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Atlantic salmon (*Salmo salar*, L.) as raw material for the smoking industry. I: effect of different salting methods on the oxidation of lipids

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Abstract

The changes in total fat content, fatty acid composition, tocopherol, ascorbic acid, pH and oxidation were analysed in response to different salting methods, either dry or brine, in cold-smoked (20 or 30 °C) Atlantic salmon (*Salmo salar*, L.). The fish were lean ocean-ranched salmon caught at Iceland in June 1998 and farmed Norwegian salmon slaughtered in November 1998 and April 1999, differing in fresh fillet fat content from 84 to 169 g kg^{-1} wet weight. The total fat content decreased in all groups during processing, whereas the relative fatty acid composition of the fillets was not severely affected during salting and cold-smoking. The most conspicuous process consumption of antioxidants in all the groups was the relative ascorbic acid loss (58–82%). Generally, no clear effect of different salting methods was observed on the tocopherol loss during processing, but brine salting had a stronger effect on both fat and ascorbic acid loss than dry salting. The fattiest fish showed the highest oxidation during processing and they lost more tocopherol, but the final oxidation levels were generally low (thiobarbituric acid reactive substances, TBARS: 6.0–14.7 μmol kg^{-1}), reflecting the antioxidative protection offered by the vitamins during processing.

Keywords: Atlantic salmon; Ocean-ranched; Farmed; Fresh fat content; Brine salted; Dry salted; Smoking losses

1. Introduction

Traditionally, the wild Atlantic salmon (*Salmo salar*, L.) was used in the European smoking industry but during the last few decades it has been replaced by farmed salmon. Wild salmon grows more slowly than farmed salmon because both the feed composition and the feeding regimes in aquaculture have been improved with the aim of promoting maximal growth. This intensive fish production may affect the composition of the fish as well as its suitability for processing. The chemical composition of salmon shows annual variation (Corraze & Kaushik, 1999); it is affected by the nutritional stage (Bell, McEvoy, Webster, McGhee, Millar, & Sargent, 1998; Hemre & Sandnes, 1999; Lie, Sandvin, & Waagbø, 1993; Sigurgisladottir, Parrish, Ackman, & Lall, 1994) as well as by the body weight of the fish (Fauconneau, Andre, Chmaitilly, LeBail, Krieg, & Kaushik, 1997; Storebakken, Hung, Calvert, & Plisetskaya, 1991). Moreover, farmed salmon has grown fattier during the last decade due to the use of high energy feed (Einen & Roem, 1997; Hemre & Sandnes, 1999; Lie, Waagbø, & Sandnes, 1988) as well as by the intensive feeding strategies used in aquaculture (Kiessling, Johansson, & Storebakken, 1989). The fat content may also vary due to non-dietary factors such as genetic strain, environment, sexual maturation and life-cycle stages (Gjedrem, 1997). Complaints about higher losses of fat during processing, high frequency of gaping, low and uneven distribution of colour, have increased with the increased fat content in the fillets. Therefore, it is of importance to gain more knowledge about interactions between the fresh fat content and other chemical changes during the processing step both in farmed salmon and in the slower growing wild salmon.

The most common salting methods used by the processing industry have been by brine and by dry salting. The present study examines the effects of brine and

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dry-salting on changes in the total fat content, the fatty acid composition and oxidation, as well as protection against oxidation through the loss of the vitamins tocopherol (vitamin E) and ascorbic acid (vitamin C) in the fillets during salting and cold-smoking. The fish tested were lean ocean-ranched salmon, representing wild fish, and farmed salmon, slaughtered during the spring and during the autumn, thus differing in fat content due to seasonal variation.

2. Materials and methods

2.1. Fish material

Ocean-ranched Atlantic salmon (2.6 ± 0.3 kg mean round weight) were caught at Iceland in June 1998. The farmed salmon were supplied by commercial Norwegian fish farms. Fish slaughtered at the beginning of November 1998 were from the northern part of Norway (4.0 ± 0.2 kg mean round weight) and fish slaughtered in late April 1999 were from the south-west coast of Norway (3.7 ± 0.5 kg mean round weight).

The ocean-ranched salmon were slaughtered in a commercial slaughtering plant in Iceland. The Norwegian farmed salmon were fed commercial feeds (Biomar: Bio optimal^{TM}). The salmon slaughtered in November were starved for 14 days at a seawater temperature between 9.6 and 11.0 °C, while those slaughtered in April were starved for 30 days at a seawater temperature of 6 °C prior to slaughter. After slaughtering, the fish were filleted, individually tagged and the left fillet was analysed fresh, while the right fillet was processed and analysed after smoking. The temperature in the fillets post slaughtering was 2.5 and 6 °C in fish slaughtered in April and November, respectively. The following processing methods were used: dry- or brine-salting, followed by smoking at either 20 or 30 °C. Dry-salted fillets were salted by hand with refined salt and left for 6 h at 12 °C, rapidly rinsed in water (15 °C) and stored at 2 °C until smoking. The brine salted fillets were submerged in saturated brine, 360 g l^{-1}, at 12 °C for 6 h, rinsed in water and stored at 2 °C until smoking, which was done in a tunnel with an air velocity of 2 m/s^{-1}. The smoking time was 2.5 h and chips of beech were used (Cardinal et al., 2001). Following processing, the Norwegian quality cut (NQC) of both the fresh and the smoked samples from each treatment group were analysed and the changes in tocopherol and ascorbic acid, in total fat content and fatty acid composition, as well as in pH and in oxidation status during processing, were studied.

2.2. Chemical methods

Ascorbic acid was analysed in fresh and smoked salmon by reverse phase high-performance liquid chromatography (RP-HPLC) using electrochemical detection. A fillet (0.5 g) was homogenised in 5% meta-phosphoric acid. Ascorbic acid released from the homogenates was stabilised by the addition of 0.54% EDTA. Ascorbic acid was separated by use of an ODS Hypersil (C18, 5 μm, 100×4.6 mm) column (Hewlett Packard), equipped with a similar quality 20×4 mm guard column. Ascorbic acid was detected at 0.6 V by an electrochemical detector (Hewlett Packard). A standard curve of ascorbic acid was used for quantification (Sandnes, Hansen, Killie, & Waagbø, 1990).

Tocopherol was analysed in both fresh and smoked fillets by normal phase HPLC with fluoroscence detection (excitation: 289 nm, emission: 331 nm). A 0.5 g sample was homogenised and saponified in 4 ml ethanol, 0.5 ml saturated EDTA and 0.5 ml 20% KOH and extracted in 2×2 ml hexane. Ascorbic acid and pyrogallol were added before saponification to prevent oxidation during the process (Lie, Sandvin, & Waagbø, 1994).

The total fat content in the fresh and the smoked fillets was determined gravimetrically after extraction with ethyl acetate (Losnegård, Bøe, & Larsen, 1979).

In both the fresh and the smoked fillets, lipids were extracted in chloroform:methanol (2:1, v/v) and the fatty acid composition of total lipids was obtained by saponification of the extracted lipids, esterification by 12% BF_3 in methanol and the methyl esters separated by capillary gas chromatography (50 m, 0.3 mm i.d., CP-sil 88 fused silica capillary column; Chromopack). Peaks were identified by reference to a standard mixture of methyl esters (Rønnestad, Finn, Lein, & Lie, 1995).

Thiobarbituric-reactive substances (TBARS) were determined in both the fresh and the smoked salmon to evaluate the oxidation stability during processing of the salmon fillets. Samples were homogenised under nitrogen with chloroform:methanol (2:1, v/v). Two parts of water were then added to make a two-phase, system, and an aliquot of the methanol/water phase, containing the short chain aldehydes, was heated in the presence of excess thiobarbituric acid in trichloroacetic acid. The complex created was measured spectrophotometrically at 532 nm, using malondialdehyde as standard (Schmedes & Hølmer, 1989).

2.3. Statistical methods

The values for the chemical composition of the fresh fillets and for the smoked fillets at different stages of processing were compared by analysis of variance (ANOVA). If significant differences ($P < 0.05$) between means were obtained, Tukey's honest significant test was used to differentiate between means. Statistics were performed using CSS Statistica (Ver. 5.5: 99, Statsoft Inc, 1991, Tulsa, USA). The mean chemical values, as well as the respective percentual changes through processing, were also explored by principal component

Table 1
Fresh fillets mean values of total fat contents (g kg^{-1}), sum of saturated (SFA), mono unsaturated (MUFA) and poly unsaturated fatty acids (PUFA), n-3:n-6 fatty acid ratio, EPA, DHA, tocopherol (mg kg^{-1}), ascorbic acid (mg kg^{-1}), pH and TBARS (μmol kg^{-1}) in ocean-ranched (June) and farmed salmon, slaughtered either in November or in April[a]

Fish material	Ocean-ranched June	Farmed November	Farmed April
Total fat content	84.8±4.7 C	168.8±3.6 A	132.7±7.1 B
Sum of SFA	23.5±0.3 A	22.0±0.1 B	21.7±0.2 B
Sum of MUFA	47.1±0.5 A	46.3±0.3 A	40.8±0.3 B
Sum of PUFA	26.0±0.4 C	29.0±0.2 B	34.5±0.3 A
Ratio of n-3:n-6	11.9±0.2 A	6.5±0.1 B	3.5±0.2 C
EPA	5.4±0.1 B	7.2±0.1 A	7.6±0.1 A
DHA	13.7±0.3 A	9.8±0.1 C	10.5±0.1 B
α-Tocopherol	10.8±0.4 B	27.8±0.8 A	25.4±1.3 A
Ascorbic acid	32.8±1.5 A	21.6±0.9 B	13.0±0.7 C
Fillet pH	6.42±0.01 A	6.27±0.01 B	6.39±0.01 A
TBARS	5.5±0.5 B	7.5±0.5 A	4.2±0.5 B

[a] All values (means±S.E.M.) are per wet weight. Fatty acids are given as g 100 g^{-1} total fatty acids. 'A' denotes the highest values, whereas 'B' and 'C' denote significantly lower values. EPA, eicosapentanoic acid; DHA, docosahexanoic acid; TBARS, thiobarbituric acid-reactive substances.

analysis, PCA (Martens & Naes, 1989). This was done to visualise, and thus improve, the interpretation of the relative changes in chemical composition observed in the different fillets. PCA was carried out by the Sirius (Ver. 6.5, Pattern Recognition Systems Ltd., Bergen, Norway) software (Kvalheim & Karstang, 1987).

3. Results and discussion

The ocean-ranched fish was leaner (84.8 g kg^{-1}) than the farmed salmon, and the fish slaughtered in November contained more fat (168.8 g kg^{-1}) than those slaughtered in April (132.7 g kg^{-1}, Table 1). Thus, the farmed salmon contained 1.5 to 2 times as much total fat as the ocean-ranched fish, depending on season. The higher fat content in farmed fish compared with wild has been previously reported (Bell et al., 1998). In the present experiment the lower body weight of the ocean-ranched fish might also have affected the total fat content in the fresh salmon, as body weight is known to affect the fat content of fillets (Bell et al., 1998). The ocean-ranched salmon had grown in the Atlantic Ocean feeding on natural prey, while the farmed fish had been fed commercial high energy feeds thus depositing more lipids (Bell et al., 1998; Hemre & Sandnes, 1999; Lie et al., 1988, 1993; Sheehan, O'Connor, Sheehy, Buckley, & FitzGerald, 1996). The ocean-ranched salmon contained a significantly higher ratio of n-3:n-6 fatty acids than did the farmed salmon whereas, in the farmed salmon groups, the lower the mean fat content the lower was the n-3:n-6 ratio (Table 1). The ocean-ranched salmon contained significantly less of the EPA (eicosapentanoic acid, 20:5 n-3) and more of the DHA (docosahexanoic acid, 22:6 n-3) relative to total fatty acids, than the farmed salmon. Further, the farmed salmon slaughtered during the autumn, being the fattiest, contained lower amounts of DHA than farmed salmon slaughtered in April (Table 1). The higher content of DHA and the lower content of EPA, found in the ocean-ranched compared with the farmed salmon, have also previously been reported in studies comparing farmed and wild salmon (Bell et al., 1998; Cronin, Powell, & Gormley, 1991).

The ratio of tocopherol:ascorbic acid was four to six times lower in the ocean-ranched (0.33) than in the farmed fish (1.29–1.95). The tocopherol content in fillets is affected by the addition of tocopherol in the commercial feeds (Hamre, 1995). Moreover, the content of tocopherol was higher the fattier the fillet (Table 1). A tocopherol content of about 30 mg kg^{-1} has previously been reported in farmed fish with a body weight of 2.5–5.0 kg (Bell et al., 1998). Ascorbic acid showed the opposite trend to tocopherol, as the ocean-ranched fish contained about twice as much ascorbic acid as the farmed salmon (Table 1). The difference in antioxidative vitamins might be due to different feeding and to different lipid deposition, but neither the ocean-ranched nor the farmed fish fillets were oxidised, as the TBARS values were below 8 μmol kg^{-1}. The fattiest fish, those slaughtered in November, also showed significantly lower fillet pH than either the leaner farmed fish or the ocean-ranched salmon (Table 1).

The chemical composition (wet wt.) of the smoked fillets changed during salting and cold smoking, due partly to changes in the wet weight of the fillets. The fatty acid composition of the total lipids in the smoked fillets generally showed the same values as in the fresh fillets. On the other hand, the processing methods used were found to affect both the total fat content and the ascorbic acid content (Table 2). Generally, those fillets being brine salted contained less fat than those being dry-salted; this was most evident at the lowest smoking temperature. Furthermore, the content of ascorbic acid

Table 2
Smoked fillets mean values of total fat content, tocopherol, ascorbic acid, TBARS, pH, n-3:n-6 fatty acid ratio, EPA and DHA in ocean-ranched and farmed Atlantic salmon, slaughtered in either November or April after being brine-salted (Br) or dry-salted (Ds), followed by cold-smoking at 20 or 30 °C[a]

Raw material/ processing groups	Total fat (g kg^{-1})	Tocopherol (mg kg^{-1})	Ascorbic acid (mg kg^{-1})	TBARS (μmol kg^{-1})	pH	Ratio n-3:n-6 fatty acids	EPA (g 100 g^{-1} total FA)	DHA (g 100 g^{-1} total FA)
Ocean ranched/June								
Br20	48±6 Bb	10.0±0.4 C	7.2±1.4 Aab	14.7±1.5 *a*	6.3±0.03 a	12.3±0.3 A	5.4±0.3 C	14.5±0.6A
Br30	46±4 Bab	9.9±0.7 C	5.4±0.8 Ab	7.4±0.7 *b*	6.1±0.02 cd	11.1±0.2 A	5.5±0.3 C	13.5±0.5A
Ds20	52±6 Ba	11.3±0.7 C	10.3±1.9 Aa	7.6±0.8 *b*	6.3±0.02 *a*	12.0±0.4 A	5.1±0.3 C	13.4±0.6A
Ds30	61±5 Ba	13.0±0.9 C	8.2±0.9 Aab	6.0±0.6 *b*	6.1±0.02 *c*	12.1±0.4 A	5.7±0.4 C	13.8±0.4A
Farmed/November								
Br20	125±10 Ab	23.6±1.1 A	6.2±1.8 Aab	8.3±0.6 *b*	6.1±0.01 *c*	6.7±0.1 B	7.2±0.1 B	9.9±0.1C
Br30	150±8 Aab	25.1±1.2 A	4.3±0.8 Ab	9.5±0.9 *ab*	5.9±0.01 *d*	6.2±0.2 B	7.2±0.2 B	9.5±0.3C
Ds20	143±7 Aa	24.5±1.0 A	9.0±0.8 Aa	6.0±0.8 *b*	6.1±0.01 *c*	6.5±0.2 B	6.9±0.1 B	9.7±0.3C
Ds30	162±7 Aa	24.6±1.4 A	5.4±0.5 Aab	7.0±0.5 *b*	6.0±0.01 *d*	6.4±0.3 B	7.0±0.2 B	9.4±0.3C
Farmed/April								
Br20	119±12 Ab	22.8±3.3 B	3.2±0.5 Bab	7.1±1.1 *b*	6.2±0.01 *b*	2.7±0.7 C	7.5±0.2 A	9.9±0.8B
Br30	144±10 Aab	22.2±0.6 B	2.8±0.4 Bb	7.7±0.4 *b*	6.1±0.01 *cd*	3.8±0.2 C	7.3±0.1 A	11.2±0.2B
Ds20	137±5 Aa	22.3±0.8 B	3.4±0.3 Ba	9.3±1.2 *ab*	6.0±0.01 *cd*	3.9±0.2 C	7.6±0.1 A	10.8±0.1B
Ds30	127±8 Aa	19.9±0.7 B	3.6±0.3 Bab	7.6±1.1 *b*	6.2±0.01 *b*	3.8±0.2 C	7.7±0.1 A	11.0±0.1B

[a] Values are means±S.E.M. Means followed by different capital letters differ in raw materials, whereas different small letters indicate significant effects from variable processing methods and italic letters indicate interactions between salting method and raw materials ($P<0.05$). TBARS, thiobarbituric acid-reactive substances; EPA, eicosapentanoic acid; DHA, docosahexanoic acid.

was generally lower in those fillets being brine-salted than in those being dry-salted prior to smoking. In contrast to the findings for the fat contents, ascorbic acid contents were lower in the fish smoked at the higher temperatures. A three-component PCA model of the mean fresh values of total fat, TBARS, pH, tocopherol and ascorbic acid, and their respective changes under processing, explained 83.3% of the variance in the data matrix (Fig. 1). A high loading value, in the plot for variables coding for relative changes, reflects a large change in the actual parameter during the processing steps, and vice-versa. The ocean-ranched fish separated from the farmed fish along PC1, in showing higher relative loss (27–52%) of total fat during processing, while both the farmed fish groups were observed close to the fresh fat variable, confirming their higher fat contents (Fig. 1). The fish groups slaughtered during the spring were negatively correlated to the 'change in fat' variable along both the first and the second principal components, showing a low relative loss (0–10%) of fat during processing. The higher original fat content (169 g kg^{-1}) and the higher relative changes (4–26%) in the groups slaughtered in the autumn, resulted in the highest absolute loss of fat in these groups (range: 7–44 g kg^{-1}). The highest absolute loss of fat during processing was observed in the brine salted, low temperature (20 °C) groups. Rørå, Kvåle, Mørkøre, Rørvik, Steien and Thomassen (1998) reported that the smoking loss in farmed salmon decreased as the fat content increased, but claimed the smoking loss to be due to increased trimming loss, i.e. not actually fat loss in trimmed fillets. The trimming loss from their investigation adds to the fat loss reported in the present paper, in which trimmed fillets were analysed before and after smoking.

All groups lost substantial amounts of ascorbic acid under processing. The ocean-ranched fish had a higher content of ascorbic acid in the fresh fillets and also showed the highest loss (22.5–27.4 mg kg^{-1} or 69–84%). The brine salting had on average a 7% stronger effect on vitamin C loss than the dry-salting method. The farmed salmon contained more tocopherol (denoted 'fresh vitamin E' in Fig. 1), whereas the ocean-ranched fish group was negatively correlated to the change in tocopherol, indicating that this group showed stable tocopherol values through processing. The changes in vitamin E during processing were most conspicuous in the groups slaughtered during the spring, varying between 10 and 22% loss (2.6–5.5 mg kg^{-1}). Generally, no clear effect of the different salting methods was observed on the tocopherol loss during processing.

For both the pH and the oxidation, interactions between raw materials and the processing methods were found. All the smoked groups showed slightly higher TBARS values than the fresh fillets, although the levels could not be characterised as oxidised (Table 2). pH values in smoked fillets were generally one to two decimal logarithmic units higher in the fish smoked at the lower temperatures. The only exceptions were the fish slaughtered in the spring, where the dry-salted groups showed pH = 6.2 at 30 °C and 6.0 at 20 °C.

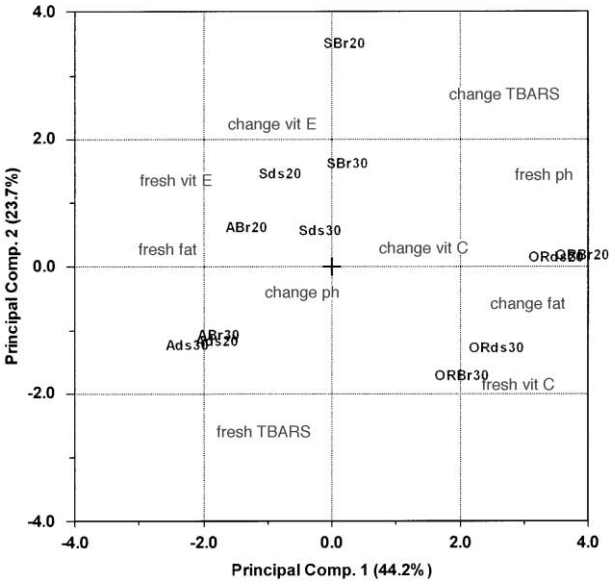

Fig. 1. Biplot from principal component analysis (PCA) of the ocean-ranched (OR) and farmed salmon (slaughtered in either A, the autumn, or S, the spring), and their respective chemical fresh values and relative changes during dry-salting (Ds) or brine salting (Br), followed by cold-smoking, at either 20 or 30 °C. The samples are dark coloured and the variables are light coloured. Explained variance along each principal component is given in brackets. The plot clearly separates the ocean-ranched and the farmed salmon. See Section 3 for further details.

4. Conclusion

The fat content of the fresh fillets significantly affected the smoking loss of nutritive components. The leaner ocean ranched salmon showed the highest relative loss (27–52%) of fat, whereas the fattiest farmed autumn group showed the highest absolute fat loss (44 g kg^{-1}), but also the highest variation between groups (range: 7–44 g kg^{-1}). The highest absolute loss of fat during processing was observed in the brine salted, low temperature (20 °C) groups. All groups lost substantial amounts of ascorbic acid during processing. The brine salting had, on average, a 7% stronger effect on vitamin C loss than the dry salting method. Generally, no clear effect of the different salting methods was observed on the tocopherol loss during processing.

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