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Nutritional value and storage stability in commercially produced organically and conventionally farmed Atlantic salmon (*Salmo salar* L.) in Norway

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ABSTRACT

Salmon feeds have changed over the years, leading to changed biochemical composition of the fish. The main aim of this study was to compare biochemical compositions and storage stability of commercially produced organic and conventional salmon. Organically (n = 40) and conventionally farmed salmon (n = 39) were sampled. The fish were anesthetized, killed by gill cutting and bled before filleting. Fish samples were subjected to proximate analysis, fatty acid and amino acid composition, along with colour and TBARS analyses. The lipid content of organically and conventionally farmed salmon was 13% and 17 %, respectively. Organic fish contained approximately 48 % more EPA and DHA than did the conventional fish, 17.2 g kg^{-1} vs. 11.6 g kg^{-1}, respectively. The organic salmon had lower colour saturation than the conventional, and TBARS were higher in the organic than in the conventional salmon. To conclude, the main differences between fresh organic and conventional salmon were related to lipid content and fatty acid composition. The high energy level in both groups should be considered when making dietary recommendations. Organic salmon is less stable due to its high content of long-chained unsaturated fatty acids, and appears similar to conventionally farmed salmon some years ago.

1. Introduction

Seafood consumption has long been associated with a healthy lifestyle and reduced risk of several lifestyle-related diseases, such as cardiovascular diseases (CVD). The health benefits have mainly been credited to the high amounts of the long-chained omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish and seafood are also rich in good quality proteins, along with micronutrients such as iodine, selenium, vitamins D and B_{12} (Weichselbaum et al., 2013). However, in recent years it has been questioned whether today's farmed fish are still as health-promoting, due to increased use of vegetable feed components.

Traditionally, aquaculture feeds contained mainly marine ingredients, such as fish meal and fish oils from small fish species not suited for human consumption. Until around 1990, aquaculture was a marginal industry in Europe, contributing to approximately 7 % of the total fish production. Today, the aquaculture share has grown to 18 % (FAO, 2018), and the traditional feed ingredients have become scarce.

During these years, aquaculture has also become an important industry in Norway, with a production of 1.35 million tonnes in 2018 and a value of 67.8 billion NOK (approximately 8.3 billion USD). Atlantic salmon (*Salmo salar* L.) is by far the most important species, accounting for 1.28 million tonnes and 64.5 billion NOK (approximately 7.9 billion USD), an 8.8-fold increase from 1990, when the production was 145990 tonnes (Statistics Norway, 2020). The remarkable increase in aquaculture production led to increased demand for feed ingredients and following this, increased prices (Olsen et al., 2014). In order to fulfil the demand for feed at a reasonable cost, the proportion of marine ingredients has gradually been reduced and switched with plant ingredients. A recent study showed that the proportion of marine ingredients has been reduced from 90 % to 30 % during the period 1990 to 2016 (Aas et al., 2019). However, a shift from mainly marine feed ingredients to terrestrial feed ingredients will inevitably lead to a change in the biochemical composition of the fish muscle and the specific biochemical composition of farmed seafood may be very different from its wild counterparts due to the formulation of the feeds (Jensen et al., 2012).

In recent years, this has raised an increased interest for organic production of salmon. Organic aquaculture is quite new in Norway, as the first organically produced salmon reached the Norwegian market in 2011 (SalMar, 2020). The production of organic salmon is still quite low,

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with only two commercial producers and a production around 16000 tonnes per year. Organic aquaculture production is subjected to stricter regulations than is conventional aquaculture. The regulations cover issues regarding the environment, fish welfare and nutrition. Among the demands for eco-certification is that the proportion of marine ingredients in the feed must be higher than in conventional farming. Further should the marine ingredients primarily originate from trimmings of fish from sustainable fisheries and synthetic antioxidants and amino acids are not allowed (Lovdata, 2017; The European Commission, 2008).

Due to the demands regarding marine ingredients and additives, it is believed that the composition and quality of the organic salmon of today is quite similar to that of the conventional salmon 10-20 years ago. It is also a common perception that the commercially available organic salmon is less fatty and less red than the conventional salmon. The last few years, some studies have been published comparing effects of different processing techniques on organically and conventionally farmed salmon (Lerfall et al., 2016a; Lerfall et al., 2016b). However, literature available on the complete biochemical composition of commercially available organic salmon of today is still scarce.

The aim of this study was thus to compare the nutritional value and storage stability of commercially available organically and conventionally produced salmon in Norway today.

2. Materials and Methods

2.1. Experimental conditions

Conventional and organic salmon (*Salmo salar L.*) were both deployed to sea in September 2016. For conventional fish, smolt of the Rauma strain reared at Follafoss (n = 172879, average weight 77 g) were deployed to sea at Oterneset, Troms county, Norway (68.9°N, 16.7°E). For organic salmon, smolt of the Aquagen strain reared at Aquafarm (n = 91467, average weight 60 g) were deployed to sea at Årberg, Troms county, Norway (69.2°N, 16.9°E). At both locations, feeding was automated and handled from a remote central. The sea cages were monitored by underwater cameras to control the feeding based on the fish's behaviour to ensure feeding until apparent satiation. Standard commercial feeds were used for both conventional and organic salmon. The protein content in feed used for conventional salmon was 31 %, and the lipid content was 29 %, while feed for organic salmon had 28 % protein and 33 % lipids. The sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were 5.3 and 12.8 % of total lipid in feed for conventional and organic salmon, respectively. Conventional salmon were slaughtered after 525 days, while organic salmon were slaughtered after 560 days in sea.

2.2. Sampling procedures

For compositional analyses, fish (n = 20) were randomly sampled from each location 2-3 days before regular slaughtering. The fish were bath anesthetized using benzocaine (30-40 mg L^{-1}), killed by gill cutting and bled out in circulating ice water for 30 min. Weights and lengths of whole fish were recorded, before the fish were filleted. Fillets were stored skin-on in ice for 5-8 h before freezing at -50°C until analyses.

Prior to analyses, the fish were thawed at room temperature and skin was removed. Visible fat was removed from the belly flaps and dorsal fin areas and the whole fish fillet was minced using a Bosch Pro Power MFW68660 (Robert Bosch GmbH, Gerlingen, Germany). Minced fish samples were subjected to proximate analysis (water, ash, lipid, and protein), fatty acid composition and amino acid composition.

For studies of lipid oxidation and colour stability, fish were sampled from the regular slaughtering lines (n = 20 for organic salmon and n = 19 for conventional salmon). Slaughtering was performed according to standard regulations. Immediately after bleeding, the fish were gutted and put in ice for transportation to the Norwegian College of Fishery Science. Upon arrival, weights and lengths were recorded and fish were filleted. Colour measurements were performed on each of the fillets. Thereafter, the fillets were packed in plastic bags and stored in ice until further analyses. After 7 days on ice, the right fillet was weighed, the drip loss was recorded and collected, and colour was measured. A sample of the fillet in the region corresponding to the Norwegian quality cut (NQC) was frozen for later analysis of thiobarbituric acid reactive substances (TBARS). On day 18, the same procedures were performed on the left fillet.

All reagents used in these analyses were of analytical grade. Dichloromethane and methanol were purchased from BDH (Poole, Dorset, UK). All other solvents and chemicals were purchased from Merck (Darmstadt, Germany), unless otherwise stated.

2.3. Analytical methods

2.3.1. Water and ash

Water and ash were determined gravimetrically using modified versions of AOAC methods 925.04 and 938.08, respectively (Latimer, 2019). In short, approximately 5 g of homogenized fish fillet was dried for 48 hours at 105°C. The water-free material was thereafter combusted at 500°C for 16 h.

2.3.2. Lipid content and fatty acid composition

Lipids were extracted according to Folch et al. (1957), with the adjustments described by Maehre et al. (2014). Approximately 1 g of homogenized fish fillet was mixed with 20 mL dichloromethane (DCM): methanol (2:1 v/v). Heptadecanoic acid (C17:0) was added to serve as internal standard. The mixture was shaken for 20 minutes, followed by filtration. The filtrate was washed with 4 mL 0.9% NaCl, followed by centrifugation at 2000 *x g* for 10 min. The water phase was removed, and the lipid phase was evaporated under N_2 until dryness. Weight was recorded and lipid content calculated. The extracted lipid was redissolved in DCM: methanol (2:1 v/v) to a final concentration of 10 mg mL^{-1}.

Prior to fatty acid analysis trans-methylation was performed according to Stoffel et al. (1959), with the modifications described by Maehre et al. (2013). In short, 100 µL of the 10 mg mL^{-1} lipid solutions were mixed with 900 µL DCM and 2 mL acidified methanol (2% v/v H_2SO_4 in methanol). The mixture was boiled for 1 h, followed by addition of 3.5 mL heptane and 3.5 mL 5% NaCl and thorough mixing. The heptane phase was collected and concentrated to 100 µL under N_2. Thereafter the samples were subjected to fatty acid analysis as described by Maehre et al., (2013), using an Agilent 6890N gas chromatograph with a flame ionization detector (FID) (Agilent Technologies Inc., Santo Clara, CA, USA). A Varian CP7419 capillary column (Varian Inc., Middelburg, the Netherlands) was used for separation. Identification of single fatty acids was performed by comparison with commercial fatty acid standard purchased from Sigma (Sigma Chemicals Co., St. Louis, MO, USA) and Nu-Chek (Nu-Chek Prep Inc., Elysian, MN, USA).

2.3.3. Protein content and amino acid composition

Amino acids were determined according to Maehre et al. (2013). Approximately 200 mg of the homogenized fish fillet was mixed with 500 µl of 20 mM norleucine (internal standard) and 700 µl distilled water. Concentrated hydrochloric acid was added to a final concentration of 6 M. The samples were flushed with N_2 for 15 s before hydrolysis at 110°C for 24 h, according to Moore & Stein (1963). After hydrolysis, 100 µl of the hydrolysate was evaporated under N_2 to complete dryness, and thereafter re-dissolved in 1 mL lithium buffer pH 2.2. All amino acids were analyzed chromatographically using a Biochrom 30 amino acid analyzer equipped with a lithium ion exchange column (Biochrom Co, Cambridge, UK) as described by Maehre et al. (2013). Protein content is given as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water) as recommended by the Food and Agriculture Organization of the United Nations (FAO, 2003).

2.3.4. Colour and thiobarbituric acid reactive substances (TBARS)

Colour of fillet was measured according to instrumental colour analysis (CIE Lab 1976) using Minolta Chromameter CR-200 (Minolta, Osaka, Japan) calibrated to a white standard. The L^*, a^* and b^* values were measured on the loin area of each fillet (from 6 cm to 20 cm from the anterior of fillet) in triplicate. Chroma (C^*) was calculated by using formula $C^* = \sqrt{(a^{*2} + b^{*2})}$ to determine the colour saturation. Hue (h^*) represents the colour angle between a^* and b^*, where $h^* = 0^\circ$ for reddish hue and $h^* = 90^\circ$ for yellowish hue. Hue was calculated using formula $h^* = \tan^{-1}(b^*/a^*)$.

Thiobarbituric acid reactive substances (TBARS) were determined as described by Ke et al. (1984) by weighing approximately 4 g of fillet in a 50 mL centrifuge tube and adding 15 mL 10 % trichloroacetic acid with 0.1 % EDTA and 0.1 % propyl gallate. The samples were homogenised using an Ultra Turrax T25 homogeniser (IKA Werke GmbH, Staufen, Germany) for 1 min at 6000 rpm, boiled for 30 min and cooled. Thereafter, the samples were filtered and mixed 1:1 with 6 g L^{-1} thiobarbituric acid. This mixture was then boiled for another 30 min. Absorbance was read at 532 nm using a Visible Spectrophotometer Genesys 20 (Thermo Scientific^{TM}, Waltham, MA, USA) and compared to a standard curve made from malondialdehyde (MDA) with concentrations ranging between 0 and 10 nmol L^{-1}.

2.4. Statistics

Statistical analysis was performed using Minitab 19 (Minitab Inc., PA, USA). Tests of normality (Ryan Joiner test) was performed. For normally distributed data, homogeneity of variance was examined (F test), before performing a Student's t-test (based on mean values) for evaluation of statistical differences. Non-normally distributed data were analyzed using the non-parametric Mann Whitney test (based on median values). Means/medians were considered significantly different at $p < 0.05$.

3. Results and discussion

3.1. Proximate composition

Average weights and proximate compositions of the fish at time of sampling are shown in Table 1. The fish (n = 20) were sampled randomly from the sea cages and there was a wide weight range, 1800 – 7200 g for organic fish and 3650 – 8150 g for conventional fish. Considering the total amount of slaughtered fish in the productions, i.e. around 170 000-180 000 individuals in the conventional production and around 90000 individuals in the organic production, a sampling of 20 individuals from each site is very small and the results must thus be interpreted with care. One individual from the organically farmed salmon was identified as an outlier, defined as values ± 2SD of the mean, for several of the analytical variables and was thus excluded from the calculations.

The ash content, reflecting the mineral content, was similar between groups. For water, protein, and lipids there were statistically significant differences between the organic and the conventional fish. The calculated energy level for the salmon in this study was around 7500 kJ kg^{-1} for the organic salmon and 8700 kJ kg^{-1} for the conventional salmon, respectively. This is mainly a reflection of the higher lipid content of the conventionally farmed salmon. In this study, the lipid contents were 169 g kg^{-1} and 134 g kg^{-1} for conventional and organic salmon, respectively. In previous studies, involving conventionally farmed salmon harvested in 1994-1996, 2003, 2010 and 2012, the lipid contents were 100 g kg^{-1}, 74 g kg^{-1}, 123 g kg^{-1} and 140 g kg^{-1}, respectively (Bell et al., 1998, Blanchet et al., 2005; Jensen et al., 2012; Lundebye et al., 2017). This indicates that there has been a tendency towards increasing lipid content of conventionally farmed fish during the last decades. In organic salmon, however, the lipid content was within the same range as that of conventionally farmed fish in 2010 and 2012. Although the lipid content in organic salmon is lower than in the conventional salmon, it is still high compared to other muscle foods, such as chicken and beef (Norwegian Food Safety Authority, 2020). In times where the prevalence of obesity is steadily increasing globally, this should be considered when dietary recommendations regarding fish consumption are made. The protein content in farmed salmon harvested in 2010 (Jensen et al., 2012) was higher than both the conventional and the organic salmon in the present study, namely 183 g protein kg^{-1} fish muscle. However, reported protein is highly dependent on analytical methods, making direct comparisons between studies difficult (Maehre et al., 2018).

3.2. Fatty acid composition

Lipids in feeds are normally present as triglycerides, i.e. three fatty acids bound to a glycerol skeleton. During digestion, two of the fatty acids are detached from the glycerol skeleton and lipids are thus absorbed as one monoglyceride and two free fatty acids, without further decomposition (Thiboudeau & Patton, 1999). The fatty acid (FA) composition in the feeds will thus to a large extent be reflected in the FA composition of the salmon muscle.

Table 2 shows the amounts of the main FAs in organic and conventional salmon, reported both in compositional values (i.e. % of total FA) and nutritional values (i.e. g kg^{-1} fish muscle). When looking at the compositional values, it is seen that there are significant differences between conventional and organic salmon in all FAs. However, due to the increased lipid content in the conventional salmon compared to the organic salmon, some of these differences are equalized

Table 1

Proximate composition (water, ash, lipid and protein) in organically and conventionally farmed Atlantic salmon at time of slaughter (after 18 months in sea cages). Values are presented as mean ± SD (n = 19 for organic, n = 20 for conventional) and in g kg^{-1} fish muscle, unless otherwise stated. Different letters in the same row indicate significant differences ($p < 0.05$) between organically and conventionally farmed fish.

	Organic salmon ($n = 19$)	**Conventional salmon ($n = 20$)**	**p-value**
Fish weight [g]	5476 ± 1427	5500 ± 1113	$p = 0.954$
Water	653 ± 18^A	613 ± 12^B	$p < 0.001$
Ash[†]	12 ± 1	12 ± 1	$p = 0.403$
Lipid	134 ± 20^B	169 ± 15^A	$p < 0.001$
Protein[*]	146 ± 7^A	140 ± 7^B	$p = 0.008$
Energy [kJ kg^{-1}][**]	7478 ± 710^B	8707 ± 510^A	$p < 0.001$

[†] Data were non-normally distributed

[*] Reported as sum of amino acid residues less the molecular weight of water, as recommended by FAO (2003)

[**] Energy was calculated in accordance with EU Council Directive 1169/2011, annex XIV (The European Commission, 2011).

Table 2

Composition of the main fatty acids in organically and conventionally farmed Atlantic salmon at time of slaughter (after 18 months in sea cages). Values are presented as mean ± SD and in % for composition and g FA kg^{-1} fish muscle for amount. Different capital letters in the same row indicate significant difference ($p < 0.05$) in nutritional value between organically and conventionally farmed salmon.

	Organic salmon (n = 19)		**Conventional salmon (n = 20)**			
	Compositional value (%)	**Nutritional value (g kg^{-1} fish muscle)**	**Compositional value (%)**	**Nutritional value (g kg^{-1} fish muscle)**	***p*-value composition**	***p*-value amount**
14:0[†]	4.4 ± 0.1	6.0 ± 0.9^{A}	2.2 ± 0.1	4.1 ± 0.6^{B}	p < 0.001	p < 0.001
16:0	12.9 ± 0.3	17.5 ± 2.8	9.4 ± 0.2	17.1 ± 2.0	p < 0.001	p = 0.596
18:0	3.1 ± 0.1	4.3 ± 0.7	2.4 ± 0.1	4.4 ± 0.5	p < 0.001	p = 0.614
Sum SFA	**20.5 ± 0.4**	**27.7 ± 4.5**	**14.1 ± 0.3**	**25.5 ± 3.0**	**p < 0.001**	**p = 0.079**
16:1 n-7[†]	5.3 ± 0.1	7.1 ± 1.2^{A}	2.4 ± 0.1	4.3 ± 0.6^{B}	p < 0.001	p < 0.001
18:1 n-9[†]	16.4 ± 0.4	22.3 ± 3.7^{B}	40.2 ± 0.4	72.9 ± 8.9^{A}	p < 0.001	p < 0.001
18:1 n-7	2.7 ± 0.1	3.6 ± 0.6^{B}	3.1 ± 0.0	5.5 ± 0.7^{A}	p < 0.001	p < 0.001
20:1 n-9	8.4 ± 0.6	11.4 ± 1.4^{A}	5.0 ± 0.1	9.0 ± 1.1^{B}	p < 0.001	p < 0.001
22:1 n-11[†]	9.7 ± 0.6	13.1 ± 1.7^{A}	4.1 ± 0.1	7.4 ± 0.9^{B}	p < 0.001	p < 0.001
22:1 n-9	2.1 ± 0.1	2.8 ± 0.4	1.5 ± 0.1	2.7 ± 0.3	p < 0.001	p = 0.447
Sum MUFA	**44.6 ± 0.9**	**60.3 ± 8.7^{B}**	**56.2 ± 0.6**	**101.9 ± 12.5^{A}**	**p < 0.001**	**p < 0.001**
18:2 n-6	13.0 ± 0.3	17.6 ± 2.6^{B}	13.6 ± 0.2	24.7 ± 3.0^{A}	p < 0.001	p < 0.001
18:3 n-3†	2.4 ± 0.1	3.3 ± 0.5^{B}	5.5 ± 0.1	10.0 ± 1.3^{A}	p < 0.001	p < 0.001
18:4 n-3†	2.3 ± 0.1	3.1 ± 0.5^{A}	1.2 ± 0.0	2.1 ± 0.2^{B}	p < 0.001	p < 0.001
20:2 n-6	0.8 ± 0.1	1.1 ± 0.2^{B}	1.1 ± 0.1	2.0 ± 0.2^{A}	p < 0.001	p < 0.001
20:5 n-3†	5.0 ± 0.3	6.8 ± 1.2^{A}	2.5 ± 0.1	4.6 ± 0.5^{B}	p < 0.001	p < 0.001
22:5 n-3	1.9 ± 0.1	2.6 ± 0.5^{A}	1.1 ± 0.1	2.0 ± 0.3^{B}	p < 0.001	p < 0.001
22:6 n-3†	7.7 ± 0.3	10.4 ± 1.5^{A}	3.9 ± 0.2	7.0 ± 0.8^{B}	p < 0.001	p < 0.001
Sum PUFA	**33.2 ± 1.0**	**45.0 ± 6.9^{B}**	**28.9 ± 0.4**	**52.4 ± 6.2^{A}**	**p < 0.001**	**p = 0.001**
- where of PUFA n-3	19.4 ± 0.8	26.2 ± 4.2	14.2 ± 0.3	25.7 ± 3.0	p < 0.001	p = 0.634
- where of LC n-3-PUFA†	14.6 ± 0.7	19.8 ± 3.2^{A}	7.5 ± 0.2	13.6 ± 1.5^{B}	p < 0.001	p < 0.001
- where of EPA+DHA†	12.7 ± 0.6	17.2 ± 2.8^{A}	6.4 ± 0.2	11.6 ± 1.3^{B}	p < 0.001	p < 0.001
- where of PUFA n6	13.8 ± 0.4	18.7 ± 2.8^{B}	14.8 ± 0.2	26.7 ± 3.2^{A}	p < 0.001	p < 0.001
n-6/n-3†		**0.7 ± 0.0^{B}**		**1.0 ± 0.0^{A}**	**p < 0.001**	**p < 0.001**

[†] Data were non-normally distributed

when looking at the nutritional values. Since this is what is most relevant in a dietary perspective, nutritional values are discussed further. Among the saturated FAs there are only non-significant differences between organic and conventional salmon. When it comes to monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) composition, however, there are pronounced differences between the groups. In the conventional salmon, the MUFAs are dominated by one single FA, oleic acid (C18:1, n-9), while the organic salmon contains relatively more of the long-chain MUFAs C20:1, n-9 and C22:1, n-11. This reflects that the proportion of marine ingredients in the organic feed is higher than in the conventional feed, as these two FAs are not commonly found in plant oils (Zambiasi et al., 2007). When it comes to the PUFAs, the organic salmon contains more of the long-chain omega-3 PUFAs than does the conventional salmon, while the contents of linoleic acid (LA; C18:2, n-6) and alpha-linolenic acid (ALA; C18:3, n-3) are higher in the conventional salmon. These two FAs, along with oleic acid, are very common in plant oils, but only found in low amounts in marine organisms (Sigurgisladottir & Palmadottir, 1993). From the FA composition it is evident that the organic salmon also receives some plant oils in their feed, as the content of LA and ALA is much higher in organic salmon than in wild salmon where the content is normally around 1.0 – 1.5 g kg^{-1} (Jensen et al., 2012; Lundebye et al., 2017).

A high intake of seafood has long been associated with decreased risk of developing lifestyle-related diseases, such as type II diabetes, cardiovascular diseases and other inflammatory diseases (Weichselbaum et al., 2013). There are mainly two factors that are associated to these health benefits, namely a high content of the long-chained n-3 PUFAs eicosapentaenoic acid (EPA; C20:5, n-3) and docosahexaenoic acid (DHA; C22:6, n-3), along with the ratio between omega-6 and omega-3 FAs.

Even though EPA and DHA can be derived from ALA, this conversion rate is limited in high-trophic species, such as mammals (Brenna, 2002) and salmon (Ruyter et al., 2000). This means that they are regarded as semi-essential FAs and must be provided through the diet. The primary producers of EPA and DHA are low-trophic marine species that form the base of the marine food chain. As these species are the natural feeding source for shellfish and small fish, seafood is the best, if not only, source of EPA and DHA. As described by Aas et al. (2019), there was a substantial decrease in the proportion of marine ingredients in salmon feed from 1990 to 2016, and as a consequence of this there have been some concerns about whether the farmed salmon would still be considered a healthy food or if it would turn into a “swimming vegetable”. As a consequence of these concerns, along with reduced availability/sustainability of traditional marine ingredients (fish meal and fish oil), there are comprehensive research activities on alternative and more sustainable marine feed ingredients, such as marine microalgae and insect meal (Dineshbabu et al., 2019; Gong et al., 2019; Nogales-Merida et al., 2019; Tibbetts et al., 2020). While insect meal so far is not commonly used in commercial aquaculture feeds, some of the large feed producers have started adding microalgae meal to their products (Lerøy Seafood Group, 2016).

The regulations for organic production of salmonids state that the proportion of marine ingredients should be at least 40 % (Lovdata, 2017; The European Commission, 2008). The proportion of marine ingredients in feeds for organic salmon is thus normally higher than in feeds for conventional fish. Knowing that most of the EPA and DHA in salmon originates from the feed, the composition will be reflected in the fish muscle. As stated in the Materials and Methods section (Section 2.1), the sum of EPA and DHA were 5.3 and 12.8 % of total lipid in feed for conventional and organic salmon, respectively. And, as seen in Table 2, this is reflected in the fish muscle; the sum of EPA and DHA were 6.4 % of total lipid in the organic fish muscle, while the sum of EPA and DHA in organic fish were 12.7 %. Hence, the organic fish in this study contained approximately 48 % more EPA and DHA than did the conventional fish, 17.2 g kg^{-1} vs. 11.6 g kg^{-1}, respectively. Compared to the sum of EPA and DHA in conventional fish harvested in 2010 (10.3 g kg^{-1}; Jensen et al., 2012) and 2012 (14 g kg^{-1}; Lundebye et al., 2017), both organic and conventional salmon from the present study contain more EPA + DHA than farmed salmon harvested in 2010 (Jensen et al., 2012), while the EPA + DHA contents in the 2012 (Lundebye et al., 2017) were in between the organic and the conventional fish in this study. The EPA and DHA levels are also comparable to the levels presented in a recent study describing the fatty acid composition in farmed Norwegian salmon commercially available in the UK (Sprague et al., 2020). There are only a few producers of aquaculture feeds globally. The feed formulation may differ slightly from one producer to another, resulting in some differences in the FA composition of the fish. However, the feeds tend to be quite similar, at least in macronutrient composition. The trends in feed development is thus similar and indicate that along with increasing the lipid content and the plant oil inclusion in the feeds, there has been a focus on keeping the EPA + DHA content at a recommended level.

Official recommendations for dietary intake of EPA + DHA vary between countries and food organizations, but as presented in a previous review paper, most of them agree on an intake in the range of 250 – 500 mg per day either as seafood consumption or as dietary supplements (Maehre et al., 2015). All of the fish from the mentioned studies are good sources of EPA + DHA, as the required amount for achieving 500 mg per day is 30 g for the organic salmon in the present study (2018), 36 g for the 2012 salmon (Jensen et al., 2012), 43 g for the conventional salmon in the present study (2018), and 49 g for the 2010 salmon (Lundebye et al., 2017), all well below a regular dinner portion (150 g). In other words, one portion of conventional salmon from the present and previous studies (Jensen et al., 2012; Lundebye et al., 2017) would cover the requirements for 3 - 4 days, while one portion of the organic salmon would cover the requirements for 5 days.

The other factor related to lowering the risk of lifestyle-related diseases is the ratio between n-6 and n-3 FAs (Harris & von Schacky, 2004; Simopoulos, 2008; Stanley et al., 2007). An optimal n-6: n-3 ratio is suggested to be in the range 2:1 - 5:1. However, in western diets today the actual ratio is between 15:1 - 17:1 (Simopoulos, 2008) and should thus be reduced. It is, however, important to remember that this ratio is meant to be a measure of the complete diet and not just of single food items.

An increasing level of plant oils in the fish feed will inevitably increase the content of LA, which is an abundant n-6 FA in most plant oils. The content of ALA varies more between different plant oils, giving very different n-6: n-3 ratios between different plant oils (Zambiasi et al., 2007). The impact on the n-6: n-3 ratio in the fish will thus depend on which plant oil is used, along with the content of LC n-3 PUFAs. In this study, there was significant difference between the n-6:n-3 ratio in organic and conventionally farmed salmon, it being

1:1.4 in the organic and 1:1 in the conventional salmon. Both ratios are however comparable with the ratio of 1:1.2 in fish harvested in 2012. In the 2010 fish the ratio was 1:2.3, indicating a “healthier” FA composition. However, when comparing the contents of all the relevant FAs, it is seen that all of them has increased since 2010, not only the plant-based ones. This supports the previously mentioned focus of keeping the EPA + DHA content at a recommended level in the conventionally farmed fish. In the organic fish, the n-6: n-3 ratio and the content of LA is higher than in the 2010 fish, while lipid content, oleic acid and ALA are approximately the same. This supports the impression that the organic salmon of today is more similar to conventional fish some years ago than conventional fish of today.

3.3. Amino acids and protein quality

Without achieving the same attention as the change of lipid sources, also the protein sources of the salmon feed have changed over the years, from fish meal to plant proteins such as soy meal (Aas et al., 2019). In contrast to the lipids, the amino acid composition of the feed will not be directly reflected in the salmon flesh. This is due to a different mechanism of digestion and absorption in the body (Thiboudeau & Patton, 1999). As only free amino acids may be absorbed in the intestine, the proteins in the feed must be hydrolysed to their constituting amino acids before absorption in the small intestine. After uptake, the amino acids are re-synthesized to new proteins in the liver.

Table 3

Amino acid composition in organically (n = 19) and conventionally (n = 20) farmed Atlantic salmon at time of slaughter. Values are presented as mean ± SD and in g AA kg^{-1} fish muscle. Different capital letters in the same row indicate significant difference ($p < 0.05$) between organically and conventionally farmed salmon.

	Organic salmon (n = 19)	**Conventional salmon (n = 20)**	***p*-value**
Essential amino acids (EAA)			
Threonine	8.9 ± 0.6	8.7 ± 0.3	p = 0.168
Valine	9.1 ± 0.6	9.0 ± 0.3	p = 0.460
Methionine	5.9 ± 0.4^{A}	5.5 ± 0.4^{B}	p = 0.010
Isoleucine	7.5 ± 0.4	7.6 ± 0.3	p = 0.681
Leucine	14.4 ± 0.8	14.4 ± 0.5	p = 0.982
Phenylalanine	7.8 ± 0.5	7.6 ± 0.3	p = 0.124
Lysine[†]	17.5 ± 1.1^{A}	16.7 ± 0.6^{B}	p = 0.039
Histidine[†]	5.3 ± 0.3	4.8 ± 1.1	p = 0.565
Tryptophan	n.a.	n.a.	
Non-essential amino acids + metabolites			
Taurine	0.6 ± 0.2^{A}	0.4 ± 0.1^{B}	p = 0.001
Aspartic acid[*]	13.5 ± 0.8	13.6 ± 0.6	p = 0.603
Serine	7.3 ± 0.4	7.2 ± 0.3	p = 0.356
Glutamic acid[*]	25.6 ± 1.7	25.5 ± 1.1	p = 0.801
Proline[†]	7.4 ± 0.4^{B}	8.3 ± 1.2^{A}	p = 0.002
Glycine[†]	9.7 ± 0.7^{A}	9.3 ± 0.6^{B}	p = 0.007
Alanine	11.4 ± 0.6	11.1 ± 0.4	p = 0.100
Cysteine[†]	0.5 ± 0.1	0.4 ± 0.2	p = 0.619
Tyrosine[†]	6.0 ± 1.0	4.5 ± 2.6	p = 0.334
b-Alanine	1.5 ± 0.1^{B}	1.6 ± 0.1^{A}	p = 0.002
1-methyl Histidine[†]	3.5 ± 0.3^{B}	4.0 ± 0.8^{A}	p = 0.001
Arginine	12.6 ± 0.8^{A}	10.5 ± 0.7^{B}	p < 0.001
Sum Amino acids + Metabolites	**175.5 ± 8.5^{A}**	**169.1 ± 8.0^{B}**	**p = 0.020**
Sum EAA	76.0 ± 4.6	74.4 ± 3.1	p = 0.199
% EAA	44.7 ± 0,9^{B}	45.6 ± 0.8^{A}	p = 0.001

[*] As asparagine and glutamine are present in their deaminated forms after acidic hydrolysis, aspartic acid and glutamic acid represent the sums of aspartic acid + asparagine and glutamic acid + glutamine, respectively; n.a. = not analysed.

[†] Data were non-normally distributed.

Table 3 shows the amino acid composition of the organic and conventionally farmed salmon. There are some significant differences between the organic and the conventional farmed salmon. The contents of proline, along with the metabolites b-Alanine and 1-methyl histidine, are significantly higher in the conventional salmon, while the contents of methionine, lysine, taurine, glycine and arginine are significantly higher in the organic salmon than in the conventional salmon.

Both amount and quality of dietary protein are important in order to maintain normal growth and production of physiologically important proteins. Protein quality is often defined by its ability to cover the requirements of essential amino acids, along with their absorption and utilization in the body. The World Health Organization (WHO) has suggested a “reference protein” that contains the required amount of each of the essential amino acids (FAO/WHO/UNU, 2007). A common way of determining the quality, or the chemical score, of different food proteins is to compare them with this reference protein. The chemical score is found by calculating the ratio between each of the essential amino acids in a food protein versus the same amino acid in the reference protein, and the lowest ratio obtained equals the chemical score of the protein. Most protein of animal origin contain sufficient amounts of all essential amino acids and thus have chemical scores of 1. Plant proteins are, however, often low in one or more essential amino acids. For instance, cereals are often deficient in lysine, while legumes may be deficient in sulphur containing amino acids such as methionine. In addition, the digestibility of plant proteins may be lower than that of animal proteins due to a different cell structure (Friedman, 1996). An increased inclusion of plant proteins at the expense of animal protein sources in a feed may thus lead to lower amounts of essential amino acids available for

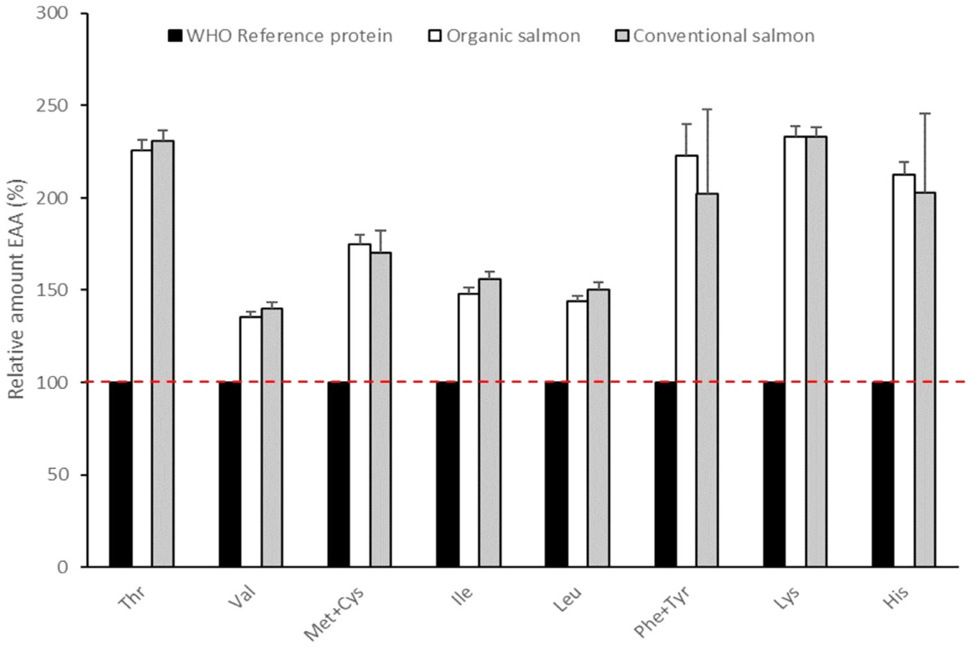

Figure 1. Essential amino acid composition in organically and conventionally farmed Atlantic salmon (Salmo salar L.) proteins relative to the reference protein set by the WHO. The values are given as mean ± SD (n = 20) and in % of the reference protein.

Table 4

Colour characteristics (L*, a*, b*, C* and h*) and thiobarbituric acid reactive substances (TBARS) in organically and conventionally farmed Atlantic salmon at 0, 7 and 18 days on ice. Values are presented as mean ± SD. Different capital letters in the same row indicate significant difference ($p < 0.05$) between organically and conventionally farmed salmon at the same sampling day. * indicate significant difference between day 0 and day 7 within each group. ** indicate significant difference between day 0 and day 18 within each group. † indicate significant difference between day 7 and day 18 within each group.

	Organic salmon (n = 20)	Conventional salmon (n = 19)	*p*-value group
L*			
Day 0	41.5 ± 1.4^{B}	45.9 ± 2.0^{A}	$p < 0.001$
Day 7	$42.6 \pm 1.5^{*; B}$	$50.6 \pm 1.6^{*; A}$	$p < 0.001$
Day 18	$45.0 \pm 1.3^{**; †; B}$	$48.8 \pm 2.1^{**; †; A}$	$p < 0.001$
p-value storage	$^{*}p < 0.001$; $^{**}p < 0.001$; $†p < 0.001$	$^{*}p < 0.001$; $^{**}p < 0.001$; $†p = 0.005$	
a*			
Day 0	8.5 ± 1.0^{B}	9.6 ± 1.1^{A}	$p < 0.001$
Day 7	$9.9 \pm 1.0^{*; B}$	$11.5 \pm 1.6^{*; A}$	$p < 0.001$
Day 18	$10.6 \pm 1.1^{**; B}$	$11.7 \pm 1.4^{**; A}$	$p = 0.011$
p-value storage	$^{*}p < 0.001$; $^{**}p < 0.001$	$^{*}p < 0.001$; $^{**}p < 0.001$	
b*			
Day 0	9.9 ± 1.3^{B}	12.2 ± 1.5^{A}	$p < 0.001$
Day 7	$11.7 \pm 1.3^{*; B}$	$14.8 \pm 2.0^{*; A}$	$p < 0.001$
Day 18	$12.6 \pm 1.1^{**; †; B}$	$15.7 \pm 1.8^{**; A}$	$p < 0.001$
p-value storage	$^{*}p < 0.001$; $^{**}p < 0.001$; $†p = 0.018$	$^{*}p < 0.001$; $^{**}p < 0.001$	
C*			
Day 0	13.1 ± 1.6^{B}	15.6 ± 1.8^{A}	$p < 0.001$
Day 7	$15.3 \pm 1.6^{*; B}$	$18.8 \pm 2.6^{*; A}$	$p < 0.001$
Day 18	$16.5 \pm 1.6^{**; †; B}$	$19.6 \pm 2.2^{**; A}$	$p < 0.001$
p-value storage	$^{*}p < 0.001$; $^{**}p < 0.001$; $†p = 0.025$	$^{*}p < 0.001$; $^{**}p < 0.001$	
h*			
Day 0	49.2 ± 1.7^{B}	51.9 ± 1.7^{A}	$p < 0.001$
Day 7	49.8 ± 1.4^{B}	52.3 ± 1.1^{A}	$p < 0.001$
Day 18	$50.1 \pm 1.3^{**; B}$	$53.4 \pm 1.8^{**;†; A}$	$p < 0.001$
p-value storage	$^{**}p = 0.037$	$^{**}p = 0.004$; $†p = 0.032$	
TBARS (μmol MDA-equivalents kg^{-1} fish muscle)			
Day 0	n.a.	n.a.	
Day 7	12.85 ± 2.07^{A}	8.72 ± 1.51^{B}	$p < 0.001$
Day 18	$22.52 \pm 6.33^{†; A}$	$14.81 \pm 4.58^{†; B}$	$p < 0.001$
p-value storage	$†p < 0.001$	$†p < 0.001$	

n.a.: not analysed

re-synthesis of physically important proteins. This may, in turn, lead to higher utilization of muscle storage proteins, and eventually to reduced growth and development. In conventional aquaculture it is thus common to add synthetic amino acids in order to ensure sufficient amounts of the most exposed essential amino acids, especially lysine and methionine. This is, however, not allowed in organic aquaculture. Here, potential lack of/low amounts of essential amino acids must be prevented through addition of ingredients naturally rich in free amino acids, such as shellfish and molluscs.

In Figure 1, the amounts of essential amino acids in organic and conventional salmon relative to the amounts of essential amino acids in the WHO reference protein are shown.

As seen, there are only small differences in the content of essential amino acids between the organic and the conventional salmon and both are well above the reference protein for humans. This means that both organic and conventional salmon are good protein sources for humans, despite a relatively high content of plant protein in the feeds.

3.4. Stability of colour and lipids during storage

In Table 4, colour measurements and lipid oxidation by means of TBARS are shown. When customers are asked what properties they consider when choosing conventional or organic salmon, colour is one of the frequently mentioned factors (Olesen et al., 2010; Anderson, 2000). A majority of the consumers state that the appearance of the fillet is important and that they are willing to pay more for organic salmon than conventional salmon if the fillet colour is similar (Olesen et al., 2010).

In this study, significant differences between conventionally and organically farmed salmon were observed in all colour measures at time of filleting, and the differences remained during the storage period. The organic salmon was significantly darker, less red and yellow, and had lower colour saturation than the conventional salmon at time of filleting. This is in accordance with the study by Lerfall et al. (2016a). During storage, both groups became lighter, more red and yellow, and the colour saturation was increased. The colour of salmon is normally associated with the content of astaxanthin and other carotenoids in the muscle. In conventional salmon feeds synthetic astaxanthin and/or other carotenoids are added, primarily as antioxidants, and these affect colour properties of the conventionally farmed fish. In organic feeds, addition of synthetic antioxidants is prohibited (Lovdata, 2017; The European Commission, 2008). However, these feeds also contain carotenoids, mainly coming from natural resources such as shellfish, bacteria and algae (Garcia-Chavarria & Lara-Flores, 2013). These species often contain a broad spectrum of different carotenoids, with variable colours and intensity. This variation will in turn affect the colour of the salmon muscle, even if the total carotenoid content is the same (Lerfall et al., 2016a).

Salmon is, due to its high lipid content, prone to lipid oxidation. Lipid oxidation is a chain reaction that inevitably will occur during storage. During this process, several metabolites associated with rancidity are formed. Thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalents per kg fish muscle, is a commonly used marker for spoilage due to lipid oxidation. There is no official limit for TBARS defining when the fish is “not eligible for consumption”, however, Ke et al. (1984)] suggested that levels below 8 mg MDA eqv. kg^{-1} fish muscle could be recognized as good quality, the range 8 – 21 mg MDA eqv. kg^{-1} fish muscle as slightly rancid, and above 21 as rancid. In this study, TBARS levels were higher in the organic salmon than in the conventional salmon at both sampling points. Using the ranges suggested by Ke et al. (1984) both fish groups could be categorized as slightly rancid after 7 days on ice, but only the organic salmon reached the level where it would be categorized as tainted at 18 days. The differences between the organic and conventional salmon is most likely linked to the higher content of LC-PUFAs in organic salmon, as these are especially prone to lipid oxidation.

4. Conclusions

In the present study, it was found that the main differences between organically and conventionally farmed salmon were related to lipid content and fatty acid composition. In addition, there were some differences in colour properties and storage stability, given by the lipid oxidation marker TBARS.

The lipid content in conventionally farmed salmon has increased in recent years, making a more energy dense product, which may be important when energy restriction is considered. The lipid content of the organic salmon seems to be more similar to that of conventional salmon some years ago. Organic salmon provides more of the health beneficial fatty acids, EPA and DHA, than does the conventional salmon. However, an increased content of these fatty acids may reduce the storage stability due to lipid oxidation.

All-in-all, both farming strategies result in products of good nutritional quality, providing good quality proteins and covering the daily recommendations of long-chained n-3 fatty acids.

Declaration of Competing Interest

None.

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