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Cold Smoking Does Not Decrease the Content of Long-Chain Polyunsaturated Fatty Acids in Siberian Whitefish Species

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Abstract. Four raw and cold-smoked whitefish species of the genus *Coregonus*, least cisco (*C. sardinella*), Arctic cisco (*C. autumnalis*), muksun (*C. muksun*) and broad whitefish (*C. nasus*), caught in the Yenisei River (Siberia, Russia) were analyzed. Content (mg per g of product) of long-chain polyunsaturated fatty acids of the omega-3 family (LC-PUFAs), namely, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), as well as fatty acid (FA) profiles, were measured. There was no significant decrease in the EPA+DHA content in smoked fish compared to raw fish. Moreover, in least cisco, a significant increase in the EPA+DHA content, from 3.22 ± 0.39 mg · g^{-1} in raw fish to 5.04 ± 0.50 mg · g^{-1} in smoked fish, occurred. Thus, smoked fish of the studied species proved to be a valuable source of LC-PUFA for human nutrition. The FA profiles of the examined fish, which reflect their diet, did not change considerably in the process of smoking. Therefore, the FA profiles of smoked fish could be used for verifying label information on species identity and catchment area.

Keywords: least cisco, Arctic cisco, muksun, broad whitefish, eicosapentaenoic acid, docosahexaenoic acid, FA profiles, cold smoking.

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Холодное копчение не снижает содержание длинноцепочечных полиненасыщенных жирных кислот в сибирских видах сиговых рыб

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Аннотация. Проведены исследования сырой и приготовленной холодным копчением рыбы четырех видов рода *Coregonus*: ряпушка (*C. sardinella*), арктический омуль (*C. autumnalis*), муксун (*C. muksun*) и чир (*C. nasus*), выловленной в реке Енисей (Сибирь, Россия). Определено содержание (мг на г продукта) длинноцепочечных полиненасыщенных жирных кислот семейства омега-3 (ДЦ-ПНЖК), а именно эйкозапентаеновой кислоты (20:5n-3, ЭПК) и докозагексаеновой кислоты (22:6n-3, ДГК), а также проанализированы общие профили жирных кислот (ЖК). В рыбе холодного копчения не обнаружено значимого снижения содержания ЭПК+ДГК по сравнению с сырой рыбой. Более того, в ряпушке наблюдалось значительное увеличение содержания ЭПК+ДГК – от 3,22 ± 0,39 мг · г$^{-1}$ в сырой рыбе, до 5,04 ± 0,50 мг · г$^{-1}$ – в копченой рыбе. Таким образом, рыба холодного копчения из исследованных видов является продуктом питания, ценным по содержанию ДЦ-ПНЖК. Процесс копчения не оказывал существенного влияния на общий профиль ЖК исследованных видов рыб, отражающий их рацион. Это означает, что профили жирных кислот в рыбе холодного копчения можно использовать для верификации сведений поставщиков о видовой принадлежности рыбы и районе вылова.

Ключевые слова: ряпушка, арктический омуль, муксун, чир, эйкозапентаеновая кислота, докозагексаеновая кислота, профили жирных кислот, холодное копчение.

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Introduction

Consumers' interest in healthy food is steadily growing. Among essential components of healthy food are long-chain polyunsaturated fatty acids of the omega-3 family (LC-PUFAs), namely, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). Indeed, EPA plays a major role as a precursor of signaling molecules (lipid mediators), prostaglandins, thromboxanes and leukotrienes, which regulate inflammatory and allergic reactions and control the cardiovascular system, while DHA regulates the synthesis of mediators and is the main fatty acid of cell membrane phospholipids in the brain and other neural tissues (SanGiovanni, Chew, 2005; McNamara, Carlson, 2006; Adkins, Kelley, 2010; Wall et al., 2010; Norris, Dennis, 2012; Calder, 2018). In general, LC-PUFAs play an important role in primary prevention of cardiovascular diseases, neural disorders and other diseases, and their required daily consumption in a healthy individual is ca. 0.5–1.0 g (Harris et al., 2009; Kris-Etherton et al., 2009; Casula et al., 2013; Nagasaka et al., 2014; Calder, 2018; Tocher et al., 2019; Jia et al., 2021).

Humans obtain EPA and DHA primarily from fish (Robert, 2006; Adkins, Kelley, 2010; Gladyshev et al., 2013, 2015; Tacon, Metian, 2013; Tocher et al., 2019). To meet their required consumption, the lower threshold content of EPA+DHA in an average serving of fish of ~200 g must be 2.5 mg · g^{-1} (Gladyshev et al., 2018a). However, the content of EPA and DHA in the biomass of many fish species is far below the threshold value of 2.5 mg · g^{-1} (Gladyshev et al., 2018b), therefore it is hardly possible to obtain the recommended daily dose of EPA+DHA by eating such fish (Kwetegyeka et al., 2008). Thus, it stands to reason to inform consumers about the quantity of LC-PUFAs in a portion of a given fish species.

In addition to the variation in EPA and DHA content between fish species, which is caused by their genetics and ecology (Gladyshev et al., 2018b), the nutritive value of fish dishes may vary because of the method of culinary treatment (Ohshima et al., 1996; Tarley et al., 2004; Mnari Bhouri et al., 2010; Zotos et al., 2013; Sampels et al., 2014; Chaula et al., 2019; Van Pamel et al., 2021). Indeed, LC-PUFA content varied ca. 40-fold in fish dishes, from 40.1 mg · g^{-1} in fried Atlantic salmon (*Salmo salar*) (Ansorena et al., 2010) to 1.0 mg · g^{-1} in stewed zander (*Sander lucioperca*) (Gladyshev et al., 2014). Consequently, information on the effects of culinary treatments on EPA and DHA content in fish species is important for producers and consumers.

Among culinary treatments, cold smoking is popular in some regions for a number of fish species. For instance, in Siberia (Russia), smoked whitefish species of the genus *Coregonus* were found to be valuable sources of EPA and DHA (Gladyshev et al., 2020). However, it was unclear whether smoking diminished the LC-PUFA content in the processed fish products compared to raw fish. Moreover, there are no data in scientific literature concerning the effects of smoking on EPA and DHA content (mg · g^{-1} of product) in fish.

In addition to the information on nutritive value, i.e., LC-PUFA content, studies of fatty acid (FA) profiles of processed fish products provide an opportunity to verify label information on the origin of fish (e.g., wild or cultivated), which considerably influences consumer choice (Fasolato et al., 2010). Thus, the aims of this study were 1) to determine whether smoking causes a decrease in EPA and DHA content in fish and 2) to evaluate whether smoking deforms FA profiles in fish and thereby does not allow detection of mislabeling via FA markers.

Materials and methods

Fish samples

Four whitefish species from the Yenisei River, Arctic cisco (*Coregonus autumnalis*), least cisco (*Coregonus sardinella*), muksun (*Coregonus*

muksun) and broad whitefish (*Coregonus nasus*), were studied. The ecological traits and feeding habits of these species are described elsewhere (Gladyshev et al., 2017; Sushchik et al., 2020). Briefly, Arctic cisco is a semi-anadromous fish that feeds mainly on zooplankton in the pelagial zone of the Yenisei Gulf of the Kara Sea, as well as in the Yenisei River. Least cisco is also a planktivorous pelagic feeder. Muksun and broad whitefish are benthivorous species that primarily consume bottom invertebrates.

Raw and cold smoked fish were obtained from a reputable local fish-processing factory. Muscle tissues without skin (fillets) below the dorsal fin were collected for subsequent analyses.

Analysis of moisture content

To measure the moisture content, approximately 10–15 g of wet weight from the fillets of each specimen was taken and dried to constant weight at 105 °C.

Analysis of fatty acids

The fatty acid analysis has been described in detail elsewhere (Gladyshev et al., 2020). In brief, the method included the following steps. Lipids were extracted from mechanically homogenized muscle tissues with a chloroform/methanol mixture (2:1, v/v) three times. The dried lipids were then hydrolyzed under reflux at 90 °C in methanolic sodium hydroxide solution (8 mg · mL^{-1}). The resulting mixture was added to an excess solution of 3 % sulfuric acid in methanol and refluxed at 90 °C for 10 min to obtain fatty acid methyl esters (FAMEs). Finally, the mixture was washed with several portions of NaCl saturated solution, and FAMEs were extracted with a portion of hexane. The chromatographic analyses of FAMEs were performed with a gas chromatograph equipped with a mass spectrometer detector (model 7000 QQQ, Agilent Technologies, USA) and a 30-m long, 0.25-mm internal diameter capillary HP-FFAP column. The instrumental conditions were the same as previously described (Gladyshev et al., 2020). Data were collected and analyzed using MassHunter Software (Agilent Technologies, USA). Peaks of FAMEs were identified by their mass spectra, which were compared to those in the integrated NIST 2008 MS LIB database (Revision Jan 2010) and to those in the standard 37-FAME mixture (U-47885, Supelco, USA). FAMEs were quantified according to the peak area of the internal standard, 19:0-FAME (Sigma–Aldrich, USA), which was added to the samples prior to lipid extraction after the addition of the first portion of the chloroform/methanol mixture.

Statistical analysis

Kolmogorov–Smirnov one-sample tests for normality, Student's *t* tests, ANOVAs and multivariate correspondence analyses were performed using STATISTICA software, version 9.0 (StatSoft, Inc., Tulsa, OK, USA). To improve assumptions of normality and homogeneity of variance, the arcsine–square root transformation of percentage data was performed for ANOVA (Childs et al., 2021), as in the similar studies of FA composition (Kainz et al., 2009; Torres-Ruiz, Wehr, 2020). Before the transformation, percentages were expressed as proportions, i.e., divided by 100 (Childs et al., 2021).

Results

Moisture content in raw fish was similar in the examined species, ranging from 75.8 ± 1.1 % in the Arctic cisco to 82.9 ± 2.3 % in the broad whitefish (Fig. 1). Moisture content in smoked fish was also similar, ranging from 65.8 ± 0.4 % in the least cisco to 75.3 ± 0.6 % in the muksun (Fig. 1). In all species, a significant decrease in moisture content of ca. 5–10 % occurred as a result of smoking (Fig. 1).

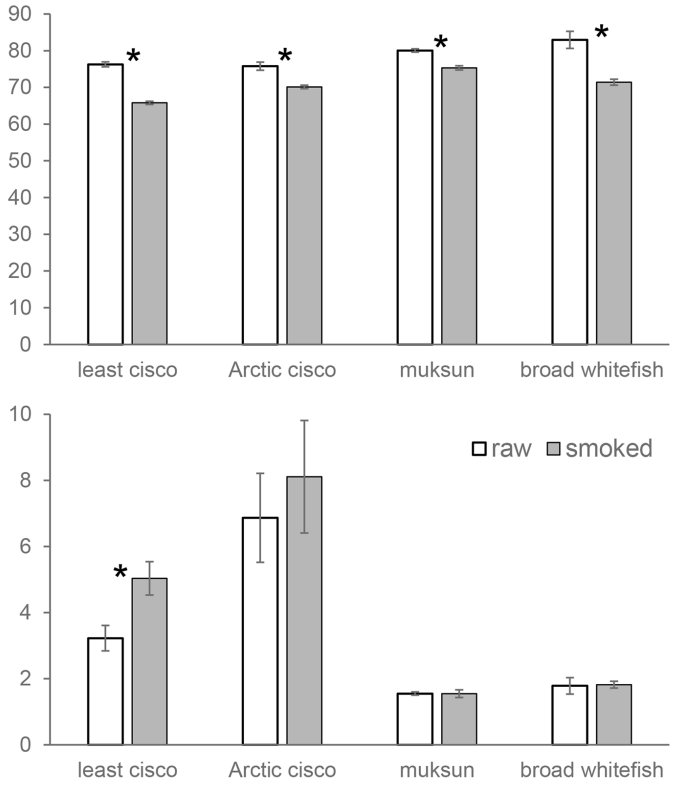

Fig. 1. Mean moisture (%) and mean content (mg g^{-1} wet weight) of the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in raw and smoked fish. Bars represent standard error. Means labeled with asterisks are significantly different at $P< 0.05$ according to Student's *t* test

In raw fish, the content of EPA+DHA varied from 1.55 ± 0.05 mg · g^{-1} of wet weight in muksun to 6.86 ± 1.35 mg · g^{-1} in Arctic cisco (Fig. 1). In smoked fish, the content of EPA+DHA varied from 1.54 ± 0.12 mg · g^{-1} in muksun to 8.11 ± 0.12 mg · g^{-1} in Arctic cisco (Fig. 1). There was no significant decrease in the EPA+DHA content after smoking (Fig. 1). Moreover, in least cisco, there was a statistically significant increase in the content of EPA+DHA, from 3.22 ± 0.39 mg · g^{-1} in the raw fish to 5.04 ± 0.50 mg · g^{-1} in the smoked fish (t = 2.85, p = 0.015, degree of freedom = 12) (Fig. 1). In Arctic cisco, the EPA+DHA content also tended to increase but not significantly in the process of smoking (Fig. 1).

The pelagic planktivorous species, least cisco and Arctic cisco, had significantly higher percentages of 14:0 and tended to have the highest percentages of 18:4n-3 but had significantly lower percentages of 18:0 and 20:4n-6 than the benthivorous species, muksun and broad whitefish; smoking did not significantly affect these patterns (Table 1).

Least cisco had significantly higher levels of 20:3n-3, 20:4n-3 and 22:4n-3 and tended to have a higher level of 20:2n-6, and these differences were not affected significantly by smoking (Table 1). Raw least cisco also had significantly higher levels of Σ24PUFAs, which increased significantly after smoking (Table 1).

Arctic cisco had significantly higher levels of 20:1n-9 and tended to have the highest levels of 16:1n-7, 16:4n-1 and Σ22:1 but tended to have the lowest percentage of 16:0; this tendency was not diminished by smoking (Table 1).

Table 1. Fatty acid composition (mean ± standard error, % of total fatty acids) of raw and cold smoked least cisco (*Coregonus sardinella*), Arctic cisco (*Coregonus autumnalis*), muksun (*Coregonus muksun*) and broad whitefish (*Coregonus nasus*). Means labelled with the same letter are not significantly different after Tukey *post hoc* test for ANOVA, carried out for log-transformed data; *n* – number of samples

Fatty acid	least cisco		Arctic cisco		muksun		broad whitefish	
	raw (*n*=7)	smoked (*n*=7)	raw (*n*=7)	smoked (*n*=6)	raw (*n*=5)	smoked (*n*=6)	raw (*n*=6)	smoked (*n*=6)
14:0	4.0 ± 0.2^{A}	4.7 ± 0.2^{A}	3.7 ± 0.3^{A}	4.0 ± 0.4^{A}	1.1 ± 0.2^{B}	1.6 ± 0.2^{BC}	2.3 ± 0.2^{C}	2.4 ± 0.2^{C}
i15:0	0.2 ± 0.0^{AB}	0.3 ± 0.0^{BD}	0.1 ± 0.0^{AC}	0.1 ± 0.0^{AC}	0.0 ± 0.0^{C}	0.1 ± 0.0^{C}	0.5 ± 0.2^{D}	0.3 ± 0.0^{BD}
15:0	0.4 ± 0.0^{A}	0.5 ± 0.0^{AC}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	0.5 ± 0.1^{AC}	0.5 ± 0.0^{AC}	0.6 ± 0.1^{AC}	0.6 ± 0.1^{C}
i16:0	0.1 ± 0.0^{AB}	0.1 ± 0.0^{B}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{B}	0.4 ± 0.1^{C}	0.6 ± 0.1^{C}
16:0	18.6 ± 0.6^{AB}	18.4 ± 0.4^{AB}	16.1 ± 0.2^{A}	16.1 ± 0.6^{A}	19.9 ± 3.6^{B}	18.3 ± 0.5^{AB}	18.8 ± 0.7^{AB}	19.3 ± 1.0^{B}
16:1n-9	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}	0.2 ± 0.0^{A}	0.3 ± 0.1^{AB}	0.3 ± 0.0^{BC}	0.4 ± 0.0^{C}	0.3 ± 0.0^{BC}
16:1n-7	9.3 ± 0.7^{A}	11.3 ± 0.6^{AB}	16.9 ± 2.3^{BC}	21.0 ± 1.8^{C}	6.5 ± 1.1^{A}	9.4 ± 0.7^{A}	10.4 ± 1.2^{A}	9.3 ± 0.3^{A}
i17:0	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}	0.1 ± 0.0^{A}	0.2 ± 0.0^{A}	0.3 ± 0.0^{A}	1.4 ± 0.3^{B}	2.1 ± 0.3^{C}
a17:0	0.1 ± 0.0^{AB}	0.1 ± 0.0^{AB}	0.0 ± 0.0^{AB}	0.0 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.2 ± 0.0^{AB}	0.9 ± 0.2^{C}	1.2 ± 0.2^{C}
16:2n-4	0.3 ± 0.0^{AC}	0.3 ± 0.0^{ABC}	0.4 ± 0.0^{AB}	0.6 ± 0.1^{B}	0.1 ± 0.0^{C}	0.2 ± 0.0^{AC}	0.4 ± 0.2^{AB}	0.3 ± 0.0^{ABC}
17:0	0.2 ± 0.0^{A}	0.1 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{B}	0.3 ± 0.1^{C}	0.3 ± 0.0^{C}	0.6 ± 0.1^{D}	0.7 ± 0.1^{D}
16:3n-4	0.3 ± 0.0^{AC}	0.3 ± 0.1^{AC}	0.3 ± 0.0^{AC}	0.4 ± 0.1^{A}	0.1 ± 0.0^{BC}	0.1 ± 0.0^{C}	0.3 ± 0.2^{AC}	0.2 ± 0.0^{AC}
17:1n-8	0.1 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{AB}	0.2 ± 0.0^{BC}	0.2 ± 0.0^{CD}	0.4 ± 0.1^{DE}	0.4 ± 0.0^{E}
16:4n-1	0.1 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.3 ± 0.1^{BC}	0.4 ± 0.1^{C}	0.0 ± 0.0^{A}	0.1 ± 0.0^{A}	0.0 ± 0.0^{A}	0.0 ± 0.0^{A}
18:0	3.5 ± 0.2^{A}	2.9 ± 0.1^{AB}	3.0 ± 0.2^{AB}	2.4 ± 0.2^{B}	5.6 ± 1.0^{C}	4.6 ± 0.1^{C}	5.0 ± 0.3^{C}	5.0 ± 0.1^{C}
18:1n-9	12.6 ± 0.6^{AD}	12.6 ± 0.7^{AD}	14.6 ± 0.9^{ACD}	13.4 ± 1.5^{AD}	6.6 ± 1.1^{B}	10.5 ± 0.7^{D}	18.5 ± 1.6^{C}	17.0 ± 1.0^{AC}
18:1n-7	5.0 ± 0.2^{A}	6.3 ± 0.4^{AB}	6.2 ± 0.3^{AB}	6.7 ± 0.5^{AB}	5.4 ± 1.1^{AB}	6.8 ± 0.3^{B}	5.2 ± 0.5^{AB}	5.0 ± 0.2^{AB}
18:1n-5	0.3 ± 0.0^{ACD}	0.4 ± 0.0^{AD}	0.4 ± 0.1^{A}	0.4 ± 0.0^{ACD}	0.1 ± 0.0^{B}	0.2 ± 0.0^{BC}	0.3 ± 0.1^{ABCD}	0.2 ± 0.0^{BD}
18:2n-6	1.6 ± 0.1^{A}	1.4 ± 0.1^{A}	0.7 ± 0.1^{B}	0.8 ± 0.1^{B}	0.5 ± 0.1^{B}	0.7 ± 0.1^{B}	2.7 ± 0.4^{C}	2.4 ± 0.2^{C}
18:3n-3	1.0 ± 0.1^{A}	1.0 ± 0.1^{A}	0.4 ± 0.1^{B}	0.3 ± 0.0^{B}	0.3 ± 0.0^{B}	0.5 ± 0.1^{B}	2.4 ± 0.4^{C}	2.2 ± 0.3^{C}
18:4n-3	1.4 ± 0.2^{AB}	1.9 ± 0.1^{A}	1.1 ± 0.1^{BD}	1.3 ± 0.1^{AB}	0.3 ± 0.1^{C}	0.6 ± 0.1^{DC}	0.7 ± 0.1^{DC}	0.7 ± 0.1^{DC}
20:1n-9	1.2 ± 0.1^{A}	0.9 ± 0.1^{A}	4.4 ± 0.8^{B}	4.1 ± 0.8^{B}	0.5 ± 0.1^{A}	1.0 ± 0.2^{A}	1.1 ± 0.2^{A}	1.3 ± 0.1^{A}
20:1n-7	1.5 ± 0.1^{A}	2.1 ± 0.2^{AC}	0.7 ± 0.2^{B}	0.6 ± 0.1^{B}	2.0 ± 0.4^{AC}	2.9 ± 0.4^{C}	0.3 ± 0.0^{B}	0.4 ± 0.0^{B}
20:2n-6	0.5 ± 0.0^{AC}	0.6 ± 0.0^{A}	0.1 ± 0.0^{B}	0.1 ± 0.0^{B}	0.1 ± 0.0^{BD}	0.2 ± 0.0^{BD}	0.4 ± 0.1^{AD}	0.3 ± 0.1^{CD}

Table 1 Continued

Fatty acid	least cisco		Arctic cisco		muksun		broad whitefish	
	raw (*n*=7)	smoked (*n*=7)	raw (*n*=7)	smoked (*n*=6)	raw (*n*=5)	smoked (*n*=6)	raw (*n*=6)	smoked (*n*=6)
20:4n-6	1.1 ± 0.1^{A}	0.7 ± 0.0^{A}	0.6 ± 0.1^{A}	0.5 ± 0.1^{A}	3.1 ± 0.6^{B}	2.3 ± 0.2^{B}	3.0 ± 0.5^{B}	3.3 ± 0.2^{B}
20:3n-3	0.4 ± 0.0^{A}	0.6 ± 0.0^{A}	0.1 ± 0.0^{B}	0.0 ± 0.0^{B}	0.1 ± 0.0^{B}	0.1 ± 0.0^{B}	0.2 ± 0.0^{B}	0.1 ± 0.0^{B}
20:4n-3	1.5 ± 0.1^{A}	1.8 ± 0.1^{A}	0.6 ± 0.1^{B}	0.6 ± 0.1^{B}	0.3 ± 0.0^{C}	0.5 ± 0.1^{BC}	0.3 ± 0.0^{BC}	0.4 ± 0.0^{BC}
20:5n-3	7.5 ± 0.3^{AC}	6.6 ± 0.3^{AE}	9.8 ± 0.3^{B}	8.9 ± 0.3^{BC}	13.8 ± 2.5^{D}	10.5 ± 0.4^{B}	5.3 ± 1.0^{E}	4.8 ± 0.4^{E}
Σ22:1	0.4 ± 0.0^{AE}	0.5 ± 0.0^{AB}	1.7 ± 0.4^{BC}	1.8 ± 0.6^{C}	0.0 ± 0.1^{D}	0.1 ± 0.1^{AD}	0.1 ± 0.0^{DE}	0.1 ± 0.0^{AD}
22:5n-6	0.3 ± 0.0^{A}	0.1 ± 0.0^{AB}	0.1 ± 0.0^{B}	0.0 ± 0.0^{B}	0.3 ± 0.1^{A}	0.2 ± 0.0^{A}	0.8 ± 0.2^{C}	0.9 ± 0.1^{C}
22:4n-3	0.9 ± 0.1^{A}	1.3 ± 0.1^{A}	0.1 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}	0.0 ± 0.0^{B}
22:5n-3	2.7 ± 0.0^{A}	2.4 ± 0.1^{AB}	1.9 ± 0.2^{BC}	1.6 ± 0.1^{C}	2.7 ± 0.5^{A}	2.4 ± 0.2^{AB}	1.9 ± 0.2^{BC}	2.3 ± 0.2^{AB}
22:6n-3	18.3 ± 1.5^{AC}	12.6 ± 0.7^{AB}	12.2 ± 1.6^{AB}	10.1 ± 2.1^{B}	26.4 ± 4.6^{C}	20.6 ± 1.9^{C}	9.1 ± 1.6^{B}	10.1 ± 1.0^{B}
Σ24PUFA	1.9 ± 0.3^{A}	3.3 ± 0.3^{B}	0.6 ± 0.1^{C}	0.5 ± 0.1^{C}	0.2 ± 0.1^{C}	0.3 ± 0.1^{C}	0.2 ± 0.1^{C}	0.4 ± 0.1^{C}

Muksun had a significantly higher level of 20:5n-3, which decreased significantly after smoking (Table 1). It also tended to have the highest level of 22:6n-3 but the lowest levels of 16:2n-4 and 16:3n-4, and these distinctions were not significantly diminished by smoking (Table 1). In addition, raw muksun had the lowest level of 18:1n-9, which increased significantly after smoking (Table 1).

Broad whitefish had the highest levels of i16:0, i17:0, a17:0, 17:0, 17:1n-8 and 22:5n-6 and tended to have the highest percentages of i15:0 and 15:0, and this tendency was not significantly diminished by smoking (Table 1). In addition, broad whitefish had the significantly highest levels of 18:2n-6 and 18:3n-3 and tended to have the highest percentage of 16:1n-9, and this tendency was not significantly diminished by smoking (Table 1). In contrast, broad whitefish tended to have the lowest level of 20:5n-3, which did not significantly decrease after smoking (Table 1).

According to the correspondence analysis (CA) of FA profiles, samples of raw and smoked specimens of the same species were grouped into distinct clusters separated from those of other species (Fig. 2).

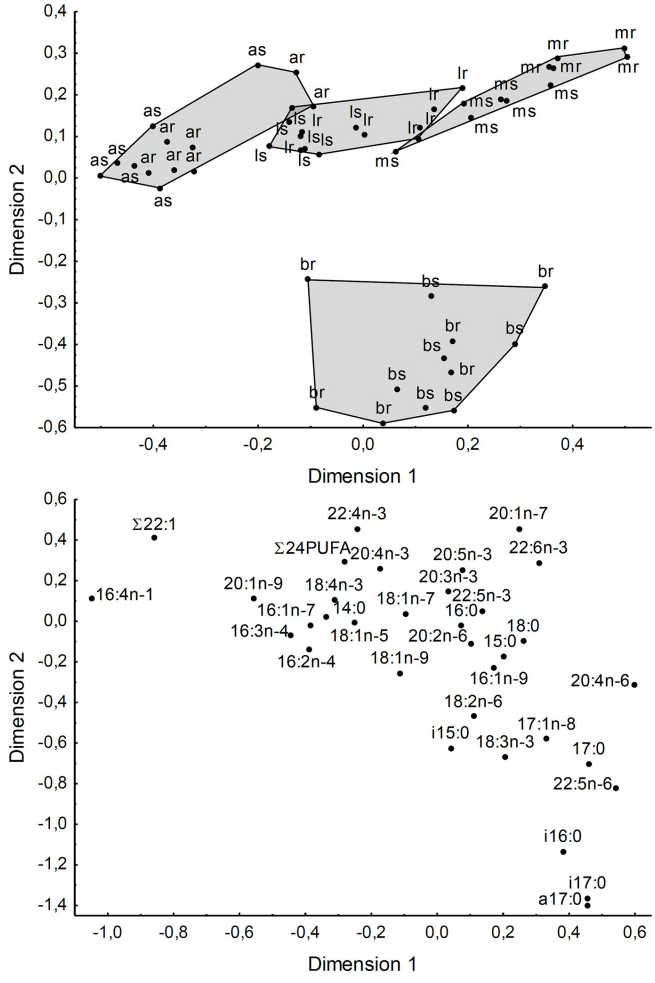

Fig. 2. Canonical correspondence analyses of fatty acid (FA) composition (% of the total) in fish: lr and ls, ar and as, mr and ms, br and bs – raw and smoked least cisco *Coregonus sardinella*, Arctic cisco *C. autumnalis*, muksun *C. muksun* and the broad whitefish *C. nasus*, respectively. Dimension 1 and Dimension 2 explain 31.3 % and 30.1 % of the variance, respectively

The contributions of the two factors, species identity and smoking, to the variations in FA composition were evaluated using two-way ANOVA. Species identity made the most significant contribution to the variability in the percentage levels of all FAs, except for 18:1n-7, for which the treatment contributed more strongly than the species identity (Table 2).

Table 2. Results of two-way ANOVA comparing contribution of two factors, species identity (Species, degree of freedom d.f. = 3) and treatment (raw or cold smoked, d.f. = 1) and their interaction (Species × Treatment, d.f. = 3) on the levels of fatty acids (% of total FAs) of least cisco (*Coregonus sardinella*), Arctic cisco (*Coregonus autumnalis*), muksun (*Coregonus muksun*) and broad whitefish (*Coregonus nasus*). *MS* – mean square effect for independent variables, *F* – Fisher's test. Significant values with $P < 0.05$ are given in bold. FAs with insignificant ANOVA are not shown

Fatty acid	Species		Treatment		Species × Treatment	
	MS	F	MS	F	MS	F
14:0	0.0230	71.9	0.0018	**5.6**	0.0002	0.7
i15:0	**0.0033**	**40.8**	0.0000	0.3	0.0002	2.1
15:0	**0.0014**	**31.9**	0.0001	1.8	0.0000	1.1
i16:0	0.0066	88.5	**0.0004**	**5.6**	0.0001	1.4
16:0	**0.0042**	**10.5**	0.0002	0.5	0.0003	0.9
16:1n-9	**0.0010**	**26.3**	0.0000	0.8	**0.0001**	**3.3**
16:1n-7	0.0621	**29.3**	**0.0128**	**6.0**	0.0034	1.6
i17:0	**0.0290**	**120.1**	0.0009	3.9	**0.0007**	**2.9**
a17:0	**0.0167**	**98.8**	0.0006	3.3	0.0003	2.0
16:2n-4	**0.0018**	**11.8**	0.0003	2.1	0.0002	1.5
17:1n-8	**0.0030**	**48.6**	0.0000	0.9	0.0000	0.4
16:4n-1	**0.0047**	**27.4**	**0.0013**	**7.6**	0.0000	0.1
18:0	**0.0123**	**78.6**	**0.0021**	**13.1**	0.0003	1.9
18:1n-9	**0.0377**	**29.4**	0.0007	0.5	**0.0051**	**4.0**
18:1n-7	**0.0019**	**4.2**	**0.0033**	**7.3**	0.0009	2.0
18:1n-5	**0.0011**	**14.5**	0.0000	0.1	0.0001	1.4
18:2n-6	**0.0171**	**74.3**	0.0000	0.0	0.0004	1.7
18:3n-3	**0.0228**	**93.6**	0.0000	0.2	0.0003	1.4
18:4n-3	**0.0093**	**34.6**	**0.0025**	**9.2**	0.0004	1.5
20:1n-9	**0.0361**	**37.2**	0.0003	0.3	0.0010	1.1
20:1n-7	0.0255	69.7	0.0021	**5.8**	0.0005	1.5
20:2n-6	**0.0045**	**36.6**	0.0001	0.6	0.0001	0.9
20:4n-6	**0.0333**	**78.2**	0.0012	2.9	0.0008	1.9
20:3n-3	0.0056	48.9	0.0000	0.1	0.0001	0.8
20:4n-3	**0.0136**	**87.4**	**0.0008**	**5.0**	0.0001	1.0
20:5n-3	**0.0369**	**67.4**	0.0067	12.2	0.0010	1.9
Σ22:1	0.0323	**34.3**	0.0017	1.8	0.0001	0.1
22:5n-6	**0.0010**	**65.5**	0.0001	0.8	0.0004	2.8
22:4n-3	**0.0343**	**212.1**	0.0002	1.1	0.0003	1.8
22:5n-3	**0.0025**	**13.9**	0.0002	1.1	**0.0005**	**2.8**
22:6n-3	**0.0850**	**28.1**	**0.0206**	**6.8**	0.0061	2.0
Σ24PUFA	0.0364	86.0	0.0026	6.2	0.0018	4.2

Discussion

Cold smoking did not decrease the EPA+DHA content in the filets of the four studied species of the genus *Coregonus* from the Yenisei River. In a number of studies, no decrease in the levels of these LC-PUFAs in fish during culinary treatments was detected (Candela et al., 1998; Sioen et al., 2006; Gladyshev et al., 2006, 2007, 2014; Amira et al., 2010; Ansorena et al., 2010; Leung et al., 2018; de Brito et al., 2019). Moreover, there was a significant increase in the LC-PUFA content in smoked filets of least cisco (*C. sardinella*) compared to raw fish. This increase was likely caused by the moisture loss of approximately 10 %. Indeed, increases in EPA and DHA content in processed fish products compared to those in raw fish were reported in other studies, and moisture loss was identified as a possible mechanism for these changes (Sioen et al., 2006; Ansorena et al., 2010; Gladyshev et al., 2014). However, in the other studied fish species, Arctic cisco, muksun and broad whitefish, there was no significant increase in EPA or DHA content despite moisture loss.

The following may explain the above patterns in the EPA and DHA content change resulting from smoking. Evidently, during culinary treatment, namely, heating, LC-PUFAs undergo oxidative effects, which result in the degradation and loss of these polyunsaturated acids. For instance, a significant increase in reactive aldehydes derived from n-3 PUFAs was found in panfried and boiled salmon (Leung et al., 2018). On the other hand, cooking induces water loss in food, which leads to an increase in lipid content, including that of LC-PUFAs. A balance between these antagonist processes, oxidation and moisture loss, results in either increase or decrease, or zero change in the EPA and DHA content in cooked fish. Most likely, the balance of oxidation and moisture loss is species-specific, as found in our present study.

It is worth noting that the nutritive value of processed fish must be estimated based on units of mass, i.e., mg of EPA+DHA per g of product, rather than in relative units, % of the LC-PUFAs of total FAs (reviewed by Gladyshev, Sushchik, 2019 and references therein). Accordingly, the changes in the EPA+DHA content of diverse fish species under certain culinary treatments must be also measured in units of mass. Here, we regard the 'nutritive value' of fish as the content of two essential fatty acids, EPA and DHA. Fish is also a valuable nutritive source of proteins (amino acids), vitamins and micronutrients for humans. However, fish constitute only ~6 % of the protein consumed by humans (Tacon, Metian, 2013), while fish EPA+DHA supply in the human diet is more than 97 % (Gladyshev et al., 2015). Thus, the essential nutritive value of fish for humans consists in EPA and DHA content in their biomass.

In our previous study of cold smoked fish of the genus *Coregonus* from the Yenisei River, their high nutritive value regarding EPA and DHA content was revealed (Gladyshev et al., 2020). In the present study, the high nutritive value of the four species was confirmed. The EPA+DHA content in the Arctic cisco (*C. autumnalis*) and least cisco (*C. sardinella*) exceeded the threshold value of 2.5 mg · g^{-1}. The two other species, the broad whitefish (*C. nasus*) and muksun (*C. muksun*), had the EPA+DHA content slightly below the threshold value but close to it. To obtain the recommended daily dose of EPA+DHA of 0.5 g, one needs to consume ~320 g of smoked muksun and ~270 g of smoked broad whitefish. These daily portions exceed the common average portion of fish per serving (~200 g) but still seem to be reasonable.

The second aim of the present study was to compare the FA profiles of raw and smoked fish. Although the levels of some FAs were changed by smoking, the overall FA profiles were not affected

considerably; i.e., they were close to the profiles of raw fish and remained species- and site-specific. For instance, the pelagic planktivorous species, least cisco and Arctic cisco, tended to have higher levels of markers of planktonic cryptophyte algae (18:4n-3) (Desvilettes et al., 1997) than the benthivorous species, muksun and broad whitefish, and smoking did not significantly affect these patterns. In turn, the two benthivorous species had significantly higher levels of the terrestrial organic matter marker 20:4n-6 (Feniova et al., 2015) than the two planktivorous species, and this distinction did not diminish significantly after smoking. Least cisco had significantly higher levels of 20:3n-3, 20:4n-3 and 22:4n-3 that might be intermediate compounds indicative of the conversion of C 18 to C 20–22 PUFA by this species (Tocher et al., 2019; Sushchik et al., 2020), and these differences were not affected significantly by smoking (Table 1). Arctic cisco tended to have the highest levels of markers of diatom algae, 16:1n-7 and 16:4n-1 (Dijkman, Kromkamp, 2006), and this tendency was not diminished by smoking. In addition, semi-anadromous Arctic cisco had significantly higher levels of the marker of marine copepods (20:1n-9) and tended to have higher levels of another marker of the copepods, Σ22:1. It was not significantly diminished by smoking and indicated its feeding on marine zooplankton, i.e., migration to the estuary of the Yenisei River, which is characteristic of this species (Gladyshev et al., 2017). Both smoked and raw broad whitefish had distinctly high levels of bacterial FAs, a17:0, i17:0 and i16:0 (Fig. 2). It indicated their feeding on bottom sediments that is characteristic of these species (Gladyshev et al., 2017).

This means that the FA profiles of smoked fish can confirm a species' identity and catchment area. Thus, they could be used for verifying the label information provided by vendors.

Conclusion

Smoking did not decrease the EPA+DHA content (mg · g^{-1} of product) in the four *Coregonus* species compared to that in raw fish. Moreover, in some cases, their content increased, probably due to moisture loss. Smoking did not considerably change the FA profiles of fish. Therefore, the FA profiles of smoked fish could be used for verifying label information on species identity and catchment area.

References

Adkins Y., Kelley D. S. (2010) Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. *Journal of Nutritional Biochemistry*, 21(9): 781–792

Amira M. B., Hanene J. H., Madiha D., Imen B., Mohamed H., Abdelhamid C. (2010) Effects of frying on the fatty acid composition in farmed and wild gilthead sea bream (*Sparus aurata*). *International Journal of Food Science & Technology*, 45(1): 113–123

Ansorena D., Guembe A., Mendizabal T., Astiasaran I. (2010) Effect of fish and oil nature on frying process and nutritional product quality. *Journal of Food Science*, 75(2): H62–H67

Calder P. C. (2018) Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *Proceedings of the Nutrition Society*, 77(1): 52–72

Candela M., Astiasaran I., Bello J. (1998) Deep-fat frying modifies high-fat fish lipid fraction. *Journal of Agricultural and Food Chemistry*, 46(7): 2793–2796

Casula M., Soranna D., Catapano A. L., Corrao G. (2013) Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, double blind, placebo controlled trials. *Atherosclerosis Supplements*, 14(2): 243–251

Chaula D., Laswai H., Chove B., Dalsgaard A., Mdegela R., Hyldig G. (2019) Fatty acid profiles and lipid oxidation status of sun dried, deep fried, and smoked sardine (*Rastrineobola argentea*) from Lake Victoria, Tanzania. *Journal of Aquatic Food Product Technology*, 28(2): 165–176

Childs D.Z., Hindle B.J., Warren P.H. (2021) *APS 240: Data analysis and statistics with R*. Downloaded from https://dzchilds.github.io/stats-for-bio/

de Brito B.M., Lira G.M., Pinheiro A.G. A., Santana C.M. A. S., Amaral I.L. (2019) Effect of cooking with interesterified margarine in the chemical composition of fish. *Food Science and Technology*, 39(suppl. 2): 640–645

Desvilettes C., Bourdier G., Amblard C., Barth B. (1997) Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. *Freshwater Biology*, 38(3): 629–637

Dijkman N.A., Kromkamp J.C. (2006) Phospholipid-derived fatty acids as chemotaxonomic markers for phytoplankton: Application for inferring phytoplankton composition. *Marine Ecology Progress Series*, 324: 113–125

Fasolato L., Novelli E., Salmaso L., Corain L., Camin F., Perini M., Antonetti P., Balzan S. (2010) Application of nonparametric multivariate analyses to the authentication of wild and farmed European sea bass (*Dicentrarchus labrax*). Results of a survey on fish sampled in the retail trade. *Journal of Agricultural and Food Chemistry*, 58(20): 10979–10988

Feniova I., Dawidowicz P., Gladyshev M.I., Kostrzewska-Szlakowska I., Rzepecki M., Razlutskij V., Sushchik N.N., Majsak N., Dzialowski A.R. (2015) Experimental effects of large bodied *Daphnia*, fish and zebra mussels on cladoceran community and size structure. *Journal of Plankton Research*, 37(3): 611–625

Gladyshev M.I., Anishchenko O.V., Makhutova O.N., Kolmakova O.V., Trusova M.Y., Morgun V.N., Gribovskaya I.V., Sushchik N.N. (2020) The benefit-risk analysis of omega-3 polyunsaturated fatty acids and heavy metals in seven smoked fish species from Siberia. *Journal of Food Composition and Analysis*, 90: 103489

Gladyshev M.I., Glushchenko L.A., Makhutova O.N., Rudchenko A.E., Shulepina S.P., Dubovskaya O.P., Zuev I.V., Kolmakov V.I., Sushchik N.N. (2018a) Comparative analysis of content of omega-3 polyunsaturated fatty acids in food and muscle tissue of fish from aquaculture and natural habitats. *Contemporary Problems of Ecology*, 11(3): 297–308

Gladyshev M.I., Makhutova O.N., Gubanenko G.A., Rechkina E.A., Kalachova G.S., Sushchik N.N. (2015) Livers of terrestrial production animals as a source of long-chain polyunsaturated fatty acids for humans: an alternative to fish? *European Journal of Lipid Science and Technology*, 117(9): 1417–1421

Gladyshev M.I., Sushchik N.N. (2019) Long-chain omega-3 polyunsaturated fatty acids in natural ecosystems and the human diet: assumptions and challenges. *Biomolecules*, 9(9): 485

Gladyshev M.I., Sushchik N.N., Gubanenko G.A., Demirchieva S.M., Kalachova G.S. (2006) Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*). *Food Chemistry*, 96(3): 446–451

Gladyshev M.I., Sushchik N.N., Gubanenko G.A., Demirchieva S.M., Kalachova G.S. (2007) Effect of boiling and frying on the content of essential polyunsaturated fatty acids in muscle tissue of four fish species. *Food Chemistry*, 101(4): 1694–1700

Gladyshev M. I., Sushchik N. N., Gubanenko G. A., Makhutova O. N., Kalachova G. S., Rechkina E. A., Malyshevskaya K. K. (2014) Effect of the way of cooking on contents of essential polyunsaturated fatty acids in filets of zander. *Czech Journal of Food Sciences*, 32(3): 226–231

Gladyshev M. I., Sushchik N. N., Makhutova O. N. (2013) Production of EPA and DHA in aquatic ecosystems and their transfer to the land. *Prostaglandins and Other Lipid Mediators*, 107: 117–126

Gladyshev M. I., Sushchik N. N., Makhutova O. N., Glushchenko L. A., Rudchenko A. E., Makhrov A. A., Borovikova E. A., Dgebuadze Y. Y. (2017) Fatty acid composition and contents of seven commercial fish species of genus *Coregonus* from Russian Subarctic water bodies. *Lipids*, 52(12): 1033–1044

Gladyshev M. I., Sushchik N. N., Tolomeev A. P., Dgebuadze Y. Y. (2018b) Meta-analysis of factors associated with omega-3 fatty acid contents of wild fish. *Reviews in Fish Biology and Fisheries*, 28(2): 277–299

Harris W. S., Mozaffarian D., Lefevre M., Toner C. D., Colombo J., Cunnane S. C., Holden J. M., Klurfeld D. M., Morris M. C., Whelan J. (2009) Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids. *Journal of Nutrition*, 139(4): 804S-819S

Jia G., Qiong Z., Yong-Hua W. (2021) Health effects of omega-3 polyunsaturated fatty acids in common diseases. *International Food Research Journal*, 28(6): 1098–1108

Kainz M. J., Perga M.-E., Arts M. T., Mazumder A. (2009) Essential fatty acid concentrations of different seston sizes and zooplankton: a field study of monomictic coastal lakes. *Journal of Plankton Research*, 31(6): 635–645

Kris-Etherton P. M., Grieger J. A., Etherton T. D. (2009) Dietary reference intakes for DHA and EPA. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 81(2–3): 99–104

Kwetegyeka J., Mpango G., Grahl-Nielsen O. (2008) Variation in fatty acid composition in muscle and heart tissues among species and populations of tropical fish in lakes Victoria and Kyoga. *Lipids*, 43(11): 1017–1029

Leung K. S., Galano J. M., Durand T., Lee J. C. Y. (2018) Profiling of omega-polyunsaturated fatty acids and their oxidized products in salmon after different cooking methods. *Antioxidants*, 7(8): 96

McNamara R. K., Carlson S. E. (2006) Role of omega-3 fatty acids in brain development and function: Potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 75(4–5): 329–349

Mnari Bhouri A., Jrah Harzallah H., Dhibi M., Bouhlel I., Hammami M., Chaouch A. (2010) Nutritional fatty acid quality of raw and cooked farmed and wild sea bream (*Sparus aurata*). *Journal of Agricultural and Food Chemistry*, 58(1): 507–512

Nagasaka R., Gagnon C., Swist E., Rondeau I., Massarelli I., Cheung W., Ratnayake W. M. N. (2014) EPA and DHA status of South Asian and white Canadians living in the National Capital Region of Canada. *Lipids*, 49(10): 1057–1069

Norris P. C., Dennis E. A. (2012) Omega-3 fatty acids cause dramatic changes in TLR 4 and purinergic eicosanoid signaling. *Proceedings of the National Academy of Sciences of the United States of America*, 109(22): 8517–8522

Ohshima T., Shozen K.-I., Ushio H., Koizumi C. (1996) Effects of grilling on formation of cholesterol oxides in seafood products rich in polyunsaturated fatty acids. *LWT – Food Science and Technology*, 29(1–2): 94–99

Robert S. S. (2006) Production of eicosapentaenoic and docosahexaenoic acid-containing oils in transgenic land plants for human and aquaculture nutrition. *Marine Biotechnology*, 8(2): 103–109

Sampels S., Zajíc T., Mráz J. (2014) Effects of frying fat and preparation on carp (*Cyprinus carpio*) fillet lipid composition and oxidation. *Czech Journal of Food Sciences*, 32(5): 493–502

SanGiovanni J. P., Chew E. Y. (2005) The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Progress in Retinal and Eye Research*, 24(1): 87–138

Sioen I., Haak L., Raes K., Hermans C., De Henauw S., De Smet S., Van Camp J. (2006) Effects of pan-frying in margarine and olive oil on the fatty acid composition of cod and salmon. *Food Chemistry*, 98(4): 609–617

Sushchik N. N., Makhutova O. N., Rudchenko A. E., Glushchenko L. A., Shulepina S. P., Kolmakova A. A., Gladyshev M. I. (2020) Comparison of fatty acid contents in major lipid classes of seven salmonid species from Siberian Arctic lakes. *Biomolecules*, 10(3): 419

Tacon A. G. J., Metian M. (2013) Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in Fisheries Science*, 21(1): 22–38

Tarley C. R. T., Visentainer J. V., Matsushita M., de Souza N. E. (2004) Proximate composition, cholesterol and fatty acids profile of canned sardines (*Sardinella brasiliensis*) in soybean oil and tomato sauce. *Food Chemistry*, 88(1): 1–6

Tocher D. R., Betancor M. B., Sprague M., Olsen R. E., Napier J. A. (2019) Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. *Nutrients*, 11(1): 89

Torres-Ruiz M., Wehr J. D. (2020) Complementary information from fatty acid and nutrient stoichiometry data improve stream food web analyses. *Hydrobiologia*, 847(2): 629–645

Van Pamel E., Cnops G., Van Droogenbroeck B., Delezie E. C., Van Royen G., Vlaemynck G. M., Bekaert K. M., Roldan-Ruiz I., Crivits M., Bernaert N., De Block J., Duquenne B., Broucke K., De Ruyck H., Herman L. (2021) Opportunities within the agri-food system to encourage a nutritionally balanced diet– Part II. *Food Reviews International*, 37(6): 573–600

Wall R., Ross R. P., Fitzgerald G. F., Stanton C. (2010) Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutrition Reviews*, 68(5): 280–289

Zotos A., Kotaras A., Mikras E. (2013) Effect of baking of sardine (*Sardina pilchardus*) and frying of anchovy (*Engraulis encrasicholus*) in olive and sunflower oil on their quality. *Food Science and Technology International*, 19(1): 11–23