A comparative study of organic- versus conventional farmed Atlantic salmon. I. Pigment and lipid content and composition, and carotenoid stability in ice-stored fillets

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Abstract

The aim of the present study was to investigate retention of pigment´s and composition of fatty acids (FA) in farmed organic- and conventional Atlantic salmon fed commercial feed adapted to organic and conventional salmon farming, respectively. Moreover, stability of pigments, FAs and colour was investigated throughout the fillet shelf life. No significant differences were observed in fish weight between organic- and conventional salmon (5.44 and 5.40 Kg, respectively). However, the average condition factor (Cf) was significantly lower in organic (1.00) as compared to conventional salmon (1.15). The fillet characteristics of the organic salmon investigated were; 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 as compared to conventional salmon. Only small differences were however found regarding stability of carotenoids, Vitamin E, FAs and colour during 22 days ice storage. Hence, the pigment stability for both groups was regarded as good.

1. Introduction

Organic farming and production has been regulated at EU level since 1991. The principles defined by the International Federation of Organic Agriculture Movements (IFOAM) form the basis for the production of organic food. Today the European requirements for organic production are set by Council Regulation (EC) No 834/2007 defining the official EU aims, objectives and principles of organic farming and production, and by implementing regulations (EC No. 889/2008, 710/2009 and 1358/2014), detailing the organic production including requirement to feed and feed raw material. All products labelled as organic and sold in the EU must be produced in accordance with these regulations.

The characteristic pink colour of wild salmonid muscle is a result of the deposition of naturally occurring carotenoid pigments, mainly to astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) as in wild rainbow trout (*Salmo gairdneri* R.) (Schiedt et al., 1986), and wild Atlantic salmon (*Salmo salar* L.) (Nickell and Springate, 1999). Fish cannot synthesize carotenoids *de novo* and therefore have to obtain these pigments from dietary sources. Astaxanthin has been widely used as an animal additive for several decades, mainly in the aquaculture industry. Almost all commercially available astaxanthin (> 90%) for aquaculture is produced synthetically from a petrochemical source. This highly stable synthetic source consists of free,

non-esterified astaxanthin, stabilized with an antioxidant (*e.g.* ethoxyquin) and embedded in a carbohydrate and gelatin matrix. In 2011, the source was the cheapest in the market with a price of around $ 2,000 per kilogram, while natural pigment sources were retailed for around $ 7,000 per kilogram (Schmidt et al., 2011). Several studies where pigment sources are compared have shown that synthetic astaxanthin has the best bioavailability (Choubert et al., 2006; Choubert et al., 1995; Foss et al., 1984). Consumer desire for fish farming to be sustainable and environmentally responsible has led to increased use of natural ingredients in the feed. It is therefore an increased focus on the development of natural pigment sources for aquaculture, such as from certain green algae, red yeast or a soil bacterium. The use of natural astaxanthin from red yeast (Sanderson and Jolly, 1994) or green algae (Choubert et al., 2006) as a replacement for synthetic astaxanthin is well documented, but has proven to be partially unsuccessful and relatively expensive when compared to synthetic sources. In 2007, the European Food Safety Authority approved the use of Panaferd-AX®, with certain modifications, for use in aquaculture of salmonids (EFSA, 2007). This natural product 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.

Deposition of carotenoids in salmonid flesh occurs because of several processes: absorption of pigments in the digestive tract, transport of pigment in the blood, retention in the muscle and metabolism of carotenoids. Achieving successful pigmentation in farmed salmonids is crucial in having a sellable product for the marked place. Although, the pigmentation has no effect on fish taste (Østerlie et al., 2001) the consumer perception relies heavily on the flesh colour (Anderson, 2000) and is in addition product freshness the most important quality criterion (Koteng, 1992).

The hue in salmon muscle after breeding is depending on several factors such as; the astaxanthin source, geometric isomers, secondary carotenoids in the source, the dose, the length of the feeding and other ingredients (*e.g.* lipids) in the feed (Bjerkeng, 2000; Bjerkeng and Berge, 2000; Bjerkeng et al., 1997; Buttle et al., 2001; EFSA, 2007; Hatlen et al., 1998; Mørkøre et al., 2001; Nickell and Bromage, 1998; Osterlie et al., 1999; Regost et al., 2001, 2004; Skrede and Storebakken, 1986; Storebakken et al., 1987; Torrissen, 1985). In addition,

astaxanthin is a very good antioxidant (Goswami et al., 2010; Stahl and Sies, 2003) as well as in fish oils (Østerlie and Lerfall, 2015) and acts as pro-vitamin A in fish (Matsuno, 1991; Miki, 1991).

Demand for all types of organic food products is highest in North America and Europe, while Scandinavian consumers and customers from the Alpes spend the most money on the organic food (Willer and Kilcher, 2009). Olesen et al. (2010) found that the Norwegian consumer was willing to pay 15% more per kilogram for organic salmon. In Switzerland, a relatively large proportion (23%) of traded salmonids is produced organically. Consumer desire for a more environmentally friendly and sustainable salmon product results in the need for product differentiation and thus a significant market opportunity for salmon producers. However, colour variation in the fillet due to improper use of pigment or new and inferior pigment sources may potentially have a negative impact on the consumer´s overall acceptance of the product. Therefore, the aim of the present study was to investigate retention of pigment´s and composition of fatty acids (FA) in commercial farmed organic- and conventional Atlantic salmon fed commercial feed adapted to organic- and conventional salmon farming, respectively. Moreover, stability of pigments, FA´s and flesh colour was investigated throughout the shelf life of the fillet. This study is the first study of two, where the second paper focuses on the suitability of the presented organic salmon as raw material for the smoking industry (Lerfall et al. AQUA-D-15-00889).

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 ambient 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 ca 1 kg in October 2013. Then the fish were 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 (fish density lower than 25 kg fish × $(m^3)^{-1}$), while fish at location Furneset (62° 63' 39" N; 7° 13' 93" E) produced in compliance with EU rules for organic production (EU-regulations 834/2007) until harvest (fish density lower than 10 kg fish × $(m^3)^{-1}$). Both fish groups were kept in large circular net pens

(circumference; 157m, net depth 30 m) at ambient rearing condition, and were fed either organically or conventional salmon extruded feeds delivered by feed manufacturers EWOS Ltd, Bathgate, and Skretting N, Averøy, respectively. Both groups of fish were fed to apparent satiation on a daily basis and fish's appetite level were judged by trained feeding operators receiving underwater images from permanently submerged cameras in all cages. The organic feeds contained a high proportion of marine ingredients (>65% over the life cycle), predominantly derived from herring trimmings, and organically certified legumes and oil seed meals, -compared to conventional feeds where a higher proportion of conventional vegetable protein and oil were used at the expense of marine protein and oil ingredients (Table 1). The digestible energy level of the organic and conventional feed were similar (20.5-21.5 MJ kg^{-1}) but dietary lipids contributed substantially more to the digestible energy in organic feeds than in conventional feeds (Table 2). The organic feed were added approximately 68 mgxkg^{-1} of natural pigment source Panaferd-AX® (Nippon oil, Japan), stabilized with natural antioxidants, whereas synthetic astaxanthin (Carophyll Pink (CP), DSM, Switzerland) was supplemented to the conventional feed at approximately 50 mgxkg^{-1} dose.

One 2 kg batch of each feed type (organic- and conventional feed, respectively) in use the days prior to harvest were obtained and later analyzed for water, protein, carotenoid and lipid content and compositions, and vitamin E content at HIST following standard analytical procedures.

On May 23 (2014), both groups of fish were starved according to normal farm practices before they were transported by a well-boat from the rearing cage to the sea cages at the processing plant where they were acclimated for 2 days before commercial slaughtering (percussive stunning combined with gill cutting followed by chilling and bleeding (seawater, 0°C, 20-30 min).

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 weighed before the right fillets were transported on wet ice in polystyrene boxes to Sør-Trøndelag University College (HiST, Trondheim, Norway). The right fillets were divided into two different groups. The first group (both organic and conventional fillets) were used to study raw fillet quality whereas the second group (both organic and conventional fillets) were used in a cold smoking trial (Lerfall *et al.* AQUA-D-15-

00889). In the first trial six randomly chosen organic and conventional fillets were stored on ice for 22 days to study the stability of flesh carotenoids, colour (CIE, 1994), fatty acids and vitamin E during storage. In the second trial, six randomly chosen fillets of each group were dry salted and cold smoked. Thereafter mass transfer (water and sodium chloride), stability of carotenoids, colour and fatty acids were assessed through the processing steps and over 14 days refrigerated storage at 4 °C.

2.2. Fillet quality

Flesh samples for fillet quality parameters except for vitamin E were obtained after 2, 7, 10, 15, 17 and 22 days ice storage, whereas vitamin E was analyzed after 2, 10 and 22 days. At each sampling date a cylindrical sample (diameter 31 mm) was punched out of the dorsal white muscle according to Figure 1. All samples were thereafter vacuum packaged and frozen at -80 °C until further analyses.

2.3. Experimental methods

Total content of protein was calculated in feed samples from nitrogen measurements (NCFA, 2003) using the formula: %protein=%nitrogen×6.25. The water content of both the feeds and the organic- and conventional fish white muscle samples were calculated gravimetrically after drying at 105 °C for 24 hours (ISO.6496, 1983).

Lipids were extracted from crushed feeds (crushed in a mortar), where the conventional feed, due to the embedding with carbohydrate and gelatine, was added liquid enzyme (Protex 6L, protease from *B. licheniformis*, Genecor International, 200 µL). Both the conventional and organic feed had water added thereafter (8.0 mL), mixed separately and put in an ultrasound bath (50 °C, 30 min). The lipids were extracted from the feed mixtures and from organic- and conventional fish muscle by a method after Bligh and Dyer (1959) with slight modifications.

Total amount of lipids was calculated by net weight and the lipids were thereafter analysed for total amounts of carotenoids together with distribution of carotenoids and fatty acids (FA).

The distribution of FA were analyzed as FA methyl-esters in the lipid fraction extracted from the feeds and the organic- and conventional fish muscle, respectively. FA 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 temperature had an isothermal temperature at 210 °C. Preparation of methyl esters of the samples was conducted as in Metcalfe et al., (1966).

Total amounts, and distribution of carotenoids were analyzed in the lipid fraction extracted from the feeds and the organic and conventional fish muscle, respectively. Approximately 0.5 g lipids were added to 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).

Vitamin E (α-tocopherol) were analysed both in the feeds, and organic and conventional fish muscle mixed with ethanol (96%) and homogenized (9500 rpm, 2 min) using an Ultra-Turrax T25, Janke & Kunkel IKA®-Labortechnik, Staufen, Germany. Samples were saponified with KOH (0.5 M in CH_3OH, BHT 0.2%) and extracted with hexane:diethyl ether (4:1;v/v) (Lerfall and Østerlie, 2011). Vitamin E content was analysed on HPLC, Agilent1100 liquid chromatograph (Agilent Technologies, Paolo Alto, CA, USA) connected to an Agilent photodiode array UV-VIS detector using a not end-capped silica gel HPLC column (Suplex PKB-100, 250×4.6 mm, 5µm, Supelco, USA). Vitamin E (α-tocopherol) was detected at 295 nm (21 °C) with methanol:methyl-*tert*-buthyl ether + water (80:20+5;v/v) as mobile phase (isocratic, flow 0.8 ml min^{-1}) and quantified by response factors (RF) prepared from standard α-tocopherol (Calbiochem, Germany).

Colorimetric assessments (CIE, 1994) were performed on raw fillets at three defined points (Figure 1) with a Minolta Chroma meter, CR200 Minolta, Japan. L^* describes the lightness of the sample, a^* intensity in red ($a^* > 0$), b^* intensity in yellow ($b^* > 0$). Average values of each fillet were used for statistical analyses.ISO.13299 (2003)

2.4. Statistics

Data were analyzed by a general linear model (GLM), one-way analysis of variance (ANOVA) 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% ($P<0.05$). All results are given as average ± standard deviation (SD), unless otherwise is stated.

3. Results

3.1. Biometrics

No significant differences were observed in average head on gutted (HOG) weight of the organic and conventional salmon (5.44±0.24 kg and 5.40±0.25 kg, respectively; $P>0.648$). The average condition factor (Cf) was however significantly lower ($P<0.001$) in the organic- (1.03±0.09) as compared to conventional salmon (1.15±0.07). The lover Cf coincided with a 3 cm longer fork length of the organic as compared to the conventional salmon.

3.2. Chemical composition of the feeds and white salmon muscle

The organic salmon feed contained significantly more protein and less lipids and water compared to the conventional feed (Table 2). The higher content of lipids present in the conventional feed (7 percentage units), did not result in statistically more lipids in the conventional as compared to the organic salmon (Table 2, $P>0.454$). The lower vitamin E content in organic versus conventional feed (Table 2) resulted in an average retention of 10.2% and 12.6% vitamin E in organic and conventional salmon, respectively. Total contents of carotenoids were found to be nearly twice as high in the organic as compared to the conventional feed. As a result a non-significantly higher content of carotenoids were found in the organic salmon (Table 2, 8.8±1.24 versus 7.8±1.37 mg kg^{-1} in conventional). Of the total amount of carotenoids, astaxanthin represented 45.3 and 86.2% in the organic and conventional feed, respectively. In the white muscle of organic salmon however, astaxanthin contributed more to the total amounts of carotenoids (56.7%) as compared to the feed (45.3%). Moreover, in conventional muscle, the distribution of carotenoids (astaxanthin 86.9% of total carotenoids) reflected the composition of the respective feed.

The composition of carotenoids (% distribution) in the organic and conventional feed and in the respective salmon are presented in Table 3. The conventional feed consists of mainly astaxanthin, but lutein (11.7%) and canthaxanthin (2.0%) were also found. In the

conventional salmon however, only traces of canthaxanthin were found whereas astaxanthin and lutein had nearly the same distribution as the feed (87.9 and 12.2%, respectively). The organic feed consists of several different carotenoids; astaxanthin (45.2%), adonirubin (20.2%), adonixanthin (9.6%), canthaxanthin (7.7%), lutein (5.9%), β-carotene (4.4%), echinenone (3.5%), asteroidenone (1.9%) and 3-hydroxyechinenone (1.4%). However, several of the carotenoids represented in the feed (β-carotene, echinenone, 3-hydroxyechinenone) were not detected in organic salmon flesh.

Organic salmon stored on ice revealed higher total pigment content relative to conventional salmon flesh and astaxanthin comprised less of total pigments than in conventional salmon throughout the storage period. Ice storage throughout the shelf life of the fresh fillets did not affect the total carotenoid content or astaxanthin concentration in either organic or conventional salmon (P>0.209, Figure 2) revealing good pigment stability in both groups of fish.

The fatty acid (FA) distribution of the organic and conventional feeds were found to be significantly different (Table 4, Multivariat GLM; P<0.001). The organic feed had significantly higher contents of C14:0, C16:0, C16:1n-7, C18:4n-3, C20:1n-9, C20:4n-6, C20:5n-3 (EPA), C22:1n-9, C22:5n-3 and C22:6n-3 (DHA) fatty acids compared to the conventional feed. The FA profile of the salmon flesh reflected the profile of the feeds, which resulted in significantly higher contents of ∑SFA and ∑PUFA in the organic as compared to the conventional salmon. The n-3/n-6 ratio was significantly higher in the organic feed as compared to the conventional ($n\text{-}3 \times n\text{-}6^{-1}$= 0.9 versus 2.1, respectively; Table 4). In the fish however, a higher n-3/n-6 ratio was observed for both groups, and organic salmon had a three times higher ratio when compared to the conventional salmon (1.2 versus 3.3, respectively; Table 4).

The distribution of ∑SFA, ∑MUFA, ∑PUFA and the ratio between n-3 and n-6 PUFAs in the salmon muscle were found to be affected by feed (organic versus conventional) and storage time (GLM, P<0.001, Figure 3). During storage a significant increase of ∑SFA was observed in conventional muscle (P=0.023). In stored organic fish muscle however, the content of ∑SFA was insignificant (P>0.101) but numerically higher. Moreover, a significant decrease of ∑MUFA was observed for both the organic and conventional salmon (4.7 versus 3.7 percentage point; P=0.002 and P<0.001, respectively) over the storage period. As a consequence of a lower

content of ∑MUFA, a relative increase of ∑PUFA was observed in both groups as affected by storage time (organic: 3.7 percentage points, P=0.001; conventional: 2.8 percentage points, P<0.001). Moreover, changes in FA profile during storage resulted in a higher n-3/n-6 ratio. The n-3/n-6 ratio increased 43 and 30 percent between day 2 and 22 for organic- and conventional salmon, respectively (P<0.001).

The content and stability of vitamin E throughout the shelf life of the organic and conventional salmon fillets is shown in Figure 4. The overall model (GLM) showed a significant effect of feed (organic- versus conventional, P<0.001) and days on ice (P=0.031). Splitting the model into separate groups of organic and conventional salmon however, showed that the observed decrease in Vitamin E during storage was insignificant (ANOVA, P>0.175 and >0.056, respectively).

3.3. Fillet appearance

The fillet appearance were found to be affected by both the storage time and the farming conditions (organic versus conventional, Multivariate GLM, P<0.001, Table 5). Organic fillets were significantly darker (lower *L**-value) compared to the conventional salmon fillets (GLM, P<0.001). During ice storage, fillet lightness increased for both groups, which resulted in a more diffuse white appearance of the fillets at end of the storage trial. Parameters *a** (redness) and *b** (yellowness) were not different between the groups. However, after storage both organic and conventional fillets had a higher redness, while there was an insignificant increase in yellowness in both groups (P>0.228 and >0.580, respectively) during storage.

4. Discussion

The fish examined in the presented study were obtained from two nearby fish farms producing organic and conventional Atlantic salmon, respectively. Both farms were located in Romsdalsfjorden, Norway with a distance of 2.5 km between them and with equal environmental conditions. The organic fish had been fed organic salmon feed from smolt input to harvest, and followed an organic production protocol, while the conventional salmon followed commercial farming procedures and had been fed conventional feeds from approximately 1kg onwards. Thus, it is likely that observed differences in condition factor, chemical composition and colorimetric perception between the organic and conventional salmon were caused by different feeding and farming strategies applied.

Retention of carotenoids in salmonids are influenced by several factors including dietary pigment type (Bjerkeng et al., 2007; Buttle et al., 2001; EFSA, 2007; Skrede and Storebakken, 1986; Storebakken et al., 1987), carotenoid level (Bjerkeng, 2000; Ytrestøyl et al., 2006), lipid level (Nickell and Bromage, 1998) and oil source (Regost et al., 2004). The presented study showed that the carotenoid content and composition of both conventional and organic salmon reflected the pigment composition of the feed. Thus, retention of carotenoids in the organic salmon fed Panaferd-AX were found to be more diverse. However, differences in carotenoids composition of white muscle together with no retention of β-carotene, echinenone and 3-hydroxyechinenone, indicated a selective retention or "competition" between the presented carotenoids in digestion, transport and/or absorption process in the fish. In a feeding trial of rainbow trout reported by EFSA (2007) cantaxanthin and adonirubin from the source Panaferd-AX were found to be dose-dependent whereas astaxanthin was not. The carotenoid deposition in muscle tissue of the control group fed synthetically produced astaxanthin (EFSA, 2007) was however found to be dose dependent. These findings shows the complexity of carotenoid retention in salmonids when several carotenoids are present in the diet. Such interactions between various pigments in natural pigment sources may create challenges when practical pigment regimes need to be established.

Both lipid content and the FA profile of the salmon muscle are affected by dietary lipid level (Bjerkeng et al., 1997; Regost et al., 2001) and oil source (Nanton et al., 2007; Torstensen et al., 2005). The organic feed used in the presented study contained predominantly herring trimmings fish oils (approximately 70% of added oils) whereas the conventional feed were higher in rapeseed oil at about 60% of dietary lipids. Introducing ingredients of plant origin as alternatives to marine meals and oils in feeds for aquaculture will affect the FA profile and the fish quality (Suárez et al., 2014). The FA profiles of the organic and conventional salmon reflected the lipid composition of the feeds. There were however minor changes observed in the distribution of FAs and higher contents of PUFAs in both groups of salmon as compared to the feeds. Animals including salmon cannot synthesis PUFAs with double bounds at the n-6 or n-3 carbon position (Cook and McMaster, 2002). It is therefore likely that significantly lower percentage of *e.g.* C22:1n-9 and higher contents of DHA (C22:6n-3) observed in both groups of salmon as compared to the contents in respective feeds were caused by a

selective digestion, transport or absorption of specific FAs, and/or differences in desaturase and elongase enzyme activity in the salmon. In fresh water, wild Atlantic salmon parr consume invertebrates that contain lots of C18:2n6 and C18:3n-3 with minor contents of C20:5n-3 and almost no C22:6n-3. After smoltification salmon enters the marine environment where their diets are naturally rich in n-3 HUFAs (C20:5n-3 and C22:6n-3). The genes encoding the desaturase and elongase enzymes responsible for the conversion of C18:3n-3 to C22:6n-3 are downregulated soon after the salmon migrate to the seawater. Several studies have however suggested that salmonids can utilize vegetable oils in seawater provided the diets containing enough C18:3n-3 to satisfy essential fatty acid requirements (Bransden et al., 2003; Polvi and Ackman, 1992). There are also evidences that no "switch off" of fatty acid-metabolizing enzymes occur in salmon post-smolts. Tocher et al. (1997) suggested that fish fed vegetable oils showed increased conversion of C18:3n-3 to C22:6n-3 and C18:2n-6 to C20:4n-6 compared with salmon fed fish oil. Moreover, Bell et al. (2001) found that a complete replacement of fish oil in the diet with rapeseed oil reduce the percentage of C22:6n-3 in dietary lipid by more than fourfold but the muscle lipid content by only twofold. In contrast to C22:6n-3, the monoenes C22:1n-9 and C18:1n-9 are discriminated against in muscle lipids relative to dietary lipids when presented in high concentrations (Bell et al., 2001).

The storage pattern of FAs in salmonids are another important factor that affect the FA distribution. A relatively large proportion of fat is stored in salmon muscle (Polvi and Ackman, 1992), but fat is also stored in the visceral cavity and elsewhere in the body (Jobling et al., 2002). The storage pattern of lipids and FAs in different parts of Atlantic salmon was elucidated in a study by Nanton et al. (2007). They reported the belly flap, myosepta and the visceral cavity to contain more triacylglycerols (TAGs) and MUFAs as well as less polar lipids and n-3 PUFAs compared to the muscle tissue.

Carotenoids are quite labile compounds where the stability of carotenoids in salmon muscle is related to the strength of the protein-carotenoid binding (Latscha, 1990). During ice storage, denaturation of proteins occurs as a result of autolysis which increases the drip loss (consisting of water, proteins, lipids and pigments) from the salmon tissue (Lerfall and Rotabakk, 2015; Lerfall et al., 2015; Ofstad et al., 1996). In the presented study however, similar amounts of pigments in the salmon muscle of both groups were observed throughout

the storage period. Hence, the muscle carotenoids in raw salmon were very stable during ice storage independent of pigment source in the diet. Carotenoids are excellent antioxidants and their properties are mainly due to physical quenching of singlet oxygen ($^1O_2$), where energy absorbed from $^1O_2$ to produce triplet oxygen ($^3O_2$) is converted to rotary and vibratory energy by the chromophore system of the carotenoid (Stahl and Sies, 2003). Another important antioxidant found in salmonids is vitamin E, which is a potential radical scavenger in both hydrophilic and hydrophobic environments (Cynshi et al., 1995). A slightly decrease of vitamin E found in conventional salmon during ice storage may be a result of the antioxidant action to protect degradation of unsaturated FA in muscle tissue. It is however not possible to explain observed changes in the FA profile during storage by degradation of vitamin E (reference of rancidity). It is therefore likely to believe that increased contents of SFAs and PUFAs related to MUFAs in the flesh during ice storage is related to other factors such as the lipid storage pattern (Nanton et al., 2007) and or different extractability properties of specific FAs.

The appearance of salmonid fillets are affected by several factors including dietary carotenoid concentration (Bjerkeng, 2000; Hatlen et al., 1998), pigment type (Buttle et al., 2001; Skrede and Storebakken, 1986; Storebakken et al., 1987), lipid level (Bjerkeng et al., 1997; Mørkøre et al., 2001; Nickell and Bromage, 1998; Regost et al., 2001) and oil source (Regost et al., 2004). Hence, it was likely to assume that organically and conventionally produced salmon would appear differently regarding flesh colour. In the presented study however, only minor differences were found between organic and conventional salmon in colorimetric characteristics. The presented organic salmon consists of significant amounts of adonirubin which is a deep red carotenoid (EFSA, 2007). Hence, it is likely to assume that the lower *L** observed in the organic salmon in the presented study is related to a higher concentration of deep red carotenoids in the organic muscle.

Several factors such as; fillet colour, fatty acid profile and the marked prize is important for consumers' willingness to buy organic produced Atlantic salmon. It is no doubt that organic salmon have higher production costs when compared to conventional. It is however difficult to predict the accurate cost differences between organic and conventional salmon since both costs and prizes will vary with the supply and demand of the marked.

5. Conclusion

It is concluded that the fillet characteristic of the organic Atlantic salmon showed similar total concentration of muscle carotenoids, but lower contents of astaxanthin, and a more diverse composition of muscle carotenoids than conventional salmon. Organic salmon appeared to have a significantly darker red appearance compared to conventional salmon. Higher contents of n-3 FA, higher contents of SFAs and PUFAs, lower contents of MUFAs were found in organic salmon. Only small differences were however found regarding stability of carotenoids, Vitamin E, FAs and colour during 22 days ice storage, revealing that both organic and conventional produced salmon is well pigmented, and that both natural and conventional pigment sources used in the present study are stable.

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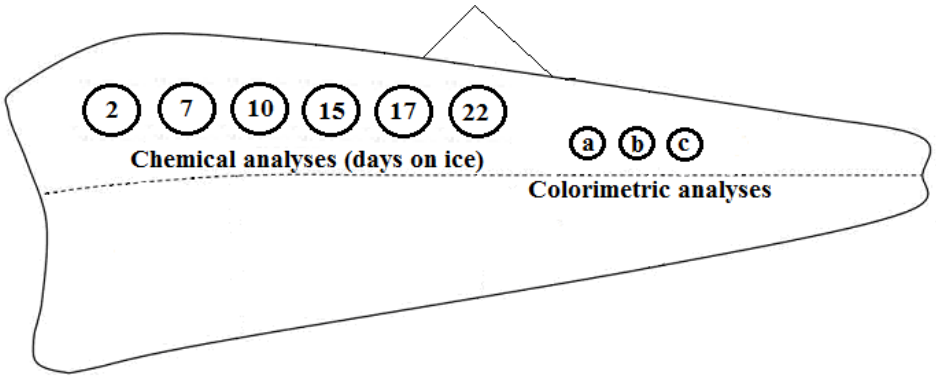

Figure 1 Schematic illustration showing the areas upon the right fillet which analyses were conducted. Areas 2, 7, 10, 15, 17 and 22 represent sampling areas for chemical analyses after respective days on ice. Areas a-c represented areas were colorimetric analyses were performed.

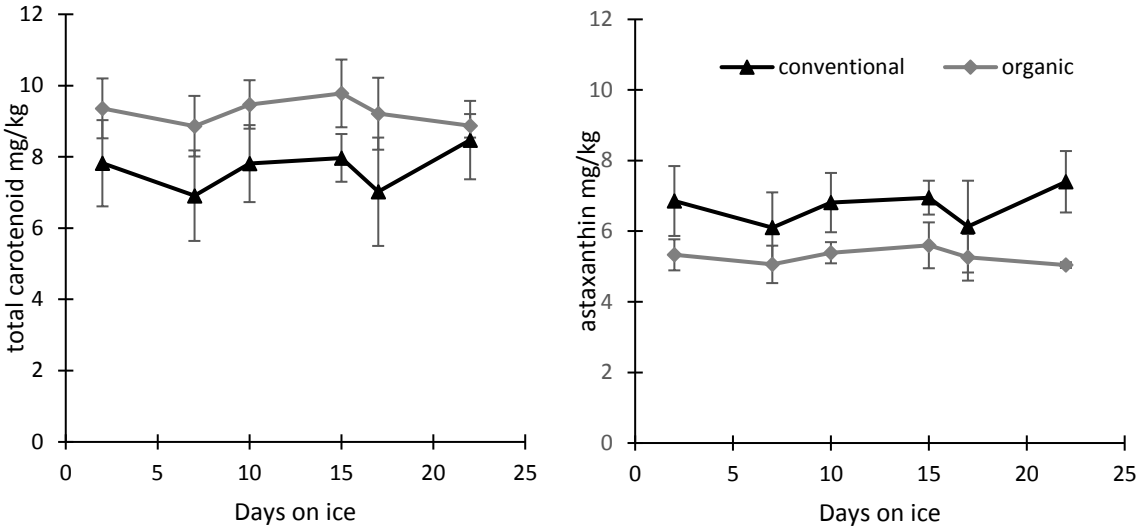

Figure 2 Contents (Mean±SD) of total carotenoids (**1A**) and astaxanthin (**1B**) in conventional versus organic Atlantic salmon stored on ice for 22 days (GLM: Corrected model, P<0.001; Intercept, P<0.001; Conventional vs. Organic, P<0.001; Days on ice, P>0.209).

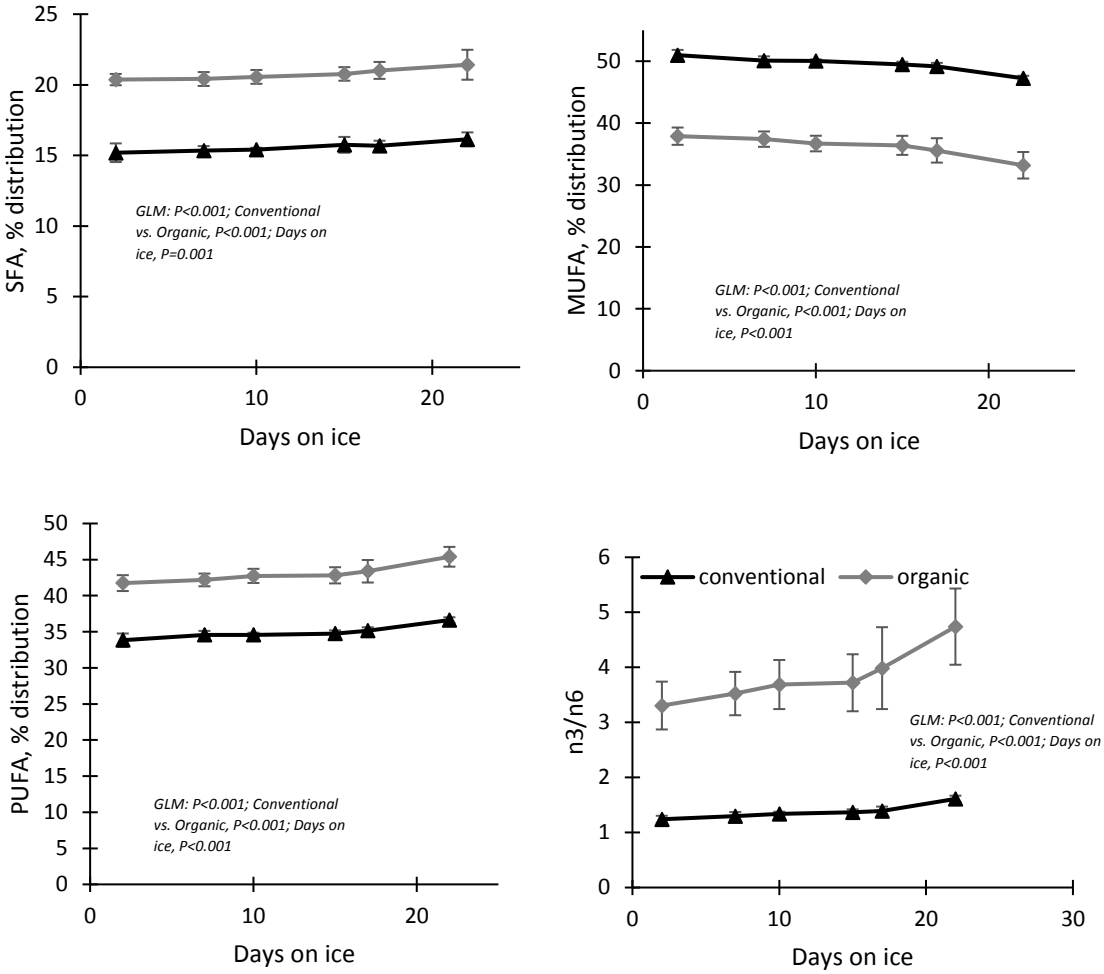

Figure 3 Distribution (%, Mean±SD) of SFA (**2A**), MUFA (**2B**) and PUFA (**2C**) and the ratio between n-3 and n-6 fatty acids (**2D**) in conventional versus organic Atlantic salmon stored on ice for 22 days.

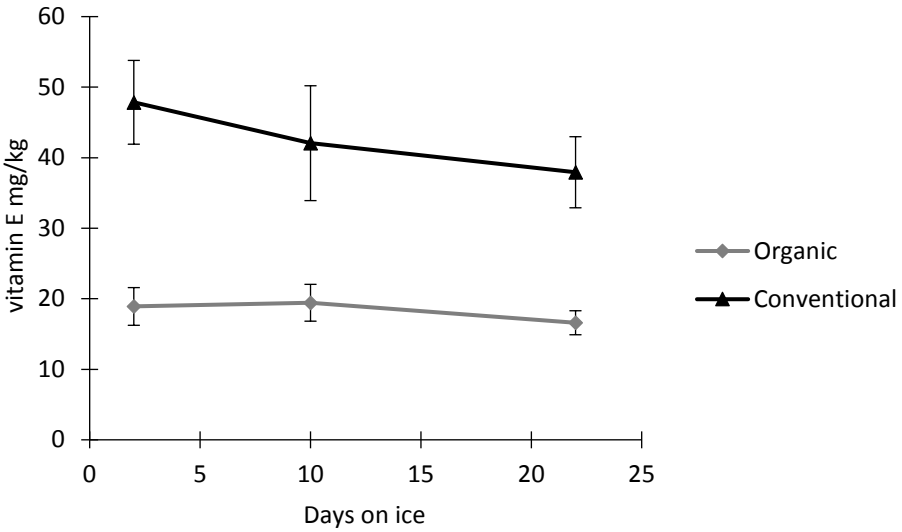

Figure 4 Contents (mg×kg^{-1}, Mean±SD) of vitamin E in conventional versus organic Atlantic salmon stored on ice for 22 days (GLM: Corrected model, P<0.001; Intercept, P<0.001; Conventional vs. Organic, P<0.001; Days on ice, P=0.031).

Table 1 List of raw materials used in the conventional and organic feed, respectively

Feed	
Conventional	**Organic**
Carophyll Pink	Panaferd-AX
Fishmeal	Trimmings fishmeal
Rapeseed oil	Trimmings fish oil
Fish oil	Organic rapeseed oil
Soya protein concentrate	Organic soya expeller
Wheat	Organic sunflower expeller
Wheat gluten	Organic wheat
Faba beans	Organic faba beans
Vitamin and mineral premixes	Organic feed peas
	Vitamin and mineral premixes

Table 2 Contents of lipids, protein, water, carotenoids and vitamin E of the conventional salmon feed, and the organic salmon feed as compared to profile of conventional and organic Atlantic salmon fed the respective feeds

	Feed		Fresh salmon	
Parameter	**Conventional**	**Organic**	**Conventional**	**Organic**
Protein, %	35.1±0.29^{B}	37.1±0.26^{A}	n.a.	n.a.
Water Content, %	7.1±0.16^{A}	6.8±0.11^{B}	68.4±2.19	68.9±2.83
Total lipids, %	40.3±0.52^{A}	33.2±1.24^{B}	9.4±2.56	8.0±3.88
Vitamin E mg kg^{-1}	380.0±44.27^{A}	184.8±14.27^{B}	47.9±5.94^{a}	18.9±2.67^{b}
Total Carotenoids mg kg^{-1}	40.3±1.83^{B}	76.4±1.73^{A}	7.8±1.37	8.8±1.24
Astaxanthin mg kg^{-1}	34.7±1.55	34.6±1.20	6.8±1.09^{a}	5.0±0.69^{b}

Different capital letter and different lowercase superscripts within each row indicate significant differences (ANOVA, $P<0.05$) within the parameter feed and fresh salmon, respectively. n.a.= not analysed

Table 3 Carotenoid composition (%) in the conventional salmon feed, and the organic salmon feed as compared to carotenoid composition (%) in conventional vesus organic Atlantic salmon fed the respective feeds

	Feed		Fresh salmon		
Carotenoid	**Conventional**	**Organic**	**Conventional**	**Organic**	***Effect***
β-Carotene	-	4.4±0.09^{A}	-	0.0±0.00^{B}	*P<0.001*
Echinenone	-	3.5±0.05^{A}	-	0.0±0.00^{B}	*P<0.001*
3-Hydroxyechinenone	-	1.4±0.03^{A}	-	0.0±0.00^{B}	*P<0.001*
Canthaxanthin	2.0±0.07^{C}	7.7±0.20^{A}	0.0±0.00^{D}	6.3±0.27^{B}	*P<0.001*
Adonirubin	-	20.2±0.13^{B}	-	23.6±0.67^{A}	*P<0.001*
Astaxanthin	86.3±0.25^{B}	45.4±0.35^{D}	87.9±1.39^{A}	56.9±1.27^{C}	*P<0.001*
Asteroidenone	-	1.9±0.11^{A}	-	1.7±0.11^{B}	*P=0.007*
Adonixanthin	-	9.6±0.09	-	10.1±0.81	*P>0.124*
Lutein	11.7±0.29^{A}	5.9±0.30^{B}	12.2±1.39^{A}	1.4±0.25^{C}	*P<0.001*

Different capital letter superscripts within each row indicate significant differences (P<0.05) between the respective groups by one-way ANOVA and Tukeys pairwise comparison test.

Table 4 Fatty acid (FA) composition (%) in the conventional salmon feed, and the organic salmon feed as compared to fatty acid profile in conventional versus organic Atlantic salmon fed the respective feeds

	Feed		Fresh salmon		
FA	**Conventional**	**Organic**	**Conventional**	**Organic**	***Effect***
C14:0	2.7±0.02^{C}	5.4±0.06^{A}	2.2±0.08^{D}	4.1±0.14^{B}	*P<0.001*
C16:0	9.5±0.08^{B}	11.2±0.08^{A}	9.8±0.56^{B}	11.9±0.30^{A}	*P<0.001*
C16:1n-7	2.7±0.03^{C}	3.6±0.01^{B}	2.6±0.06^{C}	4.2±0.24^{A}	*P<0.001*
18:0	2.4±0.07^{A}	1.6±0.00^{C}	2.2±0.19^{AB}	2.1±0.09^{B}	*P<0.001*
∑C18:1n-7 and n-9	43.2±0.45^{A}	25.0±0.12^{C}	40.6±0.69^{B}	20.7±1.95^{D}	*P<0.001*
C18:2n-6	14.8±0.10^{A}	7.8±0.01^{C}	12.3±0.19^{B}	5.9±0.61^{D}	*P<0.001*
C18:3n-3	6.3±0.06^{A}	3.0±0.02^{C}	4.9±0.09^{B}	2.3±0.24^{D}	*P<0.001*
C18:4n-3	0.8±0.02^{B}	1.9±0.02^{A}	0.3±0.03^{C}	0.2±0.01^{D}	*P<0.001*
C20:1n-9	3.4±0.01^{C}	8.6±0.05^{A}	4.3±0.13^{B}	8.9±0.43^{A}	*P<0.001*
C20:4n-6	0.3±0.01^{C}	0.3±0.03^{B}	0.4±0.03^{A}	0.4±0.03^{A}	*P<0.001*
C20:5n-3	3.2±0.08^{B}	4.8±0.04^{A}	3.0±0.19^{B}	4.8±0.23^{A}	*P<0.001*
C22:1n-9	4.0±0.04^{C}	14.3±0.09^{A}	3.1±0.36^{D}	10.0±0.12^{B}	*P<0.001*
C22:5n-3	0.6±0.03^{D}	0.7±0.01^{C}	1.3±0.05^{B}	2.0±0.09^{A}	*P<0.001*
C22:6n-3	3.2±0.09^{C}	6.8±0.10^{B}	6.3±0.51^{B}	11.6±1.09^{A}	*P<0.001*
others	3.0±0.33^{D}	4.9±0.15^{C}	6.7±1.16^{B}	10.8±0.60^{A}	*P<0.001*
∑SFA	14.6±0.13^{C}	18.3±0.10^{B}	15.2±0.66^{C}	20.4±0.40^{A}	*P<0.001*
∑MUFA	49.3±0.43^{A}	37.1±0.18^{B}	51.0±0.85^{A}	37.9±1.40^{B}	*P<0.001*
∑PUFA	33.1±0.29^{C}	39.7±0.13^{B}	33.8±0.93^{C}	41.7±1.10^{A}	*P<0.001*
n-3×n-6^{-1}	0.9±0.02^{D}	2.1±0.01^{B}	1.2±0.06^{C}	3.3±0.43^{A}	*P<0.001*

Different capital letter superscripts within each row indicate significant differences (P<0.05) between the respective groups by one-way ANOVA and Tukeys pairwise comparison test.

Table 5 Colorimetric parameters for raw conventional versus organic Atlantic salmon stored 22 days on ice

Colorimetric parameters	Day	Fresh salmon		
		Conventional	Organic	*Effect*
*L**				
	2	45.1±1.30^{cA}	42.7±1.93^{dB}	***P=0.037***
	7	45.5±1.74^{c}	42.9±2.32^{cd}	*P>0.063*
	10	46.4±1.49^{cA}	44.4±1.11^{cdB}	***P=0.032***
	15	48.6±0.84^{b}	47.4±1.27^{c}	*P>0.319*
	17	51.2±1.31^{abA}	48.3±1.11^{abB}	***P=0.004***
	22	51.7±1.23^{a}	50.6±0.72^{a}	*P>0.131*
	Effect	***P<0.001***	***P<0.001***	
*a**				
	2	9.2±1.17^{c}	8.5±0.82^{c}	*P>0.305*
	7	9.3±1.12^{c}	9.4±1.00^{bc}	*P>0.860*
	10	9.1±0.88^{c}	9.6±1.06^{bc}	*P>0.460*
	15	10.2±0.93^{bc}	10.5±0.94^{abc}	*P>0.628*
	17	11.6±0.70^{ab}	11.3±0.94^{ab}	*P>0.680*
	22	11.7±1.05^{a}	12.5±0.63^{a}	*P>0.219*
	Effect	***P<0.001***	***P<0.001***	
*b**				
	2	17.8±2.63	17.3±2.57	*P>0.732*
	7	16.8±2.95	17.3±2.65	*P>0.780*
	10	16.2±2.18	17.4±2.15	*P>0.376*
	15	17.4±1.61	18.1±2.40	*P>0.595*
	17	18.1±0.59	19.5±2.08	*P>0.139*
	22	18.6±1.69	20.3±1.34	*P>0.085*
	Effect	*P>0.580*	*P>0.228*	

Different lowercase superscripts within each column and different capital letter superscripts within each row indicate significant differences (P<0.05) between the respective groups by one-way ANOVA and Tukeys pairwise comparison test.