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NUTRITIVE COMPOSITION AND LIPID QUALITY INDICES OF COMMERCIALLY AVAILABLE FILLETED FISH

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professional paper

Summary

The importance of fish in a healthy diet is to be attributed to its low energy value, high content of proteins and essential minerals, and favourable lipid composition. Since modern consumers seek convenience, the demand for frozen fish as a nutritious food that can be quickly prepared either at home or in catering establishments is ever growing. In this study, a total of 45 fillets of three commercially important filleted frozen fish, including chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)*, were analysed for their basic nutritive composition, fatty acid profile and lipid quality indices. The obtained results showed the highest average amount of fat in hake (4.20 ± 0.85 g/100 g), while chum salmon was the richest in proteins (18.74 ± 1.48 g/100 g). Hake was found to contain the highest amount of n-3 polyunsaturated fatty acids and to have the most favourable thrombogenic index, while chum salmon had the most favourable atherogenic index and the most preferable ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids. Our results could serve as the basis for widening the scope of recommendations for lean fish dietary intake.

Keywords: filleted fish, nutritive composition, fatty acids profile, lipid quality indices

Introduction

Literature data have confirmed numerous health benefits of fish consumption, since moderate-to-high fish intake is associated with the lower prevalence of chronic diseases, such as cardiovascular conditions and diabetes, as well as with the lower prevalence of some cancers (Oehlenschläger, 2012). In general, fish represents a healthy source of energy, high-quality easily-digestible proteins, vitamins (A, D, E and B12), essential minerals and amino acids. The importance of fish in a healthy diet is to be attributed to its favourable lipid composition in terms of high level of unsaturated fatty acids (60 – 84 %), especially n-3 long-chain polyunsaturated fatty acids (LC PUFA). Among the latter acids, eicosapentaenoic acid (20:5 n-3 EPA) and docosahexaenoic acid (22:6 n-3 DHA) are considered to be the most important from health aspect due to their well-recognised pleiotropic health and disease-preventative effects (Gil and Gil, 2015); of note, these acids are mainly present in fish (Murillo et al., 2014). Although the effects of fish consumption on lipid profile have generally been studied on fatty fish, several studies have confirmed that the consumption of lean fish (containing < 5% of fat) may also have a favourable impact on triglyceride levels, blood pressure and type 2 diabetes (Leaf et al., 2009; Erkkilä et al., 2008; Rylander et al., 2014).

The amount of fat and the fatty acid profile are some of the important determinants of quality and nutritive value of fish and fish products (Krešić et al., 2017). Literature data have revealed that lipid content highly varies both between and within fish species in dependence of feed used during farming, environmental conditions at the farm location, fish size and maturity, biological variations, tissue sample profile, and fish starvation (Rueda et al., 2001). Because the world's fish stocks are limited, it has recently been suggested that farmed fish might pose as an alternative suitable for consumers. Farmed seafood has an indisputable advantage over captured fishery products because it gets to be produced and harvested under controlled conditions, therefore posing as a minimal consumption-related health risk (Pleadin et al., 2017). In order to achieve the most favourable fatty acid profile, manufacturers make numerous variations in farming conditions, mainly by altering fish feed regimens (Kris-Etherton et al., 2003; Pratoomyot et al., 2010).
As with other food products, fish consumption is expected to be influenced by consumers' need for convenience (i.e. desire to save effort and time when preparing food). Carlucci and co-workers (2015) have recently documented that frozen fish has been ever more often chosen by consumers due to its convenient preparation, immediate availability and lower price. Since lean fish, especially frozen one, represents an underappreciated source of unsaturated

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fatty acids, the aim of this study was to analyse three commercially available frozen fish fillets, that is to say, chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)*, in order to compare their chemical, in particular fatty acid, composition. Additionally, health-related lipid indices, including the atherogenic index (AI), the thrombogenic index (TI), and the ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH), were determined and compared across the analysed species.

Materials and methods

Sampling and sample preparation

Chum salmon *(Oncorhyncus keta)*, saithe *(Pollachius virens)* and hake *(Merluccius hubbsi)* fish fillets were sampled from the Croatian market during the 2017 – 2018 timeframe. Each type of fish fillets was sampled in three replicates yielding three distinctive fillet lots sampled in five pieces. In total, 45 fillet pieces were taken (15 per fish species), yielding three mutually different 15-piece lots.

After mild defrosting, samples of the same type and lot (in groups of 5; see above) were homogenized for 15 sec at 6000 rpm using a laboratory homogenizer (Grindomix GM 200, Retsch, Haam, Germany). All samples were analysed within 48 hours and stored in plastic containers at +4 °C pending analyses of basic chemical parameters. The extracted fat was stored in a refrigerator at -18 °C pending fatty acid composition analysis, carried out within the next 48 hours.

Basic chemical composition

The water content (g/100 g) was established gravimetrically (ISO 1442:1997) at the sample-drying temperature of 103 °C using a Memmert UF75 Plus drier (Memmert, Germany). The total protein content was determined using the Kjeldahl technique (ISO 937:1978) that utilised a digestion unit (Unit 8 Basic, Foss, Sweden) and an automated distillation & titration device (Vapodest 50s, Gerhardt, Germany). The total fat content was determined using the Soxhlet technique (ISO 1443:1973); to that end, ether-mediated lipid extraction was performed using an extractor (Soxtherm 416 Automatic, Gerhardt, Germany). The total ash content was ascertained via incineration in a muffle furnace (LV9/11/P320, Nabertherm, Germany) at 550 °C (ISO 936:1998). The salt content determination made use of the multiple standard addition potentiometric technique that employs an ion-selective electrode and a Na EasyPlusTM analyser (Mettler Toledo, Germany). Based on the established sodium content, the representation of sodium chloride (salt) was determined stoichiometrically. The carbohydrate content was calculated based on the sum of water, ash, protein and fat content and the difference of up to 100%. The sugar content was determined spectrophotometrically (HACH DR/6000U, Germany) using an enzyme Sucrose/D-Glucose/D-Fructose kit (R-Biopharm, Germany) according to the kit manufacturer's instructions. All chemicals used for the analyses were of an analytical grade. The means obtained from two parallel runs in form of weight percentage (%) with the accuracy of 0.01% were considered descriptive of a target sample. Quality control of the analytical methods used was performed using the Certified Reference Material (CRM) T0149 (FAPAS, York, England).

Fatty acid profile

In order to determine the fatty acid composition, fatty acid methyl esters were obtained from the extracted fat according to the standard EN ISO 12966-2:2017 technique that involves dissolution of glycerides in isooctane and trans-esterification using potassium hydroxide methanol solution, and then analysed using gas chromatography (GC) according to the ISO 12966-4:2015 and EN ISO 12966-4:2015. For this purpose, 7890BA gas chromatographer equipped with flame ionization detector (FID), a 60-m DB-23 capillary column having an internal capillary diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, USA) was used. The components were detected by FID at the temperature of 280 °C, hydrogen flow rate of 40 mL/min, air flow rate of 450 mL/min and nitrogen flow rate of 25 mL/min. The initial column temperature was 130 °C; after a minute, it was increased by 6.5 °C/min until the temperature of 170 °C was reached. The temperature was further increased by 2.75 °C/min until the temperature of 215 °C was attained. The latter temperature was maintained for 12 min and then further increased by 40 °C/min until the final column temperature of 230 °C was reached, the latter being maintained for 3 min. One mL of a sample was injected into a split-splitless injector at the temperature of 270 °C and with the partition coefficient of 1:50. The carrier gas was helium (99.9999%), flowing at the constant rate of 43 cm/sec. Fatty acid methyl esters were identified by comparing their retention times to those of fatty acid methyl esters contained by the standard mixture, as described earlier by Pleadin et al. (2015). The results are expressed as a percent-share (%) of an individual fatty acid in total fatty acids, the accuracy thereby being 0.01%. Fatty acid methyl ester values were converted into fatty acid values per 100 g of edible fish sample part according to the FAO/INFOODS Guidelines for Converting Units, Denominators and Expressions (2012). Quality control made use of the CRM BCR 163 (Institute for Reference Materials and Measurements, Geel, Belgium) that has a specified content of seven individual fatty acids.

Lipid quality indices

Data on fatty acid composition were used for the calculation of lipid quality indices including the atherogenic index (AI), the thrombogenic index (TI) and the hypocholesterolaemic/hypercholesterolaemic ratio (HH). The atherogenic index (AI) indicates the relationship between the sum of main saturates and the sum of main non-saturates. This parameter was calculated as follows: AI = [(C12:0 + (4 x C14:0) + C16:0)] / [∑ MUFA + PUFA n-6+ PUFA n-3] (Ulbritcth and Southgate, 1991). The thrombogenic index (TI) represents the relationship between pro-thrombogenic (saturated) and anti-thrombogenic FAs (MUFA, PUFA n-6 & PUFA n-3). This index was calculated as follows: TI = (C14:0 + C16:0 + C18:0) / [0.5 x ∑MUFA + 0.5 x PUFA n-6 + 3 x PUFA n-3) + (PUFA n-3/PUFA n-6)]. The ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH) takes well-known effects of certain FAs on cholesterol metabolism into account (Santos-Silva et al., 2002). It was calculated as follows: HH = (C18:1n-9 + C18:2n-6 + C20:4n-6 + C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3) / (C14:0 + C16:0) (Ulbritcth and Southgate, 1991).

Data analysis

Results of basic chemical composition and fatty acid profile were expressed as mean values obtained from two parallel runs. Data analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013).

Results and discussion

The results presented in this study provide the nutritional profile of three lean fish species (chum salmon, saithe and hake) commercially available on the Croatian market. The aim of this study was to prove that lean fish is often underappreciated and underrepresented in a daily diet in comparison with fatty fish. However, since consumer demand for frozen fish continuously increases and given that fatty fish is prone to lipid oxidation and loss of nutritional quality during freezing and frozen storage, frozen filleted lean fish emerges as an interesting market niche.

Basic nutritive composition of frozen fillets of chum salmon, saithe and hake available on the Croatian market is shown in Table 1.

Table 1. Basic nutritive composition of commercially available frozen fish fillets

Chemical parameter (g/100 g)	Chum salmon	Saithe	Hake
Water	76.63±2.60	79.73±0.75	79.80±1.38
Ash	0.89±0.16	1.53±0.05	1.04±0.02
Fat	4.13±3.86	1.04±0.10	4.20±0.85
Protein	18.74±1.48	18.63±0.75	16.03±0.37
Carbohydrates	<0.5	<0.5	<0.5
Sugar	<0.2	<0.2	<0.2
Salt	0.21±0.06	0.35±0.05	0.19±0.01

The analysis of basic nutritive parameters confirmed that fish fillets represent a valuable source of proteins (ranging from 16.03 g/100 g in hake to 18.74 g/100 g in chum salmon). The share of fat in chum salmon and hake was similar (about 4%), but significantly higher than in saithe (about 1%). Although lean marine fish species usually have high protein and low fat content, the latter content significantly depends on fish maturity and nutritional status (Oehlenschläger, 2012). Generally speaking, lean marine fish species contain less than 5% of fat, medium fatty marine species 5 – 10 %, and fatty marine species > 10% of fat (Baltić and Teodorović, 1997). Based on the above, all three fish species under study can be categorized as lean marine fish species. The water content found in all analysed species varied dependent on fat, since the two accounts for approximately 80% of the fish fillet composition.

The fatty acid composition of commercially available frozen fish fillets investigated in this study, expressed as mean values and standard deviations relative of the total fatty acid content, is shown in Table 2.

Table 2. Fatty acid composition of commercially available frozen fish filets

Fatty acid	Mean±SD (% of total fatty acids)		
	Chum salmon	Saithe	Hake
C4:0	ND	ND	ND
C6:0	ND	ND	ND
C8:0	0.10±0.15	0.06±0.01	ND
C10:0	0.06±0.10	0.04±0.03	ND
C11:0	ND	ND	ND
C12:0	0.60±0.21	0.13±0.01	ND
C13:0	0.02±0.03	0.05±0.04	ND
C14:0	8.17±1.31	6.60±1.21	4.64±2.25
C14:1	0.06±0.10	0.18±0.05	ND
C15:0	0.85±0.15	0.96±0.15	ND
C15:1	0.06±0.11	0.30±0.06	ND
C16:0	20.90±0.84	30.29±2.61	30.25±2.60
C16:1n7t	0.34±0.10	0.48±0.03	ND
C16:1n7c	6.24±1.89	7.42±1.22	3.85±1.90
C17:0	1.32±0.17	0.92±0.06	2.61±2.81
C17:1	0.38±0.33	ND	ND
C18:0	8.26±2.39	4.14±0.17	18.64±4.69
C18:1n9t	0.78±0.32	1.04±0.26	1.36±0.32
C18:1n9c	28.87±2.40	18.56±1.47	14.03±2.64
C18:1n7	2.84±0.23	3.24±0.76	4.11±0.83
C18:2n6t	0.27±0.30	0.07±0.01	ND
C18:2n6c	1.37±0.52	1.62±0.09	1.26±1.32
C18:3n6	0.59±0.52	0.35±0.25	2.79±1.51
C18:3n3	1.55±0.77	1.26±0.38	4.78±2.66
C18:4n3	0.14±0.25	0.62±0.11	ND
C20:0	0.13±0.13	0.46±0.15	ND
C20:1n9	3.11±0.37	6.59±1.22	7.53±5.33
C20:2n6	0.21±0.19	0.24±0.02	ND
C21:0	ND	ND	ND
C20:3n6	ND	ND	ND
C20:4n6	0.04±0.08	0.16±0.16	ND
C20:3n3	0.05±0.08	0.05±0.08	ND
C20:4n3	0.13±0.22	0.26±0.04	ND
C20:5n3	0.79±0.85	1.14±0.54	ND
C22:0	ND	0.21±0.08	ND
C22:1n11	5.35±1.81	4.12±0.65	4.15±4.85
C22:1n9	3.25±2.40	0.95±1.26	ND
C22:2n6	ND	ND	ND
C23:0	ND	ND	ND
C22:5n3	0.64±0.35	0.67±0.18	ND
C24:0	0.15±0.26	ND	ND
C22:6n3	1.65±1.04	5.20±0.95	ND
C24:1n9	0.73±0.11	1.63±0.29	ND

ND – not detected; limit of detection (LOD) = 0.05%

Prato and Biandolino (2012) investigated the share of fat and the fatty acid profile of 11 commercially available fish species inhabiting the Mediterranean Sea, and determined a total of 25 C12- to C22- fatty acids. In general, fish fat mainly consists of unsaturated fatty acids (60 – 84 %), among which, when it comes to the marine fish, approximately 88% are highly unsaturated fatty acids with 5 or 6 C-double bonds. About half of fish fat is made of oleic fatty acid (C18:1n9) responsible for the soft, juicy texture (Cvrtila and Kozačinski, 2006). In this study, the fatty acid most represented in chum salmon was oleic acid, followed by palmitic (C16:0) and myristic (C14:0) fatty acid. On the other hand, the fatty acid dominating in saithe and hake was palmitic acid, followed by oleic fatty acid, while myristic acid made the least of their fatty acid content.

Since saithe is one of the most common fish in northern European inshore water, and is often to be found in the vicinity of sea cages at salmon farms, Skog and co-workers (2003) conducted an interesting research into the effects of the vicinity of salmon farms on saithe fatty acid profile. They found the concentrations of palmitic, linoleic (C18:2n6) and α-linolenic (C18:3n3) acids to decrease as the distance from fish farms increases. Fatty acid profile of saithe retrieved from the nearby fish farm-free fjords was very similar to that of saithe samples under this study. From the nutritional standpoint, fatty acids containing C20 and C22 are more valuable than fatty acids containing C18, the highest importance thereby being attributed to EPA and DHA which are, at the same time, largely responsible for changes in n-6/n-3 ratio (Pleadin et al., 2017). In this study, the highest content of fatty acids detailed above was determined in saithe, followed by chum salmon, while none of them was found in hake.

The analyses revealed the highest amount of MUFA in chum salmon followed by hake, while the representation of MUFA in saithe was 10-fold lower as compared to chum salmon (Table 3). Among the analysed samples, hake proved itself as a filleted fish with the highest nutritional value due to its richness in n-3 polyunsaturated fatty acids. Hypotriglyceridaemic effect of n-3 LC PUFA has been proven beneficial in terms of reducing the percent-share of small pro-atherogenic low-density lipoprotein (LDL) particles, and possibly also in terms of ameliorating inflammatory processes associated with metabolic syndrome seen in patients with diabetes mellitus or cardiovascular conditions (Lopez-Huertas, 2012). Generally speaking, fatty acid profile was the poorest in saithe, especially given that only a small amount of fat was determined in that fish.

Table 3. Quantification of fatty acids in chum salmon, saithe and hake (absolute figures)

Groups of fatty acids	Chum salmon (g/100 g)	Saithe (g/100 g)	Hake (g/100 g)
SFA	1.04±1.23	0.31±0.04	1.64±0.22
MUFA	2.01±2.01	0.20±0.07	1.69±0.51
PUFA	0.29±0.29	0.05±0.02	0.44±0.11
∑ n-3	0.22±0.25	0.03±0.01	0.35±0.09
∑ n-6	0.07±0.05	0.02±0.01	0.09±0.02
n-6/n-3	0.32±0.20	0.67±1.00	0.25±0.22

SFA – saturated fatty acids, MUFA –monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, n-3 - omega-3 fatty acids, n-6 - omega-6 fatty acids

In the previously mentioned research by Prato and Biandolino (2012), the shares of saturated (SFA), polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) were 38.1 - 49.8 %; 27.6 - 34.7 %; and 17.8 - 32.4 %, respectively. SFAs predominated due to the high palmitic acid content of roughly 70%. The predominant MUFA was oleic acid (about 60 – 70 % of the total fat content).

In order to gain insight into the possible health effects of certain fatty acids present in fish, in addition to PUFA/SFA ratio, the effects of certain saturated fatty acids should be taken into account, since some of these acids (i.e. C12:0, C14:0 and C 16:0) have been evidenced to increase the total serum cholesterol (Ulbricht and Southgate, 1991). Lipid quality indices determined in commercially available frozen fish fillets analysed in this study are shown in Table 4.

Table 4. Lipid quality indices determined in analysed farmed fish species

Lipid indices	Chum salmon	Saithe	Hake
AI	0.92±0.15	1.12±0.09	1.02±0.20
TI	0.86±0.21	1.53±0.09	0.76±0.14
HH index	1.21±0.17	0.58±0.11	0.79±0.14
PUFA/SFA	0.18±0.03	0.15±0.04	0.27±0.05
n-6/n-3	0.56±0.35	0.95±0.33	0.27±0.05

AI - atherogenic index, TI - thrombogenic index, HH - ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids, SFA – saturated fatty acids, PUFA – polyunsaturated fatty acids, n-3 - omega-3 fatty acids, n-6 - omega-6 fatty acids

PUFA/SFA ratio is recommended to be higher than 0.4, so as to reduce the risk of cardiovascular, autoimmune and other chronic diseases (Simopoulos, 2002). The recommended value was not determined in any of the fish fillets analysed in this study, since the PUFA/SFA ratio determined within this study frame ranged from 0.15 in saithe to 0.27 in hake. Literature data have revealed that lower n-6/n-3 ratios allow for better utilisation of n-3 fatty acids in the human body (Wood et al., 2008). All species analysed in the study by Prato and Biandolino (2012) had the n-6/n-3 ratio of 0.18 in salema to 0.40 in sea bass and black goby, i.e. similar to the ratio obtained in this study for hake, whereas for chum salmon, and especially saithe, higher ratios were established. According to health recommendations, n-6/n-3 ratio should be lower than 4, thereby reducing the incidence of chronic food-related illnesses (Cordain et al., 2005; Simopoulos, 2002); in this study, that was the case in all three fish species analysed. The lowest n-6/n-3 ratio was determined in hake, which also had the lowest PUFA/SFA ratio, while the highest n-6/n-3 ratio was obtained for saithe due to the lowest amount of PUFA.

The atherogenic index (AI) is the parameter descriptive of the ability of some saturates to exhibit pro-atherogenic effects due to the facilitation of the lipid adhesion onto cells the immune and the circulatory system are composed of, while non-saturates are considered to be anti-atherogenic as they inhibit the formation of plaques and diminish the levels of esterified fatty acids, cholesterol, and phospholipids, therefore preventing micro- and macro-coronary events (Ulbritcth and Southgate, 1991). The thrombogenic index (TI) shows the tendency towards blood clotting. Lipid quality indices determined in this study showed the best AI (<1) and HH (>1) values for chum salmon, while the best TI (<1) was determined for hake.

Given that this research made use of frozen fish fillets coming from the market, it is safe to assume that the determined PUFA levels, a substantial loss of nutritional value, and suboptimal values of lipid quality indices, come as a consequence of freezing and frozen storage. Namely, several studies that made use of salmon, hake and saithe fillets have confirmed that freezing and frozen storage may provoke lipid decomposition, PUFA content decrease and SFA content increase (Dawson et al., 2018; Saldana and Bragagnolo, 2007; Karsdotttir et al., 2014).

Conclusion

Modern consumers are looking for healthy, but easily available and ready-to-prepare food. In light of the foregoing, one of the possibilities is to consume fish in form of frozen fillets. Our results confirm that commercially available frozen chum salmon fillets contain the highest amounts of proteins and fat (above all MUFA) and have the most favourable atherogenic index and hypocholesterolaemic over hypercholesterolaemic fatty acid ratio. On the other hand, hake contains the highest portion of n-3 PUFA and has the most favourable thrombogenic index. Frozen saithe fillets have a four-fold lower total fat content as compared to other two species under study.

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