Fatty acid composition of salted and fermented products from Baikal omul (*Coregonus autumnalis migratorius*)

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Abstract The fatty acid (FA) composition of raw, salted, and fermented fish products prepared from two populations of Baikal omul (*Coregonus autumnalis migratorius*) was determined. Total lipid content in the raw, salted, and fermented fish products was 3.85, 4.04, and 3.76%, respectively. Overall, the most abundant fatty acids were 14:0 (myristic acid), 16:0 (palmitic acid), 16:1n-7 (palmi-toleic acid), 18:1n-9 (oleic acid), 20:5n-3 (eicosapen-taenoic acid), and 22:6n-3 (docosahexaenoic acid, DHA). Polyunsaturated FAs were the main fatty acid group. Among unsaturated FA, n-3 forms dominated. The highest amounts of n-3 FAs were found in raw fish, followed by fermented and salted fish. Salting significantly increased the content of some FAs (15:0, 16:2n-4, 18:3n-3, 20:3n-3) compared with raw fish and decreased the DHA content. The FA composition of fermented fish did not differ from that of raw fish. The n-3:n-6 ratio did not differ between raw, salted, and fermented fish from population A, while the ratio was higher in raw fish from population B. Overall, thiobarbituric acid reactive substances, and thereby oxida-tion, were significantly lower in raw fish than in salted

and fermented fish. Salting, but not fermentation, affected the FA composition of fish.

Keywords Baikal omul · Fatty acids · Lipid classes · Oxidation · Processing

Abbreviations

FA	Fatty acids
TG	Triacylglycerols
SFA	Saturated fatty acids
UFA	Unsaturated fatty acids
MUFA	Monosaturated fatty acids
PUFA	Polyunsaturated fatty acids
FAME	Fatty acid methyl esters
FID	Flame ionization detector
TBARS	Thiobarbituric acid reactive substances
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
TLC	Thin-layer chromatography
PL	Phospholipids
LC-PUFA	Long-chain polyunsaturated fatty acids

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Introduction

Omega-3 (n-3) fatty acids (FAs) are known to have a variety of benefits for human health. Regular adequate intake of n-3 FAs can reduce the risk of cardiovascular disease (Petsini et al. 2018), stimulates brain activity (Dyall 2015), and is important in fetal development and healthy aging (Gomez-Candela et al. 2015). Compared with tradi-tional diets, the modern Western diet is characterized by n-3 FA deficiency and altered ratio of n-6:n-3 polyunsat-urated fatty acids (PUFA). A balanced n-6:n-3 ratio (1–4:1) is an important factor for health and preventing obesity

(Simopoulos 2011). Deficiency in n-3 FA has been observed in younger generations in Japan (Okuyama et al. 1996), while in Sweden intake of n-3 fatty acids by pre-school children is lower than recommended (Garemo et al. 2007). In Russian Federation seafood n-3 FA intake by adults in 2010 was lower than recommended (Micha et al. 2014). Recommended value of seafood omega-3 fatty acids is 250 mg/day. Blood levels of the sum of eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) in Russian population was generally low (4–6% of total FA in erythrocyte equivalents), except for a region located on the Sea of Japan and Northern Russia, where these levels are high (Stark et al. 2016). In the USA due to changes in the diet during the 20th century, the n-6:n-3 ratio increased by 77%, from 5.4 to 9.6, and is higher than recommended (Blasbalg et al. 2011).

Alpha-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6) are essential FAs and must be obtained through the diet. ALA is metabolized to the LC-PUFA eicosapentaenoic acid (EPA, 20:5n-3) and to a lesser degree to docosahexaenoic acid (DHA, 22:6n-3), while LA is also metabolized to arachidonic acid (AA, 20:4n-6). Only a small amount of LC-PUFAs are synthesized in mammals, including humans (Neff et al. 2011), and the majority of the LC-PUFA requirement must be met by food. Fish products and fish and krill oils are the main dietary sources of EPA and DHA. However, the content of EPA and DHA differs in different kinds of fish and fish products, e.g., the highest content of n-3 is generally found in unprocessed fish (Aro et al. 2005).

In nature, n-3 FAs are mainly esterified, either in the form of phospholipids (PL) or triacylglycerols (TAG), or occur as free fatty acids (Burri et al. 2012). It is known that bioavailability of FAs is affected by the lipid form in which LC-PUFA are consumed. For instance, there is some evidence that dietary PL-DHA may target the brain more effectively than TAG-DHA, possibly because of ingested PL-DHA enriching serum lipid pools that effectively target brain DHA (Kitson et al. 2016).

The omul (*Coregonus autumnalis migratorius*), also known as Baikal omul, is a whitefish species of the subfamily Coregonidae in the salmon family (Salmonidae) that is indigenous to Lake Baikal in Siberia, Russia. It is considered a delicacy, and is a so-called 'brand' of Lake Baikal and the object of one of the largest commercial fisheries on Lake Baikal (Nikiforova et al. 2017). Omul inhabits the pelagic zone of Lake Baikal (350–400 m depth) and displays the specific morphological features typical of pelagic ciscoes, namely a terminal mouth, a large number of rakers, and body parameters characteristic of pelagic fish. Baikal omul is represented by different populations which are reproductively isolated as they reproduce in different tributaries to Lake Baikal. The four main populations of Baikal omul,

named according to reproduction site, are the Severobaikalsk, Posolsk, Selenginsk, and Chivyrkuisk populations. These populations differ in some morphological features. Additionally, Baikal omul can be separated into three morpho-ecological groups (or morphotypes): Pelagic, near-bottom abyssal, and coastal (Glyzina et al. 2010). In other sources these are referred to as littoral, pelagic, and deep-water groups (Grahl-Nielsen et al. 2011).

Only limited information is available on the FA composition of raw Baikal omul, but it has been shown that the FA composition in the different morpho-ecological groups is similar (Glyzina et al. 2010). No data are available on the FA composition of processed Baikal omul, which mainly comprises salted products. Salted fish is one of the most widely consumed fish products in the world and in many countries it is regarded as traditional food. Fermentation, another of the oldest traditional techniques in food processing, has several benefits for the quality characteristics of products. It extends the shelf-life as a result of formation of antimicrobial substances, enhances the flavor, and improves the texture (Marco et al. 2017). It is often combined with salting and/or drying to reduce water activity and retard or eliminate growth of proteolytic and putrefying bacteria (Dincer et al. 2010). In addition, some fermented food products are suggested to promote human health (Marco et al. 2017). For instance, it has been suggested that kimchi (fermented vegetables) has anti-diabetic and anti-obesity potential. Consumption of fermented milk is reported to decrease the risk of type 2 diabetes, cardiovascular disease, and overall mortality (Marco et al. 2017).

The fermented fish product 's dushkom' (literally 'with odor') made from Baikal omul is a delicacy of Baikal's mountainous eastern shore and a traditional dish of local people (Nikiforova et al. 2017). It is made using a specific production process that involves degutting, mild salting, and storage for at least four weeks to get the necessary characteristics. The traditional method involved placing the fish with salt in wooden barrels and burying it in the ground.

Despite salted and fermented omul products being an important source of PUFA and other important nutrients, there have been few studies on their nutritional value. Therefore, the aim of the present study was to determine and compare the FA composition of raw, salted, and fermented products prepared from two populations of Baikal omul.

Materials and methods

Fish preparation

Analyses were performed on a total of 65 Baikal omul captured by commercial fishers during summer (late July-

Fig. 1 Map of Lake Baikal, showing the location of populations of Baikul omul used in this study: the Severobaikalsk population (population A) and the Posolsk population (population B). (Created using Natural Earth data in QGIS Desktop 3.2.1)

early August) 2015–2016 from two populations in Lake Baikal: the Severobaikalsk population, which lives in the northern part of the lake (population A), and the Posolsk population (population B), which lives in the southern end of the lake (Fig. 1).

Only mature fishes of both sexes were taken for the study. The age of fish was determined according to existing methods. Samples of raw fish were frozen at $-30\text{ }^{\circ}\text{C}$ immediately after capture.

Salted fish (20 fish) was prepared according to standard procedures used at processing plants with the use of mixed salting method, when both salt and brine were used for the salting. The NaCl used for the production of salted fish was 25% to the fish mass. The duration of salting was 4 days with the temperature $4\text{--}6\text{ }^{\circ}\text{C}$.

Fermented fish (17 fish) was produced under laboratory conditions, according to traditional methods. Dry method of salting was used. It included the use of 7% NaCl to the mass of fish. The duration of salting and fermentation was 1 month. The temperature for the first 24 h was $18 \pm 2\text{ }^{\circ}\text{C}$,

and $6\text{--}8\text{ }^{\circ}\text{C}$ afterwards. No other additives were used during production of salted and fermented Baikul omul.

All samples were frozen at $-30\text{ }^{\circ}\text{C}$ immediately after preparation and stored until analysis.

Lipid extraction

Lipid extraction was performed as previously described by Hara and Radin (1978) with modifications (Pickova et al. 1997). In brief, 1 g of muscle from each fish was taken for analysis. These samples (red and white muscles together) were taken from similar parts of fish, around the dorsal fin, and homogenized for $3 \times 30\text{ s}$ in a laboratory homogenizer (Ultra-Turrax, Janke and Kunkel, IKA Werke, Germany). A 10 mL aliquot of 3:2 v/v hexane-isopropanol (HIP) was added during homogenization, and the homogenizer was rinsed with 5 mL HIP. Then 6.5 mL Na_2SO_4 (water-free) solution (6.67%) were added and the samples were shaken vigorously and centrifuged at 4000 rpm, $18\text{ }^{\circ}\text{C}$, for 5 min. The lipid phase was transferred to a glass

tube and 1 mL hexane was added to the non-lipid phase, which was shaken vigorously and centrifuged again at 4000 rpm, 18 °C, for 5 min. The hexane phase was transferred to the glass tube and then the combined hexane extract was evaporated under nitrogen flushing using a warm water bath.

The dry lipid samples were dissolved in 1 mL hexane and transferred to glass vials for further analysis. The total lipid composition of samples was determined by weighing the lipid dissolved in 1 mL hexane on a microbalance (Mettler Toledo, UMT 2) for more exact measurement. In further analyses, all samples were analyzed in duplicate.

Separation and quantification of lipid classes by thin layer chromatography (TLC)

Lipid classes were separated using thin layer chromatography (TLC) plates coated with silica gel (Pickova et al. 1997). Analyses of lipid class composition was performed according to Pickova et al. (1997) with minor modifications. Extracted lipid samples dissolved in hexane (conc. 1 µg/µL) were applied by a Camag ATS 4 automatic TLC sampler (Camag, Muttentz, Switzerland) in 20 mm lines on 60TLC plates (20 × 20, 0.20 mm layer; Merck, Darmstadt, Germany). The lipid classes were separated using hexane-diethyl ether-acetic acid (85:15:1, v/v/v) as mobile phase and identified by comparison against an external standard (TLC 18-4A; Nu-Check Prep, Elysian, Minnesota, USA). The different lipid classes were then scraped off and extracted with chloroform:methanol gradients, before drying and final addition of hexane.

Preparation of fatty acid methyl esters (FAME)

Preparation of fatty acid methyl esters (FAME) was performed as described by Appelqvist (1968). First, 2 mL NaOH in dry methanol (0.01 M) were added to each tube, and all samples were shaken vigorously and placed in a heating block at 60 °C for 10 min. Then 3 mL of 14% boron trifluoride–methanol complex (BF₃) were added as an esterification agent and the vials were re-heated at 60 °C for 10 min. All samples were cooled in ice water before addition of 2 mL of 20% NaCl solution and 2 mL hexane. The tubes were shaken vigorously and left to stand at 4 °C for 20 min. The upper phase, containing the FAME, was transferred to a glass tube and evaporated under nitrogen gas until dryness. The dry samples were dissolved in hexane, vortexed, and stored at – 20 °C until gas chromatography analysis.

The FAME were analyzed with a Varian (Stockholm, Sweden) CP3800 gas chromatograph equipped with FID and split injector, and fitted with a 50 m length, 0.22 mm i.d., 0.25 µm film thickness BPX 70 fused-silica capillary column (SGE, Austin, TX, USA) .

Samples (1 µL) were injected in split mode with a CP8400 autosampler (Varian). The split ratio was 1:10. Column temperature was programmed to start at 158 °C for 5 min, then increase from 158 to 220 °C over 2 min, and remain at 220 °C for 8 min. Injector and detector temperature was 230 and 250 °C, respectively.

Fatty acids were identified by comparison of samples against the GLC-68A reference standard (Nu-check Prep, USA). The carrier gas was helium (22 cm/s, flow rate 0.8 mL/min). Make-up gas was nitrogen. The peaks were identified using Galaxie software.

All solvents and chemicals were of analytical grade and were purchased from Merck unless otherwise stated.

Moisture content

Moisture content was determined by oven-drying to constant weight (AOAC).

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances (TBARS) were analyzed as previously described by Ulu (2004). First, 1 g of fish was homogenized with 0.5 mL of 0.2% butylated hydroxytoluene (Sigma, USA) and 5.0 mL of 5% trichloroacetic acid (Sigma, USA) for 30 s, using a laboratory homogenizer (Ultra-Turrax, Janke and Kunkel, IKA Werke, Germany). The homogenate was filtered using Munktell filter paper (00 K), 1 mL of the filtrate was placed in a clean glass tube, and 1 mL of 20 mM 2-thiobarbituric acid (TBA) solution (Sigma, USA) was added. All tubes were heated in a water bath at 85 °C for 45 min and then cooled for 10 min with cold tap water.

The absorbance was determined at wavelength 532 nm against a blank containing 1 mL of deionized water (Milipore Synergy 185, Germany) and 1 mL of 20 mM TBA solution. Malondialdehyde (MDA) concentration within the range 0–2.5 µM was calculated using 1,1,3,3-tetramethoxypropane (TMP) as a standard.

Statistical analysis

Statistical analysis of the chemical composition and FA composition of products was performed using Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC, USA). The fixed effects of treatment (raw, salted, or fermented fish), population, and interactions between treatment and population were evaluated using procedure mixed. Year was included in the model as random factor.

Statistical analyses of lipid classes in products from Baikal omul was performed with R Statistics, version 3.2.3 (The R Foundation for Statistical Computing). The values of each fatty acid and lipid class were compared by

analysis of variance (ANOVA). The influence of treatment, fish population, and their interaction on FA composition was analyzed. When significant differences ($P < 0.05$) between mean values were found, a Tukey test was performed.

Results

Total lipids and moisture content

No significant effect of treatment (raw, salted, fermented) or population (A, B) on fat content was found (Table 1). As expected, the moisture content was highest in raw fish. However, there were significant interactions between treatment and population, with a similar moisture content in raw and fermented products from both populations, but a higher moisture content in salted fish from population A compared with population B (Table 1).

The TBARS values were significantly lower in raw fish than in salted and fermented products. No significant differences were found between the TBARS values of salted and fermented fish ($P > 0.05$) (Table 1).

Fatty acid composition

The most abundant FAs in all products were myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1n-7), oleic acid (18:1n-9), eicosapentaenoic acid (EPA) (20:5n-3), and docosahexaenoic acid (DHA) (22:6n-3).

Salting significantly increased the content of some FAs (15:0, 16:2n-4, 18:3n-3, 20:3n-3) compared with raw fish and decreased the DHA content ($P < 0.05$) (Table 2). The FA composition of fermented fish was similar to that of raw fish. Population significantly affected 18:2n-6 level ($P < 0.05$), with the content being lower in population B than in population A.

The n-3:n-6 PUFA ratio differed between the populations when measured in raw fish. It did not differ between treatments in population A, but in population B the n-3:n-6 ratio was higher in raw fish compared to salted and fermented fish of population B and differently treated fish of population A (Table 2).

The distribution of FAs in the form of TAG and PL in different products from Baikal omul is shown in Tables 3 and 4. The main FAs in TAG form were 16:0, 16:1n-7, and 18:1n-9. The dominant FAs in PL form were 16:0, 18:0, 18:1n-9, and 22:6n-3. The percentage of PUFA was higher in PL than in TAG.

Fish processing caused only slight changes in the FA composition of PL and TAG in different products. For example, the content of oleic acid in PL and the content of 17:0, 18:0, 20:1n-9, ALA, 22:4n-6, EPA, and DHA in TAG

Table 1 Effect of salting and fermentation on the chemical composition and thiobarbituric acid reactive substances (TBARS) values of products from two populations (A, B) of Baikal omul (least-square (LS) mean $\pm$ SE)

Population	Raw		Salted		Fermented		<i>P</i> value	
	A	B	A	B	A	B	Treatment	Treatment* population
Fat content	3.3 $\pm$ 0.69		4.1 $\pm$ 0.73		3.2 $\pm$ 0.73		0.195	0.316
Moisture content	76.1 ^a $\pm$ 0.67	76.6 ^a $\pm$ 0.67	67.3 ^b $\pm$ 0.67	63.6 ^c $\pm$ 0.92	71.1 ^d $\pm$ 0.79	71.9 ^d $\pm$ 0.63	< 0.0001	0.006
TBARS	0.4 ^a $\pm$ 0.29		1.2 ^b $\pm$ 0.30		1.0 ^b $\pm$ 0.30		< 0.0001	0.053

LS means with different superscripts differ significantly ($P < 0.05$). If interaction between treatment and sampling site was $P < 0.1$, data for each sampling site are presented separately. If interaction was $P > 0.05$, data are presented for treatment only

Table 2 Effect of treatment on the fatty acid (FA) composition of total lipids in raw, salted, and fermented products from two populations (A, B) of Baikal omul [least-square (LS) mean $\pm$ SE, expressed as % of total FA]

	Raw		Salted		Fermented		P value	
	A	B	A	B	A	B	Treatment	Population
<i>Fatty acid</i>								
14:0	6.3 ^a $\pm$ 0.18		6.9 ^b $\pm$ 0.22		6.9 ^b $\pm$ 0.21		0.050	< 0.001
15:0	0.54 ^a $\pm$ 0.018		0.59 ^b $\pm$ 0.020		0.55 ^a $\pm$ 0.020		0.007	< 0.001
16:0	17.6 $\pm$ 0.53		16.9 $\pm$ 0.55		16.8 $\pm$ 0.55		0.060	< 0.001
17:0	1.0 $\pm$ 0.05		1.1 $\pm$ 0.06		1.1 $\pm$ 0.06		0.808	< 0.001
18:0	2.8 $\pm$ 0.11		2.7 $\pm$ 0.12		2.8 $\pm$ 0.12		0.780	0.174
SFA	28.4 $\pm$ 0.57		28.3 $\pm$ 0.59		28.2 $\pm$ 0.59		0.932	< 0.001
16:1n-9	0.48 $\pm$ 0.032		0.49 $\pm$ 0.037		0.42 $\pm$ 0.037		0.250	< 0.001
16:1n-7	8.0 $\pm$ 0.32		7.9 $\pm$ 0.39		8.6 $\pm$ 0.39		0.390	< 0.001
18:1n-9	11.9 $\pm$ 0.59		13.1 $\pm$ 0.64		12.6 $\pm$ 0.64		0.118	< 0.001
18:1n-7	2.9 $\pm$ 0.07		3.0 $\pm$ 0.09		2.9 $\pm$ 0.09		0.512	0.001
20:1n-9	3.9 $\pm$ 0.22		4.3 $\pm$ 0.25		4.0 $\pm$ 0.25		0.360	< 0.001
MUFA	24.0 $\pm$ 0.81		25.3 $\pm$ 0.91		24.0 $\pm$ 0.81		0.280	0.003
16:2n-4	0.63 ^a $\pm$ 0.042		0.68 ^b $\pm$ 0.044		0.61 ^a $\pm$ 0.044		0.028	< 0.001
18:2n-6	5.7 ^a $\pm$ 0.13	4.5 ^b $\pm$ 0.18	5.5 ^a $\pm$ 0.15	5.3 ^{ac} $\pm$ 0.22	5.5 ^a $\pm$ 0.19	4.8 ^b $\pm$ 0.18	0.355	< 0.001
18:3n-3	4.1 ^a $\pm$ 0.41		4.5 ^b $\pm$ 0.41		4.1 ^a $\pm$ 0.41		0.013	< 0.001
20:4n-6	3.0 $\pm$ 0.07		2.9 $\pm$ 0.08		2.8 $\pm$ 0.08		0.101	0.075
20:3n-3	0.28 ^a $\pm$ 0.030		0.35 ^b $\pm$ 0.031		0.29 ^a $\pm$ 0.030		0.001	< 0.001
20:4n-3	1.1 $\pm$ 0.05		1.1 $\pm$ 0.05		1.1 $\pm$ 0.05		0.235	0.171
20:5n-3	6.9 $\pm$ 0.14		6.6 $\pm$ 0.16		6.6 $\pm$ 0.16		0.295	< 0.001
22:4n-6	2.0 $\pm$ 0.09		2.0 $\pm$ 0.10		2.2 $\pm$ 0.10		0.298	< 0.001
22:5n-3	2.0 $\pm$ 0.03		2.0 $\pm$ 0.04		1.9 $\pm$ 0.04		0.854	0.001
22:6n-3	14.5 ^a $\pm$ 0.84		12.1 ^b $\pm$ 0.93		13.3 ^{ab} $\pm$ 0.92		0.031	< 0.001
PUFA	40.9 $\pm$ 0.59		39.0 $\pm$ 0.71		39.4 $\pm$ 0.71		0.093	0.033
n-3	28.9 $\pm$ 0.52		26.7 $\pm$ 0.63		27.3 $\pm$ 0.63		0.026	0.008
n-6	11.4 $\pm$ 0.17		11.6 $\pm$ 0.21		11.6 $\pm$ 0.21		0.727	0.826
n-3/n-6	2.3 ^a $\pm$ 0.06	2.8 ^b $\pm$ 0.08	2.3 ^a $\pm$ 0.07	2.3 ^a $\pm$ 0.10	2.3 ^a $\pm$ 0.09	2.4 ^a $\pm$ 0.08	0.010	0.011

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. LS means with different superscripts differ significantly ($P < 0.05$). If interaction between treatment and sampling site was $P < 0.1$, data for each sampling site are presented separately. If interaction was $P > 0.05$, data are presented for treatment only

Table 3 Fatty acid composition of phospholipids in raw, salted, and fermented products from two populations (A, B) of Baikal omul [least-square (LS) mean $\pm$ SE, expressed as % of total FA]

	Raw		Salted		Fermented		<i>P</i> value	
	A	B	A	B	A	B	Treatment	Population * Population
<i>Fatty acid</i>								
14:0	3.1 $\pm$ 1.75		3.0 $\pm$ 0.56		3.5 $\pm$ 0.78		0.865	0.026
15:0	0.44 $\pm$ 0.24		0.53 $\pm$ 0.13		0.39 $\pm$ 0.23		0.696	0.115
16:0	25.0 $\pm$ 3.32		27.0 $\pm$ 2.53		26.2 $\pm$ 2.15		0.473	0.037
17:0	0.30 $\pm$ 0.32		0.18 $\pm$ 0.21		0.10 $\pm$ 0.15		0.270	0.001
18:0	10.7 $\pm$ 5.13		8.6 $\pm$ 1.69		9.7 $\pm$ 3.84		0.759	0.630
SFA	39.6 $\pm$ 6.86		39.3 $\pm$ 3.54		39.9 $\pm$ 4.56		0.991	0.529
16:1n-9	0.19 $\pm$ 0.24		0.13 $\pm$ 0.14		0.0 $\pm$ 0.00		0.190	0.026
16:1n-7	3.9 $\pm$ 1.87		2.7 $\pm$ 0.41		4.5 $\pm$ 1.58		0.244	0.001
18:1n-9	10.5 ^{ab} $\pm$ 1.69		7.9 ^a $\pm$ 0.33		12.6 ^b $\pm$ 1.54		0.002	0.596
18:1n-7	2.7 $\pm$ 1.07		2.2 $\pm$ 0.30		2.9 $\pm$ 0.45		0.610	0.937
20:1n-9	1.17 $\pm$ 0.81		0.94 $\pm$ 0.21		0.89 $\pm$ 0.81		0.653	0.004
MUFA	18.6 $\pm$ 4.23		13.8 $\pm$ 1.09		21.0 $\pm$ 3.61		0.051	0.067
16:2n-4	0.44 $\pm$ 0.48		0.61 $\pm$ 0.02		0.38 $\pm$ 0.31		0.775	0.949
18:2n-6	3.0 $\pm$ 1.28		2.5 $\pm$ 0.18		2.9 $\pm$ 0.59		0.809	0.012
18:3n-3	1.9 $\pm$ 0.91		1.8 $\pm$ 0.11		1.6 $\pm$ 0.88		0.739	0.002
20:4n-6	3.6 $\pm$ 2.14		3.4 $\pm$ 0.33		2.4 $\pm$ 0.63		0.504	0.265
20:4n-3	0.26 $\pm$ 0.28		0.54 $\pm$ 0.19		0.19 $\pm$ 0.19		0.123	0.005
20:5n-3	4.8 $\pm$ 1.38		6.3 $\pm$ 0.45		4.3 $\pm$ 1.13		0.113	0.300
22:4n-6	2.2 $\pm$ 0.80		2.4 $\pm$ 0.49		2.3 $\pm$ 0.32		0.944	0.569
22:5n-3	1.38 $\pm$ 0.78		1.51 $\pm$ 0.18		0.99 $\pm$ 0.56		0.461	0.021
22:6n-3	24.1 $\pm$ 6.25		27.5 $\pm$ 2.84		23.7 $\pm$ 5.76		0.583	0.011
PUFA	40.0 $\pm$ 5.93		45.0 $\pm$ 3.75		37.5 $\pm$ 5.39		0.214	0.340
n-3	32.5 $\pm$ 5.32		37.7 $\pm$ 2.82		30.8 $\pm$ 5.33		0.182	0.099
n-6	9.0 $\pm$ 2.38		8.6 $\pm$ 1.04		7.9 $\pm$ 0.71		0.518	0.007

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. LS means with different superscripts differ significantly ($P < 0.05$). If interaction between treatment and sampling site was $P < 0.1$, data for each sampling site are presented separately. If interaction was $P > 0.05$, data are presented for treatment only

Table 4 Fatty acid composition of triacylglycerols in raw, salted, and fermented products from two populations (A, B) of Baikal omul [least-square (LS) mean $\pm$ SE, expressed as % of total FA]

	Raw		Salted		Fermented		P value	
	A	B	A	B	A	B	Treatment	Population * Population
<i>Fatty acid</i>								
14:0	8.8 $\pm$ 3.57		9.1 $\pm$ 0.59		7.5 $\pm$ 1.14		0.555	0.002
15:0	0.67 $\pm$ 0.18		0.66 $\pm$ 0.04		0.53 $\pm$ 0.10		0.239	0.244
16:0	17.5 $\pm$ 3.15		14.9 $\pm$ 2.89		17.1 $\pm$ 1.23		0.395	0.504
17:0	1.5 ^{ab} $\pm$ 0.64		2.2 ^a $\pm$ 0.75		1.1 ^b $\pm$ 0.37		0.018	< 0.001
18:0	4.2 $\pm$ 1.74		2.8 $\pm$ 0.64		3.6 $\pm$ 1.56		0.375	0.443
SFA	34.3 ^a $\pm$ 3.03	28.5 ^b $\pm$ 1.47	29.7 ^{ab} $\pm$ 2.33	NA	29.5 ^b $\pm$ 1.48	29.9 ^{ab} $\pm$ 4.51	0.058	0.001
16:1n-9	0.74 ^a $\pm$ 0.30		0.64 ^{ab} $\pm$ 0.17		0.36 ^b $\pm$ 0.21		0.003	< 0.001
16:1n-7	10.8 $\pm$ 1.43		10.3 $\pm$ 1.45		10.9 $\pm$ 2.09		0.822	0.033
18:1n-9	13.4 $\pm$ 3.45		10.5 $\pm$ 3.46		16.0 $\pm$ 2.99		0.058	0.006
18:1n-7	3.2 $\pm$ 0.62		2.8 $\pm$ 0.74		3.7 $\pm$ 0.30		0.060	0.001
20:1n-9	5.0 ^{ab} $\pm$ 1.47		7.1 ^a $\pm$ 2.7		4.1 ^b $\pm$ 1.55		0.032	0.012
MUFA	33.9 $\pm$ 3.19		31.7 $\pm$ 2.16		35.8 $\pm$ 1.74		0.182	0.311
16:2n-4	0.66 $\pm$ 0.26		0.48 $\pm$ 0.28		0.65 $\pm$ 0.14		0.539	0.679
18:2n-6	6.6 $\pm$ 1.11		6.0 $\pm$ 0.32		5.9 $\pm$ 1.16		0.281	0.022
18:3n-3	5.0 ^{ab} $\pm$ 1.03		6.1 ^a $\pm$ 1.92		3.9 ^b $\pm$ 1.05		0.029	0.047
20:4n-6	2.5 $\pm$ 0.65		2.5 $\pm$ 0.19		2.5 $\pm$ 0.22		0.994	0.431
20:4n-3	1.0 $\pm$ 0.38		1.4 $\pm$ 0.47		1.1 $\pm$ 0.26		0.248	0.719
20:5n-3	6.1 ^a $\pm$ 1.09		8.2 ^b $\pm$ 1.45		5.4 ^a $\pm$ 1.59		0.016	0.735
22:4n-6	1.7 ^a $\pm$ 0.49		1.7 ^{ab} $\pm$ 0.69		2.2 ^b $\pm$ 0.37		0.034	0.004
22:5n-3	1.9 $\pm$ 0.52		2.3 $\pm$ 0.52		1.9 $\pm$ 0.15		0.394	0.997
22:6n-3	5.8 ^a $\pm$ 1.81	11.0 ^b $\pm$ 2.83	9.3 ^{ab} $\pm$ 1.26	NA	9.9 ^{ab} $\pm$ 3.36	9.9 ^b $\pm$ 2.58	0.035	< 0.001
PUFA	28.3 $\pm$ 4.22		32.5 $\pm$ 2.60		30.5 $\pm$ 2.29		0.106	0.008
n-3	20.1 ^a $\pm$ 3.61	24.5 ^{ab} $\pm$ 2.67	27.4 ^b $\pm$ 3.76	NA	23.4 ^{ab} $\pm$ 4.58	21.3 ^{ab} $\pm$ 4.52	0.038	0.064
n-6	11.4 $\pm$ 1.43		10.7 $\pm$ 1.17		11.7 $\pm$ 1.56		0.697	0.677

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. LS means with different superscripts differ significantly ($P < 0.05$). If interaction between treatment and sampling site was $P < 0.1$, data for each sampling site are presented separately. If interaction was $P > 0.05$, data are presented for treatment only

were influenced by treatment method. The proportions of 17:0, 20:1n-9, ALA, and EPA were lower in fermented fish than in salted products. Fish population (A, B) had a more pronounced effect on FA composition of both PL and TAG (Tables 3 and 4).

Discussion

To the best of our knowledge, this is the first study to compare the FA composition of raw, salted, and fermented Baikal omul from different populations. Fish processing method is known to be an important factor in determining the FA composition in the final product. For example, salting generally results in an increase in lipid content, due to water losses during the production of salted fish. Sedova and Boyko (1990) found that total lipid content in salted Baikal omul increased from 3.0 to 6.0%.

In contrast, a decrease in lipid content is usually observed in fermented fish compared with raw fish most likely due to lipolytic enzymatic reactions that take place during fermentation (Yankah et al. 1993). In the present study, the fat content in different fish products was not affected by treatment, suggesting minimal effects of traditional salting and fermentation methods in the Baikal area. It can be due to short duration of salting during the production of salted Baikal omul and relatively low concentration of NaCl used for manufacturing of salted and fermented omul.

The moisture content was highest in raw fish and lowest in salted fish, which in agreement with previous findings for Baikal omul (Sedova and Boyko 1990).

It has been suggested that lipids from freshwater fish contain higher proportions of saturated fatty acids (SFA) and C18 PUFA, but lower levels of C20 and C22 unsaturates, compared with marine fish lipids (Ackman 2002). Lake Baikal fish are unique in terms of the composition of their tissue lipids, which are characterized by high levels of long-chain PUFA, a feature otherwise typical of marine species (Morris 1984).

It has been reported previously that PUFAs (38.5–53.8% of all FAs) and SFAs (31.8–36.5% of all FAs) are the dominant classes in Baikal omul (Glyzina et al. 2010). This was confirmed by findings in the present study.

Some studies have attributed the high content of PUFAs in fish from Lake Baikal to low water temperature, as the thermal regime of the lake is harsh compared with that in many other aquatic environments (Morris 1984). There are also high seasonal fluctuations in water temperature in Lake Baikal, with the temperature in summer ranging from 14–16 °C at the surface to 3.1–3.8 °C below 250 m. This results in a high degree of unsaturation in FAs in membranes (Morris 1984). It is claimed that water temperature

can affect the acyl chain melting point and fluidity of lipids in fish (Kakela et al. 2008). However, other authors suggest that low water temperature has just a minor influence on FA composition (Grahl-Nielsen et al. 2011).

There are reported to be no major differences in the FA composition of major lipids between the morpho-ecological groups of Baikal omul (Glyzina et al. 2010). In the present study, only slight changes in FA composition were observed between the two populations studied.

It is known that the FA composition and lipid content of fish are affected by multiple factors, such as maturity, species, sex, age, temperature, season, pollution, and nutritional condition (Ju et al. 1997; Shirai et al. 2002; Glyzina et al. 2010). The dietary lipid content and FA composition of fish meal, an important component of fish feed, is also an important determinant of FA composition (Shirai et al. 2002).

The predominant FAs in omul tissues analyzed in this study were identified as 16:0, 18:1n-9, and DHA, confirming findings in previous studies (Glyzina et al. 2010). However, some differences in FA composition were found between Baikal omul from the two different populations studied. One of the reasons could be the water temperature in different parts of the lake. The diet of the fish can be another reason for this difference, e.g., resulting from different prey organisms in different parts of the lake, and differences in spawning time may also contribute to the difference between geographically isolated populations.

The method of fish processing was found to have a minor influence on FA composition. Similarly, it has been found previously that FAs in salted, gutted herring and in salted herring fillet show minimal changes compared with the raw material, although marinating fish causes a decrease in total levels of FAs (Aro et al. 2005).

Fermentation of traditional fish products is a combination of the action of enzymes (proteolytic and lipolytic) and microorganisms (Dincer et al. 2010; Anggo et al. 2015). Previous studies have reported that fermentation greatly affects the FA composition of fish (Yankah et al. 1993; Dincer et al. 2010; Mohamed 2015). For instance, Elsebaïy and Metwalli (1989) observed that the amount of SFA (g/100 g fish lipids) was higher and the amount of PUFA was lower in fermented Bouri fish compared with the raw fish. Similarly, Mohamed (2015) reported a higher percentage of SFA and lower percentage of MUFA and PUFA in fermented tiger fish compared with the raw fish. Different components often used during fish fermentation, such as garlic, glucose, and red pepper, can also influence the FA content in fish sauces (Dincer et al. 2010). In the present study, however, no significant differences in SFA, MUFA, and PUFA content were found between fermented and raw fish.

The processing period affects the FA composition of fish. Longer fermentation (32 days) of anchovy (*Stolephorus* sp.) has been found to decrease the concentrations of most FA, except 18:0, DHA, and EPA, compared with shorter fermentation (8 days) (Anggo et al. 2015).

In the present study, TBARS values were higher in salted and fermented fish compared with raw fish. This is most likely due to the pro-oxidant action of salt (NaCl), which is a catalyst of oxidation and is capable of displacing iron ions from binding macromolecules. This results in more free iron ions being left to interact with the lipid fraction (Aro et al. 2005). Increased oxidation rates as a result of fermentation have been reported previously (Yankah et al. 1993). Brine pre-treatment of fish causes oxidation of lipids. The higher the NaCl concentration in the brine, the higher the rancidity of fish (Aubourg and Ugliano 2002). However, high salt concentrations can also have a protective effect by inhibition of oxygenase-type enzymes (Montano et al. 2001). A previous study on fresh untreated and salt-incubated alamang (*Acetes* spp.) found that NaCl had no effect on the FA profile and that, apart from ALA, the content of long-chain fatty acids was similar in both products (Montano et al. 2001).

Phospholipids, the main lipids in membranes, and TAG, the main storage lipids, are the two major lipid classes in fish (Ju et al. 1997). Various factors are responsible for the distribution of these lipid classes in fish, e.g., the proportions in Baikal omul are dependent on the age of fish, with the relative abundance of TAG increasing with maturity (Ju et al. 1997). Season can be also an important variable. Phospholipids normally contain a high percentage of PUFA (Shirai et al. 2002).

Another factor affecting the FA composition of lipid classes in fish is the fish diet. In the case of TAG, which has a storage function, FAs such as 16:0 and 18:1n-9, which have a high content of TAG, are consumed for energy production. The FA composition of dietary oil in the diet of farmed cod affects the FA composition of neutral lipids and PL (Lie et al. 1992). However, differences in PL FA composition in wild fish were not directly related to dietary intake, as shown from subpopulations of several other species (Lie et al. 1992).

The FA composition of feed fed to gilt-head sea bream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) influenced the FA composition of TAG in liver, muscle, and adipose tissues, but not the composition of PL in tissues and plasma (McClelland et al. 1995). A study in which the FA composition of lipid classes in farmed and wild turbot (*Scophthalmus maximus*) was analyzed showed a decrease in n-3 LC-PUFA and 20:4n-6 and an increase in 20:1 and 22:1 in farmed turbot compared with its wild counterpart (Sérot et al. 1998). An influence of fish sex and

season on PL and TAG in fish has also been reported in some studies (Kaçar et al. 2016).

In the present work the changes in TAG fraction were more significant compared to PL fraction. This is an indicator of higher oxidation in TAG. Slight changes in FA composition of PL and TAG of differently treated fish indicate low degree of oxidation caused by salting and fermentation.

Conclusion

In this study, salting altered the FA composition of Baikal omul compared with raw fish. In contrast, despite a longer processing period compared with salting, fermenting did not change the FA composition. Raw fish showed lower TBARS values than salted and fermented fish, indicating a higher degree of oxidation in the latter due to the pro-oxidant action of NaCl, used for both salting and fermentation, and storage effects on lipid degradation.

Levels of some FAs differed between the two omul populations studied, possibly due to differences in fish diet and variations in water temperature in different parts of Lake Baikal. In addition, the adaptations to niche in Lake Baikal by the different subpopulations have probably resulted in differences in the membrane FA profile.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests

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