.

salmon stored at high temperatures than in fresh salmon and salmon stored at −30 °C. The odorants formed in stored salmon were characterized as fishy, iodine, buttery, sweet, and green (results not shown).

Oil content, fatty acid composition, concentration of astaxanthin and tocopherols, peroxide value, and free fatty acid content were measured for each salmon before and after storage (Table 3). Canthaxanthin could also be detected in the salmon flesh. For astaxanthin significant effect of storage temperature was found (Table 3). The concentration of α-tocopherol decreased in salmon stored at −10 °C, and the concentration was lower than in salmon stored at the lower temperatures (Table 3). The same tendencies were found for δ- and γ-tocopherol. For the polyunsaturated fatty acids 20:5(n-3), 22:5(n-3), and 22:6(n-3), significant time−temperature interaction effects were found (Table 3) and the concentrations decreased in salmon stored at −10 and −20 °C. As the fatty acid levels were expressed as percentages, a decrease in the amount of polyunsaturated fatty acids will be reflected as an increase in the precentages of saturated and monounsaturated fatty acids. Both for peroxide values and content of free fatty acids, significant time−temperature interaction effects were found (Table 3). These parameters indicating lipid deterioration also increased in salmon stored at −10 (Table 3) and −20 °C compared to the reference treatment.

Correlations between Sensory and Chemical Changes during Frozen Storage. Attempts were made to model each of the 16 sensory attributes by different groups of measured chemical variables by a PLS1 regression. Four groups of chemical variables were used: volatiles, fatty acids, carotenoids, and tocopherols. In addition, PLS1 regression was used to predict each sensory attribute from the peroxide values and the content of free fatty acids.

The PLS analyses showed correlations between the predicted values on the basis of the concentrations of seven volatile oxidation products and, for the raw salmon samples, earthy odor and train oil odor; for the cooked samples a correlation was found with metal taste (Table 4). Likewise, correlations were found between cucumber, train oil, and linseed oil odor for the raw samples and correspondingly predicted values based on the fatty acid composition. Predicted values for the ratio of eicosenoic acid percentages before and after storage correlated, for the cooked samples, with potato odor and

bitter taste. In Table 4 is given the number of components needed to describe the sensory attributes by the given chemical parameters, and the observed correlation coeffients are also given.

With 21 randomly sampled paired observations an absolute correlation coefficient of 0.434 is significant on the 5% level. Although the 21 paired observations used for calculations of the correlation coefficients are not randomly sampled, we may still use this as a reference point of relational strength. Not surprisingly, a correlation was found between the color of the raw samples determined by the sensory panel and the astaxanthin concentration (correlation coefficient of 0.685). Peroxide values and the content of free fatty acids were the instrumental data that showed the best correlation with the sensory attributes. The correlation coefficient between peroxide values and train oil odor of raw samples was 0.684 and between peroxide values and linseed oil odor 0.657. The correlation coefficient between free fatty acids and the sensory attributes of the cooked samples was 0.680 for earthy taste, 0.637 for train oil taste, 0.654 for metal taste, and 0.642 for bitter taste. The peroxide values were best for describing the sensory attributes for the raw samples, and the content of free fatty acids was best for the cooked samples.

DISCUSSION

As stated in the Introduction, sensory changes of salmon during frozen storage are not well-characterized. It is generally accepted that the sensory changes to a large extent are due to formation of secondary oxidation products such as volatile aldehydes and ketones. Our investigation has shown that the salmon samples need to be in the oral cavity for recognition of the sensory changes. It is therefore likely that the physicochemical conditions during mastication are of importance for release of the formed oxidation products from the muscle. Similarly, it is indicated that components of low volatility can be the key compounds causing off-flavor. Formation of less volatile oxidation products could also explain why the volatile components identified in the stored salmon did not show significant time−temperature interaction effects nor did they correlate to the sensory attributes.

Farmer et al. (1997) find no significant changes in odor or flavor of salmon stored at −24 °C for 33 weeks. This is in agreement with what we found, that only minor sensory changes occur in salmon stored at −30 °C for 34 weeks. Milo and Grosch (1996) report that the rancid off-flavor of salmon homogenate stored at −13 °C for 26 weeks is mainly caused by an increase in *(E,Z)*-2,6-nonadienal, *(Z)*-3-hexenal and *(Z,Z)*-3,6-nonadienal. In our salmon investigation, *(Z)*-3-hexenal, could not be detected by the GC-MS analysis and *(E,Z)*-2,6-nonadienal and *(Z,Z)*-3,6-nonadienal only in very low level even in salmon stored for 34 weeks at −10 °C. Due to the very low odor thresholds of these compounds, 0.02−0.05 μg/kg (Milo and Grosch, 1993), they can have sensory importance in the low concentrations detected.

Color is a very important quality parameter for salmonoids (Sigurgisladóttir et al., 1997). Significant temperature−time interaction effects were not found for the color during the frozen storage, but the color and the astaxanthin content were correlated. The lack of clear tendencies in the changes of the color during storage measured by the panelists, by use of the Roche color card and by tristimulus colorimetry, reflected the high biological variation in astaxanthin content (Refsgaard et al., 1998). Salmon fed with astaxanthin as the only dietary pigment show changes in pigment during storage until 120 h at < 4 °C (Bell et al., 1998). However, for salmon fed on a diet containing a combination of astaxanthin and canthaxanthin the pigment does not change under the same conditions. In the present investigation both astaxanthin and canthaxanthin could be detected in the salmon flesh. Bjerkeng and Johnsen (1995) and Andersen et al. (1990) reported that rancidity measured as TBARS (thiobarbituric acid−reactive substances) increases more slowly in trout and salmon with high astaxanthin content, indicating an antioxidative effect. We could not show any correlation between the astaxanthin content and flavor changes. Likewise, no correlations were found between the content of astaxanthin and the formation of volatiles or lipid hydroperoxides or the content of free fatty acids.

Changes in firmness, juiciness, and fibrousness were the significant texture changes during frozen storage. This is in agreement with the texture descriptions of frozen stored salmon reported by Nilsson and Ekstrand (1995). We did not measure any instrumental parameters known to correlate to texture assessment, but we found correlations between the content of free fatty acids and firmness (correlation coefficient, 0.458) and fibrousness (0.607).

The present study is based on a homogeneous group of farmed Atlantic salmon raised under similar conditions and on the same feed. Oil content, fatty acid composition, and concentration of astaxanthin and tocopherols not only are important for the sensory quality but could also influence the stability of salmon during storage. These parameters show high biological variation in salmon, even from the same farm (Bell et al., 1998; Refsgaard et al., 1998), and they were measured in each salmon before and after storage. However, for the parameters, the only correlations found were between the sensory changes during storage and the fatty acid composition.

The decrease in content of the highly unsaturated fatty acids is due to oxidation processes where lipid hydroperoxides will be the primary products. For α-, δ-, and γ-tocopherol a decline in the concentrations in salmon were determined after storage at −10 °C. This could indicate that the tocopherols as a consequence of their function as antioxidants have been broken down in the process.

It would be of interest if one or several of the parameters measured in the salmon before storage could be used to describe or predict the sensory quality of a salmon during storage. However neither the oil content, the concentrations of tocopherols, astaxanthin, and canthaxanthin, nor the fatty acid composition, the content of free fatty acids and lipid hydroperoxides, measured in fresh salmon could be used to predict the sensory quality during storage.

ABBREVIATIONS USED

ANOVA, analysis of variances; CVA, canonical variates analysis; GC, gas chromatography; HPLC, high-performance liquid chromatography; LSD, least significant difference; MS, mass spectrometry; PCA, principal component analysis; PLS, partial least squares; 20:5, polyunsaturated fatty acid with 20 carbons and 5 double bonds.

ACKNOWLEDGMENT

The authors are grateful to Kim Mathiasen and Inge Holmberg for technical assistance. Karin Warm, Jonas Nordahl, and the panelists are thanked for participation in the sensory assessments.

LITERATURE CITED

Andersen, U. B.; Steinsholt, K. Deep-frozen salmon: Differences in quality after storage at different temperatures following different storage periods. *Norw. J. Agric. Sci.* **1992**, *6*, 211−215.

Andersen, H. J.; Bertelsen, G.; Christophersen, A. G.; Ohlen, A.; Skibsted, L. H. Development of rancidity in salmonoid steaks during retail display. A comparison of practical storage life of wild salmon and farmed rainbow trout. *Z. Lebensm.-Unters. -Forsch.* **1990**, *191*, 119−122.

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

Bell, J. G.; McEvoy, J.; Webster, J. L.; McGhee, F.; Millar, R. M.; Sargent, J. R. Flesh lipid and carotenoid composition of Scottish farmed Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem.* **1998**, *46*, 119−127.

Bjerkeng, B.; Johnsen, G. Frozen storage quality of rainbow trout (*Oncorhynchus mykiss*) as affected by oxygen, illumination, and fillet pigment. *J. Food Sci.* **1995**, *60*, 284−288.

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

Farmer, L. J.; McConnell, J. M.; Graham, W. D. Flavor characteristics and lipid composition of Atlantic salmon. Shahidi, F., Cadwallader, K. R., Eds.; In *Flavor and Lipid Chemistry of Seafoods*; American Chemical Society: Washington, DC, 1997; pp 95−109.

Grosch, W. Reactions of hydroperoxides—Products of low molecular weight. In *Autoxidation of unsaturated lipids*; Chan, H. W.-S., Ed.; Academic Press: London, 1987; pp 95−139.

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

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

Mardia, K. V.; Kent, J. T.; Bibby, J. M. *Multivariate analysis*; Academic Press: London, 1979.

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.

Nilsson, K.; Ekstrand, B. Frozen storage and thawing methods affect biochemical and sensory attributes of rainbow trout. *J. Food Sci.* **1995**, *60*, 627−630, 635.

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.* **1998**, *46*, 808−812.

Sigurgisladóttir, S.; Torrissen, O.; Lie, Ø.; Thomassen, M.; Hafsteinsson, H. Salmon quality: Methods to determine the quality parameters. *Rev. Fish. Sci.* **1997**, *5*, 223−252.

Sylvia, G.; Morrissey, M. T.; Graham, T.; Garcia, S. Organoleptic qualities of farmed and wild salmon. *J. Aquat. Food Prod. Technol.* **1995**, *4*, 51−64.

Received for review March 24, 1998. Revised manuscript received June 26, 1998. Accepted July 9, 1998. This work was supported by The Danish Research and Development Program for Food Technology through the LMC Centre for Advanced Food Studies.

JF980309Q