# **Biological Variation of Lipid Constituents and Distribution of Tocopherols and Astaxanthin in Farmed Atlantic Salmon (***Salmo salar***)**

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The contents of fat, astaxanthin, and tocopherols and the fatty acid composition of a homogeneous group of 145 farmed Atlantic salmon (*Salmo salar*) were determined. The analytical variation of the data was stastistically separated from the biological variation. The fat content in the muscle near the head was 15.0% with a biological standard deviation of 3.0%. The astaxanthin concentration was 5.5 mg/kg of muscle with a biological standard deviation of 1.1 mg/kg of muscle, and the canthaxanthin concentration was 200  $\mu$ g/kg of muscle with a standard deviation of 47  $\mu$ g/kg of muscle. The concentrations of  $\alpha$ -,  $\gamma$ -, and  $\delta$ - tocopherols were approximately 32, 2.9, and 0.4 mg/kg of muscle, respectively, and the biological standard deviations were 4.5, 0.4, and 0.07 mg/kg (14, 14, and 20%), respectively. In another group of five salmon the distributions throughout the fillet were determined, longitudinally as well as transversally. The distribution of fat, astaxanthin, and tocopherols varied throughout the salmon. The fatty acid composition varied little between extracts from different locations of the fillet.

Keywords: Salmon; biological variation; lipid; tocopherols; astaxanthin

## INTRODUCTION

Fish lipids are characterized by high levels of polyunsaturated lipids, which are very susceptible to deterioration both by oxidation and by hydrolysis. However, the reports on deterioration of salmonoid lipids show varying results. Rancidity develops more rapidly in wild salmon steaks during 6 months of storage at -17 °C than in farmed rainbow trout with higher astaxanthin content (Andersen et al., 1990). Vacuum-packed salmon can be stored for up to 34 weeks at -24 °C without affecting odor or flavor (Farmer et al., 1995). Rancid flavor is significantly higher in salmon raised on a high n-3 polyunsaturated fatty acid and low vitamin E diet compared to a diet with low n-3 fatty acid content and high vitamin E content (Waagbø et al., 1993). These results indicate that oxidation processes are the main cause of lipid deterioration in salmon and that the oxidation rate depends on the concentration of oxygen, the degree of lipid unsaturation, and the level of antioxidants. On the other hand, Polvi et al. (1991) report an increase in lysophospholipids and in free fatty acids during frozen storage of salmon fillets for 3 months at -12 °C. Likewise, Ingemansson et al. (1995) report that the major cause of lipid deterioration in farmed rainbow trout during storage at -15 °C is hydrolysis and to a lesser extent lipid oxidation. The different results could possibly be explained by biological variation in the concentration of lipid and of antioxidants such as tocopherols and astaxanthin, together with different compositions of the fatty acids. To evaluate the significance of compositional data from analyses on small numbers of fish, as frequently reported in the literature, we found it valuable to obtain an impression of the magnitude of the biological variation by analyzing a nominally homogeneous and relatively large subpopulation of farmed salmon. Therefore, we have investigated the biological variation of lipid constituents in a group of 145 farmed salmon. Moreover, we have investigated the distribution of total lipid (including fatty acid composition), tocopherols, and astaxanthin in farmed salmon by analyzing six different locations in fillets from five salmon.

### MATERIALS AND METHODS

**Salmon.** Farmed salmon (*Salmo salar*), in two separate shipments of, respectively, 145 (January) and 5 fish (May), of 4–4.5 kg, were from Sekkingstad A/S (Skogsväg, Norway). The salmon were  $\approx$ 25 months old from time of hatching and had spent the last  $\approx$ 15 months preceding harvest in the marine environment. They had at the time of harvest been fed a pelleted diet (Vextra Omega from Ewos Aqua A/S, Bergen, Norway) for 6 months. The diet composition is given in Table 1 and the fatty acid composition in Table 4. The fish were stored for 3 days on ice during transport before samples were taken for analysis.

**Chemicals.** DL- $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol were from Merck (Darmstadt, Germany). *all-trans*-Astaxanthin and *all-trans*-canthaxanthin packed under nitrogen in sealed ampules were obtained from Roche (Basel, Switzerland). Fatty acid standards were from Nu-Chek-Prep (Elysian, MN).

**Sample Preparation.** For each sample taken from the salmon as described below, the lipid content and the concentrations of astaxanthin, canthaxanthin, and tocopherols were determined in duplicate. Single determinations were used for the fatty acid composition.

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Table 1. Composition of Diet (Grams per Kilogram)<sup>a</sup>

	-	-	0
protein	380	fiber	5
fat	330	N-free extract	150
water	65	astaxanthin	0.090
ash	70	vitamin E	0.350

<sup>a</sup> Feed supplier's data.

Biological Variation. About 100 g of muscle, representing the whole transversal cut from back to belly and including the dark muscle, from a half-steak of each of the 145 salmon (corresponding to area I in Figure 1) were used. The skin was removed and the muscle samples cut into pieces, frozen in liquid nitrogen, vacuum-packed in Riloten 40/70 bags (oxygen transmission rate 39 mL/m<sup>2</sup>  $\times$  24 h<sup>-1</sup>  $\times$  1 atm; Otto Nielsen Emballage, Lyngby, Denmark), and stored for not more than 1 month at -40 °C before analysis. All samples were minced in the frozen state; two portions of 10 g of mince from each fish were extracted with 75 mL of methanol/chloroform, 2:1 (v/v), in the first extraction step, and a further 25 mL of chloroform was added in the final extraction step. To approximate the conditions of the original one-phase system, 15 mL of water was added prior to extraction (Bligh and Dyer, 1959).

*Distribution Study.* Five salmon were filleted. From each fillet samples were taken at three locations, transversally (I, II, III, Figure 1) as well as longitudinally (IV, V, VI, Figure 1), yielding a total of six samples per fish. The skin was neatly removed, but dark muscle or fat bodies were not removed from the respective cuts. The 30 samples were cut into pieces and frozen in liquid nitrogen. Ten grams of each minced sample was extracted with methanol/chloroform as outlined above (Bligh and Dyer, 1959).

**Lipid content** was determined gravimetrically after evaporation of solvent from the Bligh–Dyer extracts (Bligh and Dyer, 1959). 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– 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; 50  $\mu$ L samples were injected. Canthaxanthin and astaxanthin were separated by isocratic HPLC using an LKB (Bromma, Sweden) 2150 HPLC pump, an LKB 2157 autosampler, and an LKB 2151 variable-wavelength monitor with a 10 mm standard cell. The separation was carried out using a LiChrosorb Si60 column (100 mm × 3 mm × 5  $\mu$ m; Chrompack; Middelburg, The Netherlands) with a Chromsep silica (s2) (10 mm × 2 mm) guard column (Chrompack), eluting with 1.2 mL min<sup>-1</sup> *n*-heptane/acetone, 86:14 v/v, and detection at 470 nm.

**Tocopherol Content.** The solvent from 4.00 g of Bligh– 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 of 15  $\mu$ L were analyzed by isocratic HPLC using an LKB 2150 HPLC pump, an LKB 2157 autosampler, and a Shimadzu (Kyoto, Japan) fluorescence HPLC monitor with a flow cell volume of 12  $\mu$ L. Sample volumes of 15  $\mu$ L were injected for analysis on a Spherisorb s5w column (250 mm × 4.6 mm; Phase Separations Ltd., Deeside, U.K.), using a flow of 1.0 mL min<sup>-1</sup> *n*-heptane/2propanol, 100:0.4 v/v, and fluorescence detection (excitation at 290 nm; emission at 330 nm) (AOCS Official method Ce 8-89; AOCS, 1994).

**Fatty Acid Composition.** *Preparation of Methyl Esters.* Fatty acids of the lipids in the Bligh–Dyer extracts were transesterified to methyl esters using a base-catalyzed transesterification followed by a boron trifluoride-catalyzed esterification according to AOCS method Ce 1b-89 (AOCS, 1994). The methyl esters were dissolved in *n*-heptane to a concentration of ~20 mg mL<sup>-1</sup>.

*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  $\mu$ m; Supelco, Bellefonte, PA). The injection volume was 0.2  $\mu$ L, 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<sup>-1</sup> to 200 °C, held for 1 min, further raised by 3 °C min<sup>-1</sup> to 220 °C, and held for 12 min. The helium carrier gas flow was 21 cm s<sup>-1</sup>.

GC/MS Analysis. GC/MS was used for the identification of contaminating compounds separated and collected from the HPLC procedure for tocopherol analysis. Eluent was collected during the period of elution of the  $\alpha$ -tocopherol peak. Aliquots were injected splitlessly into a Hewlett-Packard HP5890A IIPlus gas chromatograph connected to a Hewlett-Packard HP5972 mass selective detector. The analytes were separated isothermally at 270 °C on a Hewlett-Packard HP-5MS columm (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). The detection was performed with ionization at 70 eV and an emission of 50  $\mu$ A; scans were performed in the range from 50 to 500 amu at a rate of 2.2 scans s^{-1}.

**Statistical Analysis.** The aim of the statistical analysis of the biological variation of the data is to provide the population mean levels together with confidence intervals as well as the biological population standard deviations also with confidence intervals. We emphasize here the importance of distinguishing between the biological standard deviation and the confidence interval for the mean.

For the fatty acid composition standard normal sample calculations are applied as we had only a single determination for each of the 145 salmon. The confidence intervals for the means thus come from the Student *t* distribution and those for the standard deviations from the  $\chi^2$  distribution. In this case the measured variability includes the analytical variation.

For the determinations of oil, astaxanthin and tocopherol contents, data from duplicate extractions make it possible to separate the analytical and the biological variation. This is done using a variance component model that, apart from the overall mean, includes the two components of variation: the between-salmon biological variation and the analytical variation. For the means this amounts to standard normal sample calculations on the 145 extract averages. For the estimation of the biological component of variation, the ANOVA method (Searle et al., 1992) was used, and the corresponding confidence intervals were found by Satterthwaite's method (Burdick and Graybill, 1992). In general, confidence intervals for standard deviations are found as square-root transformations of confidence intervals for variances (Burdick and Graybill, 1992). For the data on within-fish variation a two-way additive analysis of variance (factors: salmon and area) were performed to test the significance of the area to area differences.

## RESULTS AND DISCUSSION

The present study is based on farmed Atlantic salmon fed a commercial diet. Thus, the results are likely to reflect and be representative of farmed salmon reaching the market. The mean values and the biological variation as measured by the standard deviation of fat, astaxanthin, canthaxanthin, and tocopherols in a homogeneous group of 145 farmed salmon are given in Table 2. The biological variation is the contribution to the variation between the salmon that remains after exclusion of the analytical variation as described under Statistical Analysis.

The lipid content data, for example, as given in Table 2, show a mean value for the studied population of 15% (w/w) lipid, with a standard deviation of 3.0%. A span of four standard deviations (mean  $\pm$  2 SD) comprising  $\approx$ 95% of the salmon will cover a range of lipid content from 9 to 21%. This illustrates a remarkable lack of uniformity in these salmon. A variation in lipid concentration of this magnitude may result in a similarly large variation in the texture and other functional

Table 2. Mean Values and Biological Standard Variation of Lipid Constituents in Area I (Figure 1) of 145 Farmed Atlantic Salmon (*S. salar*) with 95% Confidence Intervals in Parentheses

	mean	biological standard variation
weight	4.30 kg	0.177 kg
	(4.27, 4.33)	(0.158, 0.200)
lipid content	15.0%	3.0% (2.71, 3.44)
-	(14.5, 15.5)	
astaxanthin	5.5 mg/kg of muscle	1.1 mg/kg of muscle
	(5.3, 5.7)	(0.95, 1.22)
canthaxanthin	200 $\mu$ g/kg of muscle	47 $\mu$ g/kg of muscle
	(190, 210)	(41.8, 53.8)
α-tocopherol <sup>a</sup>	35.8 mg/kg of muscle	5.0 mg/kg of muscle
	(34.9, 36.7)	(4.41, 5.78)
$\gamma$ -tocopherol	2.9 mg/kg of muscle	0.41 mg/kg of muscle
, 1	(2.8, 3.0)	(0.35, 0.49)
$\delta$ -tocopherol	350 µg/kg of muscle	70 $\mu$ g/kg of muscle
1	(340, 370)	(56.3, 91.1)
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<sup>*a*</sup> Approximately 90% of the reported amount is  $\alpha$ -tocopherol (cf. Discussion). The rest is due to contaminating compounds (squalene and *cis*- and *trans*-octyl methoxycinnamate).



**Figure 1.** Six fillet sections used for determination of lipid constituents.

properties of the muscle. Thus, the sensory quality range of farmed salmon such as the group analyzed here could be very wide. Aursand et al. (1994) reported the fat content of specific subtissues of the muscle. Their data, therefore, are not directly comparable to ours. In addition, the salmon studied by Aursand et al. (1994) were fed a diet with a lower fat content than the salmon investigated in the present study, 21 and 33%, respectively.

In a group of five salmon, the distribution of lipid and lipid constituents was determined in the six areas shown in Figure 1. We have chosen to investigate these gross sections and not selected dark and white tissues as, for example, Aursand et al. (1994) because we were interested in determining the content of lipid and the concentrations of lipid soluble compounds in such cuts of the muscle that might be used for cooking. As can been seen from Table 3 and Figure 1, the fat content was highly variable throughout the fish with a mean fat content of 18.5% in the dorsal cut (area IV, Figure 1) and of a surprisingly low 11.8% in the longitudinal midline cut (area V, Figure 1). However, this cut comprises the dark muscle as well as a considerable contribution from the white muscle. The relatively low adiposity of the white muscle (Zhou et al., 1996) may well compensate for the higher lipid content of the dark muscle section of this cut.

The astaxanthin concentration was in agreement with the 3.1–8.1 mg/kg reported for wild *S. salar* (Schiedt

et al., 1981), and the biological standard deviation was 1.1 mg/kg of muscle (Table 2). The concentration of astaxanthin in salmon has been reported not to differ among dorsal, ventral, and tail sections (Saito, 1969). This is in contrast to our findings (Table 3). We found significant variations of the astaxanthin content throughout the salmon, with high levels in the tail (area III) and dorsal (area IV) regions and a low level near the head (area I, Figure 1).

The levels of carotenoids and tocopherols, which are supplied through the commercial diet, are of interest in relation to the appearance as well as the storage stability of the salmon muscle. For the consumer, and therefore also for the producer, the color of salmonoids is a very important parameter of quality (Ostrander et al., 1976). It is noteworthy that in the present study a relatively wide range of astaxanthin content is found, despite the rearing conditions being identical for the whole group of salmon. A difference in astaxanthin level of  $\approx 1$  mg/kg causes a marked difference from light pink to strong pink (Sigurgisladottir et al., 1994). Also, the storage stability is an important parameter in salmon retailing. Antioxidative effects are exerted not only by the tocopherols but also by astaxanthin and, to a smaller degree, by canthaxanthin (Andersen et al., 1990; Jørgensen and Skibsted, 1993; Terao et al., 1992). Considering the variations found in the concentrations of these components, in conjunction with the variations found in lipid concentrations, it is very likely that different degrees of deterioration during storage can be found among individuals from the same salmon farm.

The concentrations of astaxanthin, canthaxanthin, and  $\alpha$ - and  $\delta$ -tocopherol differed significantly between the group of 145 salmon (Table 2) and the group of 5 salmon analyzed in the distribution investigation (Table 3). The only known difference between these two groups of salmon is the time of harvest, January for the 145 salmon and May for the 5 fish. The differences indicate that seasonally varying factors such as water temperature can cause changes in the content of lipid constituents and that the mean values given in Table 2 are valid only for the investigated population.

In Table 2 mean values and biological standard deviations of the  $\alpha\text{-},\ \gamma\text{-},\ \text{and}\ \delta\text{-}$  to copherols in area I (Figure 1) are given. The determinations of  $\alpha$ -tocopherol gave somewhat higher values than usually reported, wherefore an investigation of the possible causes of this deviation was undertaken. From a number of HPLC runs the fraction corresponding to the  $\alpha$ -tocopherol peak was collected. The content of this fraction was determined by GC/MS analysis, using an HP-5 column and electron ionization. It was found to contain three contaminating compounds: squalene and cis- and transoctyl methoxycinnamate. Subsequently, an estimate was made of the contributions of these compounds to the total fluorescence detector signal obtained at the  $\alpha$ -tocopherol retention time interval. Extractions were made from nine salmon duplicate cuts both with the Bligh-Dyer method (Bligh and Dyer, 1959) as used in our main study, and with a method described by Desai (1984), applying a heptane extraction to a saponified muscle homogenate. The latter method is traditionally used specifically for analysis of tocopherol (and other unsaponifiables), whereas the former extraction method yields a complete extraction of lipid soluble compounds. It is therefore conceivable that the Bligh–Dyer extract may contain compounds that can interfere in the HPLC

Table 3. Distribution of Fat (Percent), Carotenoids (Milligrams per Kilogram of Salmon Muscle), and Tocopherols (Milligrams per Kilogram of Salmon Muscle) in Farmed Salmon (*S. salar*) (n = 5) and 95% LSD Values from the ANOVA, by Which All Tests for Area Differences Were Highly Significant<sup>a</sup>

	area I	area II	area III	area IV	area V	area VI	95% LSD value
oil % astaxanthin canthaxanthin $\alpha$ -tocopherol <sup>b</sup> $\gamma$ -tocopherol $\delta$ -tocopherol	15.8 <sup>b</sup> 3.25 <sup>a</sup> 0.832 <sup>a</sup> 39.0 <sup>bc</sup> 3.05 <sup>bc</sup> 0.533 <sup>bc</sup>	$18.3^{\rm c} \\ 3.59^{\rm bc} \\ 0.888^{\rm b} \\ 42.0^{\rm cd} \\ 3.38^{\rm d} \\ 0.582^{\rm c}$	$12.9^{a}\\3.88^{d}\\0.980^{d}\\37.6^{b}\\2.88^{b}\\0.474^{ab}$	18.5 <sup>c</sup> 3.73 <sup>cd</sup> 0.947 <sup>cd</sup> 40.2 <sup>c,d</sup> 3.18 <sup>c</sup> 0.565 <sup>bc</sup>	$\begin{array}{c} 11.8^{a} \\ 3.72^{bc} \\ 0.924^{bc} \\ 35.0^{a} \\ 2.66^{a} \\ 0.392^{a} \end{array}$	18.2 <sup>c</sup> 3.57 <sup>b</sup> 0.900 <sup>b</sup> 44.2 <sup>e</sup> 3.62 <sup>e</sup> 0.559 <sup>bc</sup>	1.2 0.15 0.044 2.5 0.19 0.094

<sup>*a*</sup> Different superscripts with the figures reflect statistically significant differences (95% confidence level). <sup>*b*</sup> Approximately 90% of this value is  $\alpha$ -tocopherol (cf. Discussion). The rest is due to the contaminating compounds squalene and *cis*- and *trans*-octyl methoxycinnamate.

fluorescence analysis. Comparison of the two sets of determinations indicates a contribution of the contaminating compounds, corresponding to  $\approx 10\%$  of the "total  $\alpha$ -tocopherol concentration" given in Tables 2 and 3.

The data reported in Table 2 for the mean value and biological variation of the  $\alpha$ -tocopherol concentration thus include contributions from the three contaminating compounds. When adjusted for the average contribution from the contamination, our results are still higher than reported earlier. A concentration on 12.5 mg/kg  $\alpha$ -tocopherol in the muscle of salmon fed on a diet with 236 mg/kg a-tocopherol has been determined (Sigurgisladottir et al., 1994) for salmon with a weight of 500-600 g, harvested at a water temperature of 14 °C. The salmon analyzed in the present study were harvested at  $\approx$ 4 °C and at a weight of 4–5 kg. They had been fed a diet with 350 mg of  $\alpha$ -tocopherol/kg, thus enabling a somewhat higher muscular tocopherol level than found by Sigurgisladottir et al. (1994). The concentration of  $\beta$ -tocopherol was low, where found, but for many of the salmon it was below the detection limit, in agreement with the data of Sigurgisladottir et al. (1994). The distribution of tocopherols varied significantly throughout the salmon, and the concentrations were high in the ventral (area VI) and lowest in the cut along the midline (area V; Table 3).

The distribution of fatty acids showed little variation, and the fatty acid compositions in area I of the 145 salmon resembled the fatty acid composition of the diet (Table 4). However, for the n-9 and n-11 monoenoic fatty acids the content was higher in the muscle than in the diet. Conversely, the eicosapentenoic acid level was considerably lower in the muscle than in the diet. The preferential deposition of docosahexenoic acid over eicosapentenoic acid in salmon muscle depot fat has been described by Polvi and Ackman (1992). The fatty acid distribution varied only little among the lipids extracted from the various locations in the five salmon (data not shown). This is also described by Aursand et al. (1994). Only for 22:1(n-11) and 22:6(n-3) were found small but statistically significant differences in content among the six areas. None of these differences are sufficiently large to be expected to cause differences in the oxidative stability among different parts of the salmon muscle.

Even though the group of 145 salmon was homogeneous with respect to strain, feed, location of farm, and time of harvest, we determined biological variation coefficients of  $\sim 25\%$  for the lipid content as well as for the concentrations of antioxidants as tocopherols and astaxanthin when comparing analytical data from the same body location in all of the salmon. Also, the concentrations of lipids, tocopherol, and astaxanthin varied significantly from location to location within the

Table 4. Fatty Acid Composition (Percent) in Feed and in Farmed Salmon (*S. salar*)  $(n = 145)^a$ 

		salmon		
fatty acid	feed	mean	standard deviation	
14:0	7.2	4.8 (4.78, 4.85)	0.20 (0.18, 0.22)	
15:0	0.5	0.4 (0.367, 0.402)	0.014 (0.013, 0.016)	
16:0	17.3	13.4 (13.4, 13.5)	0.40 (0.36, 0.45)	
16:1( <i>n</i> -7)	6.7	5.1 (5.04, 5.10)	0.18 (0.17, 0.21)	
16:2	0.3	0.2 (0.220, 0.234)	0.045 (0.040, 0.050)	
17:0	0.6	0.3 (0.299, 0.305)	0.018 (0.017, 0.021)	
16:4( <i>n</i> -3)	1.6	0.3 (0.287, 0.296)	0.027 (0.025, 0.031)	
18:0	3.4	2.7 (2.66, 2.70)	0.12 (0.104, 0.131)	
18:1( <i>n</i> -9)	9.3	15.0 (15.0, 15.2)	0.52 (0.47, 0.59)	
18:1( <i>n</i> -7)	2.6	2.7 (2.64, 2.67)	0.095 (0.085, 0.108)	
18:2( <i>n</i> -6)	1.5	4.6 (4.50, 4.62)	0.40 (0.36, 0.45)	
18:3( <i>n</i> -3)	0.8	1.4 (1.34, 1.37)	0.068 (0.061, 0.077)	
18:4( <i>n</i> -3)	2.1	1.7 (1.64, 1.67)	0.088 (0.079, 0.099)	
20:1( <i>n</i> -9)	2.1	8.4 (8.30, 8.45)	0.48 (0.43, 0.55)	
20:1( <i>n</i> -7)	0.3	0.4 (0.37, 0.46)	0.27 (0.24, 0.30)	
20:2( <i>n</i> -6)	0.2	0.5 (0.36, 0.55)	0.59 (0.53, 0.66)	
20:4( <i>n</i> -6)	1.3	0.5 (0.45, 0.46)	0.027 (0.025, 0.031)	
20:3( <i>n</i> -3)	0.1	0.2 (0.200, 0.203)	0.012 (0.010, 0.013)	
20:4( <i>n</i> -3)	0.6	1.4 (1.43, 1.46)	0.087 (0.078, 0.099)	
20:5( <i>n</i> -3)	15.6	6.0 (5.92, 6.01)	0.27 (0.25, 0.31)	
22:1( <i>n</i> -11)	2.3	8.4 (8.34, 8.50)	0.50 (0.45, 0.57)	
22:1( <i>n</i> -9)	0.3	0.9 (0.91, 0.95)	0.13 (0.12, 0.15)	
22:5( <i>n</i> -3)	2.0	2.8 (2.79, 2.86)	0.22 (0.19, 0.25)	
22:6( <i>n</i> -3)	11.4	11.0 (11.0, 11.1)	0.52 (0.46, 0.58)	
24:1( <i>n</i> -9)	0.7	0.8 (0.77, 0.80)	0.10 (0.09, 0.11)	
unidentified	8.9	6.1		
$\Sigma$ saturates	29.0	21.6		
$\Sigma$ monoenes	24.5	41.7		
$\Sigma$ di- and polyenes	37.6	30.6		

<sup>*a*</sup> For the latter the standard deviation in area I (Figure 1) is given. In parentheses 95% confidence intervals are given.

salmon, illustrating the importance of a precise reference to the location of sampling for analysis of salmon flesh.

As determined in this study a very wide range of concentrations of lipid components can be found in a nominally homogeneous population of farmed salmon. This result points to the necessity of analyzing large subsamples/large numbers of fish when an estimate of a population average is wanted, and caution should be exercised in extrapolation from literature data based on analyses of only a few individuals. The confidence intervals for the means (Table 2) indicate for each of the lipid constituents which difference may be detected using 145 obervations. A confidence band approximately twice as wide will be obtained with  $(1/2)^2 \times 145$ =  $\sim$  36 observations, whereas 4  $\times$  145 = 580 observations would be needed to reduce the interval by a factor of 0.5. The large differences observed in the content of lipid constituents, polyunsaturated lipids as well as antioxidants, may also be a part of the explanation for the differences in storage stability, reported by various authors (see the Introduction) (Andersen et al., 1990; Farmer et al., 1995; Ingemansson et al., 1995; Polvi et al., 1991; Waagbø et al., 1993). Similarly, the large variations in fat and astaxanthin content will be expected to result in a wide range of the levels of sensory attributes.

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