A comparative study of organic- versus conventional Atlantic salmon. II. Fillet color, carotenoid- and fatty acid composition as affected by dry salting, cold smoking and storage

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Abstract

The aim of the present study was to investigate the effect of dry salting, cold smoking and 14 days refrigerated storage at 4 °C on the stability of carotenoids, color and fatty acids in commercially reared organic Atlantic salmon (*Salmo salar* L.). As reference, conventionally reared Atlantic salmon was used. Pigment sources in feeds for the organic and conventional salmon were Panaferd- AX® (PAN) and Carophyll Pink® (CP), respectively. The dry salting process was found to be the main cause for losses of carotenoids throughout salting, smoking and storage, whereas no differences were found in stability of the different flesh carotenoids. The diverse composition of flesh carotenoids in organic salmon seems however to have minor influence on the color of the cold smoked product. Colorimetric characteristics of the fillet surface and liquid loss during storage of cold smoke fillets was found to be mostly affected by the fatty acid composition of the flesh which differed between the organic and conventional raw material. Moreover, dry salting and cold smoking were found to alter colorimetric differences between raw organically and conventionally reared salmon, resulting in an equal colorimetric perception of cold smoked organic and conventional salmon fillets after 14 days refrigerated vacuum storage .

1. Introduction

Consumers' perception is a key attribute for the smoking industry when it comes to product quality (Cardinal et al., 2004; Gormley, 1992; Rørå et al., 2004). For producers of organic Atlantic salmon (regulated by European Council Regulation (EC) No. 834/2007, 889/2008, 710/2009 and 1358/2014) it is important that the quality of the raw material is acceptable for the smoking industry. The reddish color of salmon flesh is caused by carotenoids (Foss et al., 1984; Skrede and Wold, 2008), and the exceptional color contributes much to the elite image of salmon products (Gormley, 1992). Visual appearance of cold-smoked salmon is one of the most important consumer purchase criterion (Gormley, 1992; Røra et al., 2004), where the consumers prefer the stronger red-colored salmon products (Bjerkeng, 2000). The flesh color of farmed salmonids is influenced by several factors including dietary lipid level (Bjerkeng et al., 1997; Nickell and Bromage, 1998; Regost et al., 2001), dietary oil source (Regost et al., 2004), fillet fat content (Mørkøre et al., 2001; Nickell and Bromage, 1998), season of harvest (Mørkøre and Rørvik, 2001), dietary carotenoid concentration (Bjerkeng, 2000; Hatlen et al., 1998) and dietary pigment type (Buttle et al., 2001; EFSA, 2007; Lerfall et

al., 2016; Skrede and Storebakken, 1986; Storebakken et al., 1987). One criterion for production of organic Atlantic salmon is to use a natural pigment source. The most commonly used available natural pigment today is Panaferd-AX® (Nippon oil, Japan). Panaferd-AX® consists of dried sterilized cells of a red carotenoid-rich soil bacterium (*Paracoccus carotinifaciens*) containing around 4 % red carotenoids, predominantly astaxanthin (2.2 %), adonirubin (1.3 %) and canthaxanthin (0.4 %) besides some more yellowish carotenoids like β,β-carotene and echinenone (EFSA, 2007). In conventional production of Atlantic salmon, synthetically produced nature identical astaxanthin is normally used. One common trademark is Carophyll Pink, produced by DSM Switzerland, which consists of approximately 6-8 % astaxanthin. In a study by Lerfall et al. (2016), from the same sampling as the present study, the fish diet was found to have significant effects on the fillet composition. Compared to conventional salmon, the investigated organic salmon were found to have; similar total content of muscle carotenoids, lower content of astaxanthin, more diverse composition of muscle carotenoids, higher contents of SFAs and PUFAs, lower contents of MUFAs and significantly darker appearance. The product quality of cold smoked Atlantic salmon are known to be affected by both the raw material characteristics (Bencze Rørå et al., 1998; Birkeland et al., 2004a; Birkeland et al., 2007; Cardinal et al., 2001; Larsson et al., 2012; Lerfall and Rotabakk, 2015) and processing conditions (Birkeland et al., 2004a; Birkeland et al., 2007; Cardinal et al., 2001; Espe et al., 2002; Lerfall et al., 2011; Sigurgisladottir et al., 2000). During primary (i.e. slaughtering and filleting) and secondary (i.e. salting and smoking) processing, both decomposition and/or extraction of carotenoids and discoloration of the fillet surface may occur (Birkeland et al., 2004b; Lerfall et al., 2011). Depigmentation of cold smoked salmon fillets is immediately apparent to the consumer and negatively affects the visual perception of color in the product. Due to their chemical structure, different carotenoids differs in extractability and stability (Lerfall et al., 2011). Introduction of “new” carotenoids (other than astaxanthin and canthaxanthin) into the salmon muscle may raise questions about carotenoid stability during processing and how these carotenoids affect the quality of the cold smoked product. Thus, the presented study was undertaken to elucidate the effect of dry salting, cold smoking and 14 days refrigerated storage on the stability of carotenoids, color and fatty acids in commercially reared organic Atlantic salmon fed commercial feed adapted to organic

salmon farming. As a reference, conventionally reared Atlantic salmon fed conventional feed was used.

2. Material and Methods

2.1. Raw material and experimental design

Atlantic salmon (*Salmo salar* L.) were reared in Romsdalsfjorden at the Norwegian west coast under standard rearing conditions. Organically produced smolt of Rauma strain were transferred to sea in September 2012 at 80 g where they were kept until live weight of 1 kg in November. The livestock were then split into two nearby (distance; 2.5 km) rearing sites in Romsdalsfjorden: Fish at location Gjermundnes (62° 64' 58" N; 7° 10' 04" E) were produced as conventional Atlantic salmon from ca. 1 kg until harvest, while fish at location Furneset (62° 63' 39" N; 7° 13' 93" E) were produced in compliance with EU rules for organic production until harvest. Both fish groups were kept in large circular net pens (circumference; 157m, net depth 30 m) at ambient light and temperature, and were fed either organically or conventionally salmon extruded feeds delivered by feed manufacturers EWOS UK, Grangemounth, and Skretting N, Averøy, respectively. The organic feed contained a high proportion of marine ingredients (>65% over the life cycle), predominantly derived from herring trimmings, organically certified legumes and oil seed meals compared to conventional feeds. The latter contained a higher proportion of conventional vegetable protein and oil at the expense of marine protein and oil ingredients. The organic feed were added approximately 60 mg×kg^{-1} of natural pigment source Panaferd-AX® (Nippon oil, Japan), stabilized with natural antioxidants, whereas synthetic astaxanthin (Carophyll Pink, DSM, Switzerland) was added to the conventional feed at approximately 50 mg×kg^{-1} dose.

On May 23 (2014), the fish were starved according to commercial procedures before they were transported by a well-boat from the rearing cage to the sea cages at the processing plant. There they were acclimated for 2 days before commercial slaughtering (live chilling at 0 °C for 30 min and percussive stunning).

After slaughtering, fifteen gutted Atlantic salmon of each group (organic: gutted weight 5.1-5.8 kg, condition factor (Cf): 0.91-1.21, and conventional: gutted weight 5.1-5.7 kg, Cf: 0.99-1.25), in total thirty salmon were filleted and fillet weight were measured before the right side fillets were transported on wet ice in polystyrene boxes to Sør-Trøndelag University

College (HiST, Trondheim, Norway). Right side fillets were thereafter divided into two different groups. The first group (both organic and conventional fillets) was used to study raw fillet quality (Lerfall et al., 2016) whereas the second group (both organic and conventional fillets) was used in a cold smoking trial. In the cold smoking trial, six randomly chosen fillets of each group were dry salted and cold smoked. Before salting and after each processing step, cylindrical samples were punched out of the salmon fillets and stored at -80 °C for later analyses (Figure 1). After smoking, all fillets were vacuum packed and stored in a refrigerated room (4 °C) for 14 days. Throughout each processing step (salting and smoking) and 14 days refrigerated storage, the following parameters were monitored; mass transfer (water and sodium chloride), stability of carotenoids, color and fatty acids. Moreover, a reflective profile of a vertical cut of the Norwegian Quality Cut (NQC) were conducted at end of storage (14 days).

2.2. Salting and smoking procedure

All fillets were covered with NaCl (fine-refined salt, minimum 99.8% NaCl, GC Rieber, Norsal, Trondheim, Norway) three days *post mortem* and stored on grids in a refrigerated room (20 h, 4 °C). All fillets were then rinsed in cold water (approximately 8 °C) to remove excess of NaCl. Salt-cured fillets of both groups were randomized on grids and dried at 22 °C for 180 min, then cold smoked for 180 min at 22-24 °C in a Kerres smoke-air® show smoker CS700 EL MAXI 1001 smoking cabinet (Germany).

2.3. Mass transfer (weight loss, dry matter and contents of NaCl)

The weight loss (WL) from the fillets during processing was calculated as the difference in fillet weight between raw, and salted and smoked fillets, respectively (Equation 1). Moreover, the WL during 14 days vacuum storage was calculated as the difference in fillet weight between smoked fillets and fillets stored 14 days (Equation 1).

Equation 1:

$$WL = \frac{m_0 - m_x}{m_0} \times 100\,\%, \text{ where}$$

m_0: fillet weight at t_0

m_x: fillet weight at t_x

The dry matter of the raw material, and after each processing step (salting and smoking), and after storage (Figure 1) was calculated gravimetrically according to (ISO.6496, 1983). Moreover, NaCl content was measured in the dried samples and analysed on a Chloride Analyser (Model 926 Sherwood Scientific Ltd.). The dried samples were added hot deionised water (30 ml), homogenized (9500 rpm, 45 sec.) by an Ultra-Turrax T25, Janke & Kunkel IKA®-Labortechnik, Staufen, Germany and heated in a water bath (100 °C, 10 min), cooled to room temperature and diluted to 100 mL in a volumetric flask before analyses.

2.4. Lipid and carotenoid composition

Lipids were extracted from muscle tissue of the raw material and after each processing step and 14 days storage by a modified method derived from Bligh and Dyer (1959). Total amount of lipids was calculated by net weight and the lipids were thereafter analysed for total amounts of carotenoids together with composition of carotenoids and fatty acids.

Total amounts, and composition of carotenoids were analyzed in the lipid fraction extracted from the muscle samples (Figure 1). Approximately 0.5g lipids were added a mixture of acetone (VWR 20067.320) : n-hexane (VWR 24575.320) (86:14, 2 mL) and analyzed by HPLC (Agilent 1100 series, Waldbronn, Germany), connected to a diode array UV-VIS detector. Carotenoids were separated using two series-coupled columns of Vakosil-2 SIL 100A, 5µm 4.8 x 250 mm by Wako el Intersil GL science. All carotenoids were quantified by using all-*E*-astaxanthin (Sigma, A-9335) as an external standard. The eluent was 65.5% n-hexane (VWR 24575.320), 32.7% tetrahydrofuran (VWR 152506X) and 1.6% methanol. The flow was 1.0 $mL \times min^{-1}$ and detection wavelength was set to 470 nm. The employed extinction coefficients, $E_{1cm,\ 1\%}$, at 470 nm in hexane containing 4% (v/v) $CHCl_3$ were 2100 for all-*E*-astaxanthin (Britton, 1995). Retention (% of initial concentration) of carotenoids after each processing step (salting and smoking), and after 14 days storage was adjusted in proportion to the mass transfer during processing (salt inn, water out).

The composition of fatty acid were analyzed as fatty acid methyl-esters in the lipid fraction extracted from the raw material and cold smoked fillets stored 14 days, respectively. Fatty acid methyl-esters were analyzed by gas chromatography (GC) (Agilent 6850 GC-system, Waldbronn, Germany) equipped with a flame ionization detector (FID, 310 °C), and a polyethylene glycol capillary column (HP-INNOWax) 30 m x 250 µm x 0.25 µm. The carrier gas

was helium and the oven had an isothermal temperature at 210 °C. Preparation of methyl esters of the samples was conducted as described by Metcalfe et al. (1966).

2.5. Colorimetric and reflective properties

Colorimetric assessments (CIE, 1994) were performed with a Minolta Chroma meter, CR200 Minolta, Japan at tree defined points (Figure 1) of the raw fillets, after each processing step (salting and smoking) and after 14 days storage, respectively. *L** describes the lightness of the sample, *a** the intensity in red (*a** > 0), *b** the intensity in yellow (*b** > 0) and H^{0}_{ab} the hue angle, (where, H^{0}_{ab} of 0° represents reddish hue and 90° yellowish hue). Chroma (C*) is the color saturation where low chroma indicate greyscale and high chroma brightness. Average values of each fillet were used for statistical analyses.

Changes (Δ) of colorimetric parameters and color difference (ΔE) between the raw fillet ($L_1a_1b_1$) and the smoked fillet ($L_2a_2b_2$) were calculated for Atlantic salmon as described by CIE (1994) and Birkeland (2004).

Multispectral imaging was carried out on a VideometerLab system (Videometer A/S, Hoersholm, Denmark) system measuring the light reflected from a vertical cut of the cold smoked fillet stored for 14 days (Figure 1). This system is based on a high-intensity integrating sphere illumination featuring light emitting diodes (LED) together with a high-resolution monochrome grayscale camera (Dissing et al., 2011). The data acquisition was done by imaging the muscle at 18 different wavelengths ranging from 405 to 970 nm. Before use, the system was calibrated radiometrically using both a diffuse white and a dark target followed by a light setup optimized to fit the object of interest. The data collected from the image at each wavelength was an average of all pixels recorded in the area of interest of each sample.

2.6. Statistics

Data were analyzed by a two-factor analysis of variance (two-way ANOVA), a student t-test and/or Pearson`s correlation coefficient, r using IBM Statistical Package for the Social Sciences statistics software (release 21, IBM corporation, USA). To compare different groups, Tukey`s pairwise comparison test was used. The alpha level was set to 5 % ($P<0.05$).

3. Results

3.1. Biometrics

The average head on gutted (HOG) weight of the salmon sampled for processing were 5.4±0.21 kg, and there were no significant differences between organically and conventionally produced salmon (P>0.738). The condition factor (Cf) were however found to be significantly lower in organic salmon (1.05±0.08) compared to conventional salmon (1.15±0.03; P=0.018).

3.2. Raw material characteristics

Average muscle dry matter content of organic and conventional salmon were 31.2±2.8 % and 31.6±2.2 %, respectively (P>0.788). Comparable contents of dry matter coincided with equal lipid contents of the respective groups (Table 1, P>0.498).

The distribution of fatty acids were different in the organic and conventional salmon (Table 1) where organically reared salmon contained of significantly higher amounts of C14:0, C16:0, C16:1n-7, C18:4n-3, C20:1n-9, C20:4n-6, C20:5n-3, C22:1n-9, C22:5n-3 and C22:6n-3 as compared to the conventional raw material. Moreover, the amount of C18:1, C18:2n-6 and C18:3n-3 fatty acids were lower. Total contents of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) were significantly higher- and monounsaturated fatty acids (MUFA) significantly lower in the organic salmon compared to conventional salmon. Moreover, organic salmon flesh contained more n-3 fatty acids and less n-6 fatty acids giving a significantly higher n-3/n-6 ratio compared to conventional flesh ($n\text{-}3 \times n\text{-}6^{-1}$= 3.3±0.15 and 1.2±0.08, respectively, P<0.001).

The total content of carotenoids in the organic and conventional raw material were 8.2 and 7.7 $mg \times kg^{-1}$, respectively (Table 2, P>0.626). The contribution of astaxanthin in the respective raw material were 4.6 and 6.7 $mg \times kg^{-1}$ and differed between the groups (P=0.012). In addition to astaxanthin, conventional muscle contained lutein (1.1 $mg \times kg^{-1}$) whereas the organic flesh contained both adonirubin (1.9 $mg \times kg^{-1}$), adonixanthin (0.8 $mg \times kg^{-1}$), canthaxanthin (0.5 $mg \times kg^{-1}$), asteroidenone (0.2 $mg \times kg^{-1}$) and minor contents of lutein (0.1 $mg \times kg^{-1}$). Tristimulus color measurements (Table 2) of the raw material showed similar color characteristics of organic and conventional raw fillets for the colorimetric parameters *a** (redness), *b** (yellowness), H^{0}_{ab} (hue) and C* (chroma). Due to *L**, which

describe the fillet translucent, organic raw fillets appeared to be less translucent (i.e. 'darker') as compared to the conventional (P=0.019).

3.3. Mass transfers during salting, cold smoking and 14d storage

The dry salting procedure resulted in an average weight loss of 5.4±0.8% (between groups: P>0.567), whereas the total weight loss after drying and smoking was 10.7±0.72% (between groups: P>0.782). After 14 days refrigerated storage however, a significantly higher liquid loss were observed in conventional as compared to organic cold smoked fillets (Figure 2, P<0.001). This loss of liquids during 14 days refrigerated storage correlated significantly with contents of saturated fatty acids (r=-0.942, P<0.001), MUFA (r=0.935, P<0.001), n-3×n-6^{-1} (r=-0.920, P<0.001), n-3 fatty acid (r=-0.930, P<0.001) and n-6 fatty acid (r=0.911, P<0.001). Moreover, an insignificant and weaker negative correlation were observed between liquid loss during storage and contents of PUFA (r=-0.565, P>0.070). The flux of salt (NaCl) into the fillet (on average: 55.5±2.34 g×kg^{-1}, between groups: P>0.828) resulted in a total weight loss of 162.0±8.22 g×kg^{-1} (between groups: P=0.761) of muscle mass (mostly water) during processing.

3.4. Stability of carotenoids and colorimetric changes throughout processing and storage

During processing, a significant total loss of carotenoid (presented as retention, Table 3) was observed (two-way ANOVA: P<0.001). Between organic and conventional fillets however, no significant differences in total carotenoid stability were found (P>0.943).

Retention of specific carotenoids presented in the organic flesh followed the same pattern as presented for total carotenoids (Table 3). This indicate that astaxanthin (P=0.001), adonirubin (P=0.013), adonixanthin (P=0.033), canthaxanthin (P=0.001), asteroidenone (P=0.002) and lutein (P=0.006) behave similar throughout the cold smoking process. The same pattern were also observed after 14 days storage for carotenoids presented in conventional salmon (astaxanthin and lutein, P=0.009 and P=0.026, respectively).

In general, organic fillets showed lower reduction of both redness (Δ*a*) and total color change (ΔE) during salting and smoking, whereas after 14 days storage these differences were found to be insignificant (Figure 3). Changes in translucence (Δ*L**) was found to differ significantly between the groups after salting (P=0.037), whereas after smoking and 14 days storage these differences were found to be insignificant although statistical tendencies towards significance

were still observed (Figure 3, P=0.087 and 0.076, respectively). Moreover, no significant differences between organic or conventional salmon in changes of yellowness (Δ*b*), hue (ΔH^0_{ab}) or Chroma (ΔC*) were observed during salting, smoking or 14 days storage. Observed colorimetric changes during processing resulted in equal colorimetric perception of the organic and conventional cold smoked product (Table 4). The only difference observed between organic and conventional fillets were a significantly higher C* of organic salmon after smoking as compared to conventional (Table 4, P=0.040). This was however not seen after 14 days storage. Moreover, significant correlations were observed between total retention of carotenoids and colorimetric parameters (*a**, *b**, *L** and C*) (r=0.577, P=0.003; r=0.408, P=0.013; r=0.487, P<0.001 and r=0.451, P=0.006, respectively).

Reflective properties of a vertical cut of stored cold smoked fillets showed that the reflection of light from the upper part (close to the fillet surface) was significantly lower in organic compared to conventional salmon muscle at both 450 nm and 470 nm (Figure 4). These differences were not observed deeper in the fillet, indicating that the smoking process affect the fillet surface differently. Moreover, a significant correlation was observed between the reflective properties at 470 nm and total contents of carotenoids in the muscle (r=-0.629, P=0.036). At 450 nm however, the correlation was insignificant (r=-0.580, P=0.066). Moreover, significant correlations were observed for both reflection of light at 450 and 470 nm and contents of SFA (r=-0.631, P=0.031 and r=-0.622, P=0.041, respectively) and contents of MUFA (r=0.624, P=0.040 and r=0.614, P=0.045, respectively).

3.5. Stability of fatty acids

Salting and cold smoke processing did not affect the fatty acid composition of neither conventional nor organic salmon fillets significantly (P>0.101-0.883). Grouping the fatty acids into SFA, MUFA, PUFA (Figure 5) did not give any further information except for a tendency for higher stability (P>0.058) of MUFAs as compared to PUFAs throughout processing and storage.

4. Discussion

The chemical composition of the organic and conventional salmon fillets used as raw material in the presented processing and storage study, reflected the dietary composition of carotenoids and fatty acids of the respective feeds (Lerfall et al., 2016). Several previous

studies has shown that both feed composition and feeding strategies affect contents of lipids (Bjerkeng et al.,1997; Regost et al., 2001) , fatty acid composition (Regost et al., 2004; Rørå et al., 2005; Shearer, 1994), contents of carotenoids (Nickell and Bromage, 1998) and composition of muscle carotenoids (Buttle et al., 2001; EFSA, 2007; Storebakken et al., 1987) in salmon flesh.

Dry salting is driven by diffusion (Dyer, 1942), i.e. salt in, solute out (Horner, 1997). Diffusion of salt into the fish muscle is affected by several factors *e.g.* flesh lipid content (Gallart-Jornet et al., 2007) and the ratio between fillet surface area and fillet thickness. In the presented study, total lipid content of the flesh did not differ between organic and conventional salmon, but the conventional salmon fillets were thicker probably due to a higher condition factor (Cf). Higher Cf and thicker fillets of conventional salmon did however not affect the diffusion of NaCl in or the flux of solutes out from the fillets during processing. During refrigerated storage, a significantly higher liquid loss was observed from vacuum packaged smoked conventional fillets as compared to the organic product. This liquid loss was found related to the fatty acid composition of the flesh, where a high liquid loss correlated strongly to high contents of MUFA and n-6 fatty acids (r=0.935 and 0.911, respectively). These connections were strengthened by strong negative correlations between the liquid loss and contents of SFA and n-3 fatty acids (r= -0.920 and -0.930, respectively).

Previous studies have shown that the stability of carotenoids in salmon flesh is affected by cold smoke processing (Birkeland et al., 2004b; Lerfall et al., 2011). It is important to consider the mass transfer when studies of carotenoid stability during processing and storage are performed. In the present study, retention (% of initial content) of carotenoids were calculated as percentage of the dry matter where the contents of NaCl were subtracted. It is therefore likely that observed losses of carotenoids during salting could be explained by either decomposing or extraction of carotenoids during the dry salting procedure. Different carotenoids have earlier been reported to withstand cold smoke processing differently. Lerfall et al. (2011) reported lutein to be more stable as compared to astaxanthin and idoxanthin in dry- and injection salted cold smoked Atlantic salmon. In the presented study however, all presented carotenoids were found to be equally stable during processing. Lerfall et al. (2011) also reported the smoking step to be the main contributor to

loss of carotenoids from the fillet surface during a cold smoking protocol. Opposite to that, Birkeland et al. (2004b) reported the salting step to be responsible for the main loss of carotenoids from the salmon flesh during cold smoking. This is in line with results from the present study.

Salting and cold smoking give darker, less reddish and more yellowish fillets as compared to the raw material (Cardinal et al., 2001; Lerfall and Rotabakk, 2015). Due to differences in flesh carotenoid composition between the organic and conventional Atlantic salmon, colorimetric differences between the groups could be expected (Buttle et al., 2001; Skrede and Storebakken, 1986). Differences in translucence of the raw material (lower L-value of organic fillets) were not observed after smoking and 14 days refrigerated storage. This observation coincided with lower reduction of redness (Δa) and total color change (ΔE) in organic fillets during processing and storage. Total color change (ΔE) is earlier reported to increase stepwise after dry salting and cold smoking (Birkeland and Bjerkeng, 2005). The present study suggests that during storage somehow, the colorimetric characteristics of the raw material seems to some extent to be restored. Earlier studies by Lerfall and Rotabakk (2015) also found that equalization of colorimetric differences occur during storage. They also reported the colorimetric properties of cold smoked salmon fillets that were stored for 14 and 28 days, to be more equal to the raw material as compared to freshly smoked fillets. This is most likely a result of changes in light scattering caused by structural changes due to alteration of the muscle structure. Moreover, lower reflection of light from the fillet surface of organic cold smoked fillets as compared to conventional fillets was observed, but these differences were not observed deeper into the fillets. The development of color during smoking is caused by a series of chemical reactions such as protein and lipid oxidation (Hidalgo and Zamora, 2000) as well as Maillard reactions (Martins et al., 2000). It is therefore likely to believe that observed differences in Δa, ΔE and reflection properties in the fillet surface of the smoked product may be related to how the smoke components react with the chemical components that differs in organic and conventional salmon flesh, *e.g.* fatty acids.

The fatty acid profile of both fresh and smoked fillets reflects those of the diets (Lerfall et al., 2016), and no higher loss of individual fatty acids have been observed after smoking and/or after storage (Rørå et al., 2003). This was confirmed in the presented study meaning that it is

possible to predict the fatty acid profile of cold smoked Atlantic salmon from the fatty acid composition of the raw material.

5. Conclusion

It is concluded that the colorimetric characteristics of the fillet surface and the liquid loss during storage of cold smoke fillets are affected by the fatty acid composition of the flesh. The diverse composition of carotenoids in the organic salmon seems however to have minor impact on the color of the smoked product. It is concluded that the dry salting process was found to be the main cause for losses of carotenoids throughout salting, smoking and 14 days refrigerated storage. No differences in stability between the presented carotenoids in neither conventional- nor organic salmon was observed. In addition, the colorimetric perception of the organic and conventional cold smoked product was equal after 14 days refrigerated vacuum storage indicating excellent carotenoid stability of both organic and conventional salmon during processing, smoking and storage.

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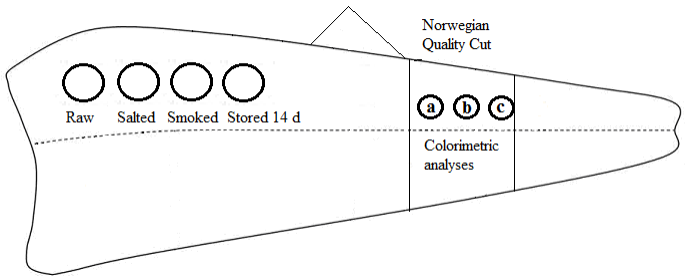


Figure 1 Schematic illustration showing the areas upon the right fillet where analyses were conducted. Areas Raw, Salted, Smoked and Stored 14 d represent sampling areas for chemical analyses after respective processing or 14 days storage. Areas a-c represent areas in the Norwegian Quality cut (NQC) where colorimetric analyses were performed.

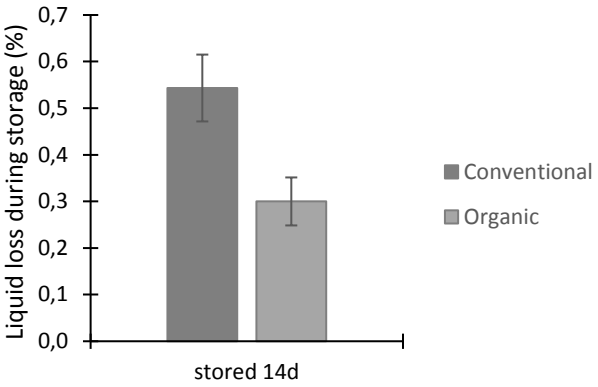
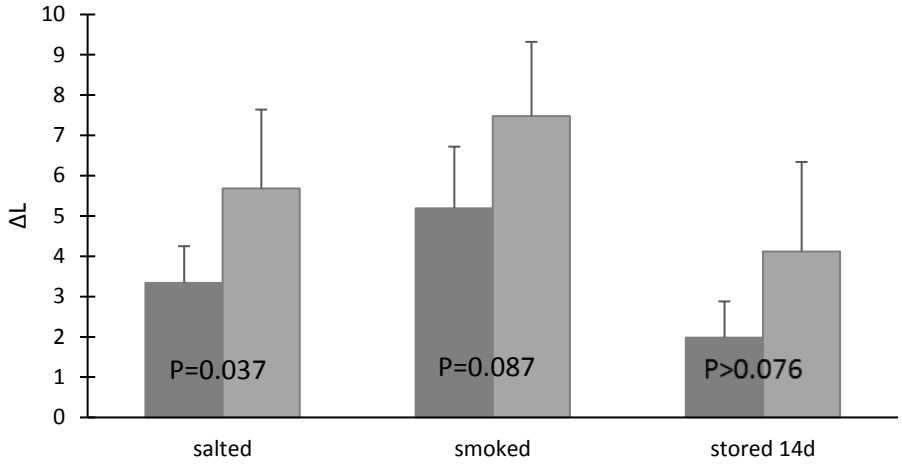


Figure 2 Weight changes (liquid loss, % of freshly smoked fillets) after 14 days refrigerated storage of organic and conventional cold smoked fillets (student t-test, $P<0.001$).



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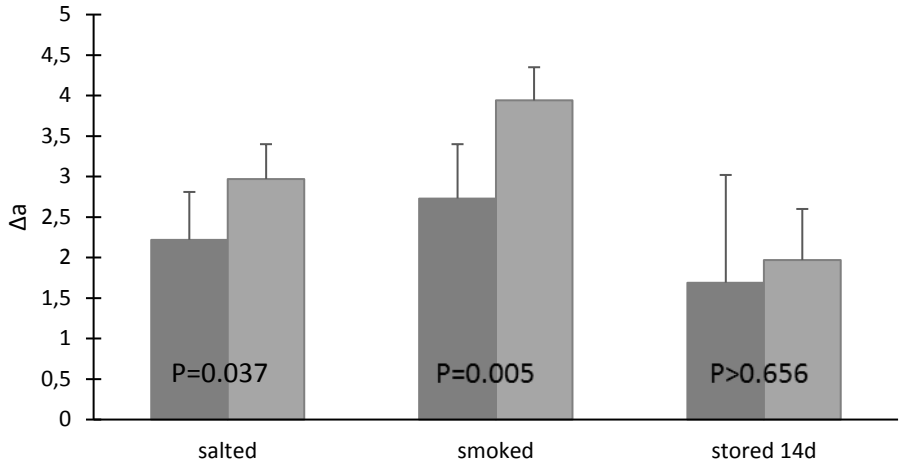

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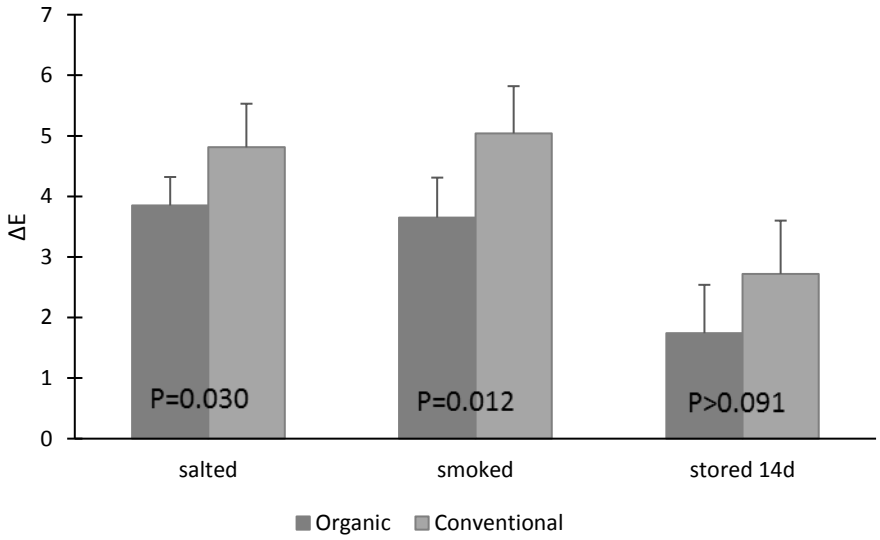

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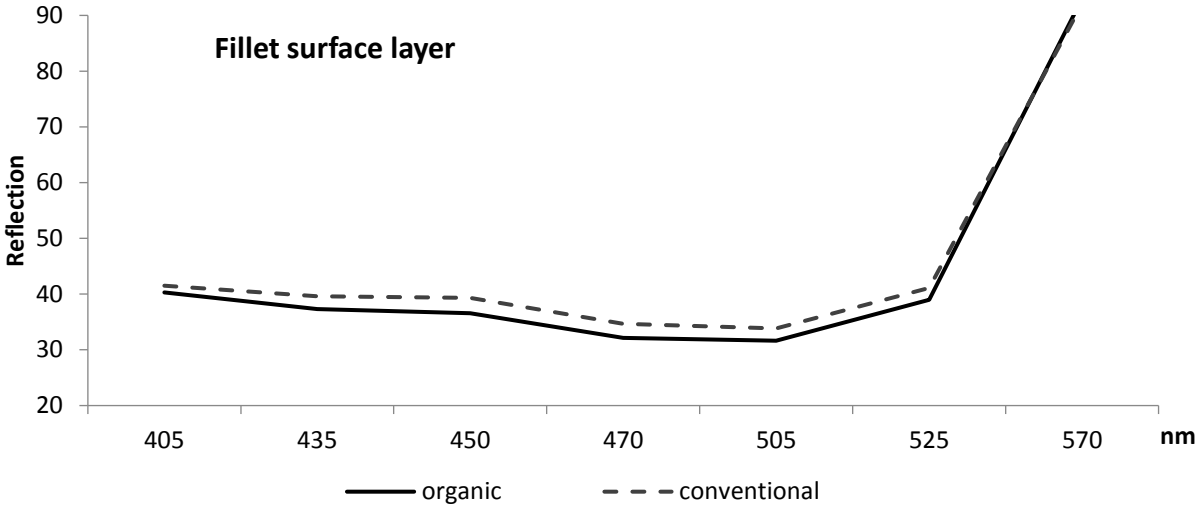
499 **Figure 3** Colorimetric changes (CIE, 1994) in the fillet surface between raw- and salted, smoked and stored (14
500 days) organic- and conventional salmon fillets. P-values were calculated by a two-factor analysis of variance
501 (two-way ANOVA) combined with Tukey`s comparison test (level of significance: P<0.05). Bars indicate one SD.

502



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504 **Figure 4** Reflective properties of a vertical cut of cold smoked fillets stored (14 days). Significant differences
505 were observed between the groups at wavelengths 450 and 470 nm (ANOVA, P=0.046 and 0.048, respectively)

506

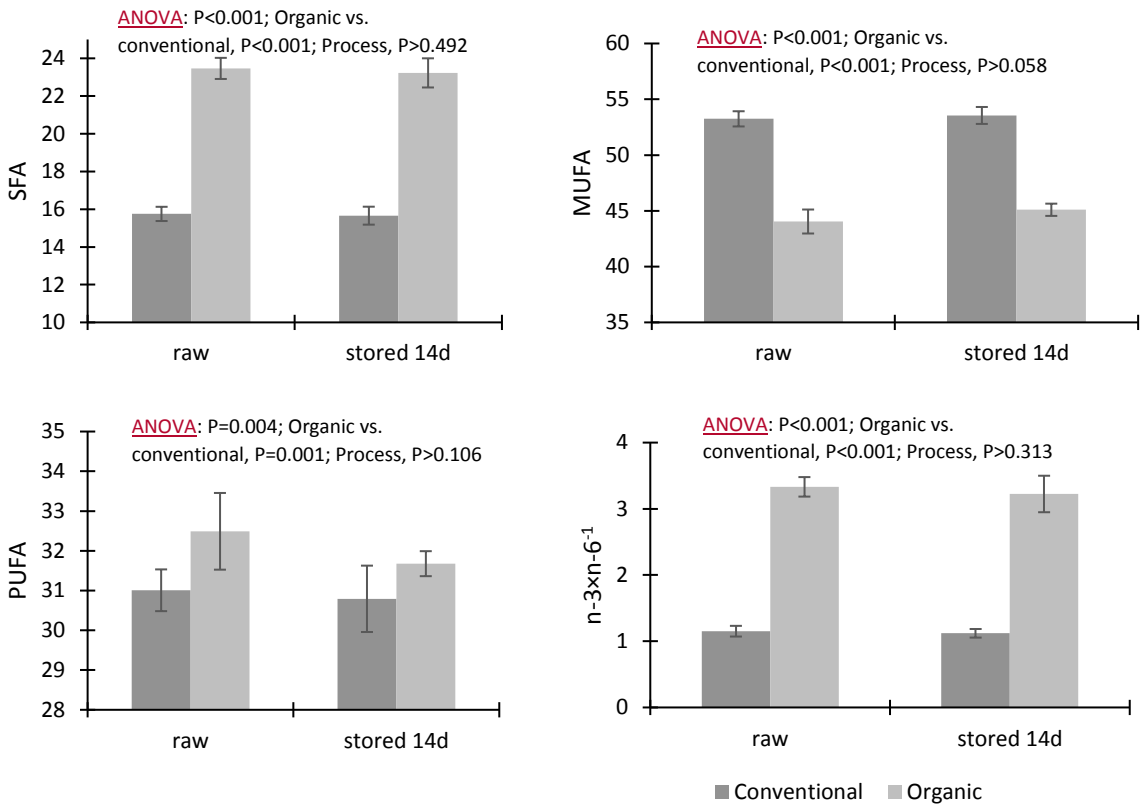

Figure 5 Distribution (%) of SFA, MUFA, PUFA and the ratio between n-3 and n-6 fatty acids in raw and cold smoked (stored 14 days) organic- versus conventional Atlantic salmon. Data were analyzed by a two-factor analysis of variance (two-way ANOVA).

Table 1 Total fat content and fatty composition in muscle from organically and conventionally reared Atlantic salmon

Parameters	Organic	Conventional	*Effect*
Total lipids (g×100g^{-1})	8.0±3.88	9.4±2.56	*P>0.498*
Composition of fatty acids			
C14:0	4.4±0.26^{A}	2.3±0.04^{B}	*P<0.001*
C16:0	11.9±0.72^{A}	9.5±0.38^{B}	*P<0.001*
C16:1n7	4.3±0.29^{A}	2.7±0.06^{B}	*P<0.001*
C18:0	2.2±0.17	2.3±0.13	*P>0.115*
∑C18:1n7 and n9	20.9±0.52^{B}	40.7±0.32^{A}	*P<0.001*
C18:2n6	5.5±0.31^{B}	12.6±0.32^{A}	*P<0.001*
C18:3n3	2.0±0.12^{B}	4.9±0.11^{A}	*P<0.001*
C18:4n3	1.4±0.12^{A}	0.7±0.03^{B}	*P<0.001*
C20:1n9	9.6±0.61^{A}	4.5±0.17^{B}	*P<0.001*
C20:4n6	0.4±0.02^{A}	0.4±0.01^{B}	*P=0.017*
C20:5n3	4.6±0.34^{A}	2.8±0.22^{B}	*P<0.001*
C22:1n9	11.3±0.78^{A}	4.0±0.47^{B}	*P<0.001*
C22:5n3	1.9±0.13^{A}	1.2±0.04^{B}	*P<0.001*
C22:6n3	9.7±0.27^{A}	5.4±0.60^{B}	*P<0.001*
others	9.8±1.49^{A}	6.0±0.76^{B}	*P<0.001*
∑SFA	23.5±0.56^{A}	15.8±0.38^{B}	*P<0.001*
∑MUFA	44.0±1.08^{B}	53.3±0.68^{A}	*P<0.001*
∑PUFA	32.5±0.96^{A}	31.0±0.52^{B}	*P=0.010*
∑n3	19.7±0.34^{A}	14.9±0.75^{B}	*P<0.001*
∑n6	5.9±0.31^{B}	13.0±0.32^{A}	*P<0.001*
n3×n6^{-1}	3.3±0.15^{A}	1.2±0.08^{B}	*P<0.001*

Different capital letters superscripts within each row indicate significant differences (P<0.05) between the respective groups by a student t-test.

Table 2 Total pigment content and composition, and colorimetric characteristics, in muscle from organically and conventionally reared Atlantic salmon

Parameters	**Organic**	**Conventional**	*Effect*
Total carotenoids (mg×kg^{-1})	8.2±1.39	7.7±1.63	*P>0.626*
Composition of carotenoids			
Astaxanthin (mg×kg^{-1})	4.6±0.75^{B}	6.7±1.26^{A}	*P=0.012*
Adonirubin (mg×kg^{-1})	1.9±0.36^{A}	0.0±0.00^{B}	*P<0.001*
Lutein (mg×kg^{-1})	0.1±0.03^{B}	1.1±0.38^{A}	*P<0.001*
Adonixanthin (mg×kg^{-1})	0.8±0.19^{A}	0.0±0.00^{B}	*P<0.001*
Canthaxanthin (mg×kg^{-1})	0.5±0.10^{A}	0.0±0.00^{B}	*P<0.001*
Asteroidenone (mg×kg^{-1})	0.2±0.03^{A}	0.0±0.00^{B}	*P<0.001*
Colorimetric parameters			
*L**	42.8±1.53^{B}	45.5±1.53^{A}	*P=0.019*
*a**	9.4±0.99	9.9±0.87	*P>0.391*
*b**	18.2±1.91	18.6±2.03	*P>0.768*
H^{0}_{ab}	62.8±0.85	62.0±1.04	*P>0.198*
C*	20.5±2.13	21.1±2.18	*P>0.676*

Different capital letters superscripts within each row indicate significant differences (P<0.05) between the respective groups by a student t-test.

522 **Table 3** Retention (% of initial) of total amounts of carotenoids in the fillet surface after each processing step

Parameters	**Organic**	**Conventional**	*Effect*
Total carotenoids			
Raw	100.0±0.0[a]	100.0±0.0[a]	
Salted	84.5±4.7[b]	85.5±8.5[b]	*P>0.841*
Smoked	84.6±5.9[b]	82.7±6.3[b]	*P>0.543*
Stored	79.4±3.9[b]	81.9±6.0[b]	*P>0.325*
Effect of processing step	P<0.001	P=0.001	

523 Different lowercase superscripts within each column indicate significant differences (P<0.05) between the
524 processing steps by a two-factor analysis of variance (two-way ANOVA) combined with Tukey`s pairwise
525 comparison test.

526

Table 4 Colorimetric characteristics (CIE, 1994) of the fillet surface of freshly cold smoked- and cold smoked fillets stored 14 days

Parameters		**Organic**	**Conventional**	*Effect*
*L**				
	Smoked	37.6±1.0^{b}	38.3±1.0^{b}	*P>0.300*
	Stored	40.8±1.5^{a}	41.3±1.3^{a}	*P>0.546*
	Effect	*P=0.006*	*P=0.002*	
*a**				
	Smoked	6.6±0.5	5.9±0.8^{b}	*P>0.138*
	Stored	7.7±1.0	7.9±1.1^{a}	*P>0.740*
	Effect	*P>0.239*	*P=0.005*	
*b**				
	Smoked	14.3±0.9^{bA}	13.8±0.7^{bB}	*P=0.034*
	Stored	17.5±0.8^{a}	17.6±1.4^{a}	*P>0.919*
	Effect	*P=0.003*	*P<0.001*	
H^{0}_{ab}				
	Smoked	65.0±1.8	65.7±1.9	*P>0.557*
	Stored	66.3±3.2	65.9±2.1	*P>0.780*
	Effect	*P>0.712*	*P>0.997*	
*C**				
	Smoked	15.7±0.9^{bA}	14.4±1.0^{bB}	*P=0.040*
	Stored	19.2±0.7^{a}	19.3±1.7^{a}	*P>0.862*
	Effect	*P=0.005*	*P<0.001*	

Different lowercase superscripts within each parameter (column) and different capital letter within each row indicate significant differences (P<0.05) by a two-factor analysis of variance (two-way ANOVA) combined with Tukey`s pairwise comparison test.