THE EFFECT OF FROZEN STORAGE ON LIPIDS AND FATTY ACIDS CONTENT IN ATLANTIC SALMON. CASE STUDY

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Abstract

Being a species appreciated in all parts of the world, the demand for salmon will always be high. Keeping frozen in the entire period of distribution and handling is a way to increase "shelf life". During cold storage, fish fat can be altered. The purpose of the paper is to determine the effect of cold preservation on the quality of salmon fat. These were evidenced by laboratory tests and by gas chromatography and mass spectrometry. It was analysed the samples of refrigerated and frozen fish (preserved at -20ºC, five days) from the same batch. The amount of determined lipids confirms the literature data (26%), respectively 24.99% in the fresh fish and 27.23% in the frozen fish. In frozen fish the amount of oleic acid (MUFA) extracted is higher, to the detriment of linoleic acid (PUFA). The same trend was observed for the amount of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Most fatty acids of frozen fish were monounsaturated fatty acids (43.99%), followed by polyunsaturated fatty acids (38.74%) and saturated fatty acids (19.56%). These data highlight that frozen fish, even for a short period of time (5 days), alters the content of essential fatty acids, especially polyunsaturated fatty acids.

Key words: fish, MUFA, PUFA, SFA

INTRODUCTION

Fish is well known for its high nutritional profile: good source of protein, high level of poli-unsaturated fatty acids (PUFA) and vitamins. It contains from 60% to 82% water and from 15% to 23% (w. w.) protein; lipid content can vary over a wide range (0.3%–45% w. w.). The PUFA compounds are essential for the human body, because they cannot be produced and therefore need to be provided via food.

The main constituents of fish lipids are triacylglycerols (TAG), phospholipids (PL), sterols, and wax esters; in addition, fish lipids contain also minor quantities of metabolic products of these, as well as small amounts of unusual lipids such as glycerol ethers, hydrocarbons, glycolipids, and sulfolipids.

But freezing and storage at freezing temperature for a long period of time determines the reducing the quality of frozen fish: decreasing of the sensorial proprieties such as rancid off-odour, orange-brown discoloration and texture changes to dry and fibrous [4].

In the present work we focused on investigating the total lipid quantity in fresh and frozen fish, then, we evaluated the free fatty acids profile of fish lipids extracted.

MATERIAL AND METHOD

The total lipids amount was evaluated by Soxhlet method [1].

The fatty acid pattern was measured in the lipids extracted from the from the whole fish fillet [2]. After the fatty acids have been converted into methyl esters, they were analysed by gas chromatography (Agilent 7890B-MSD 5977A, with GC sampler 80, using a column HP-5 MS Ultra Inert 30 m lenght, Ø=0,250 mm și fill of 0,25 µm). The chromatographic conditions were as follows: injection temperature: 250ºC; detector temperature: 150ºC; initial oven temperature 50ºC, programmed to increase gradually up to 150ºC. Helium was used as the carrier gas at a linear flow of about 0.6 mL/min. The retention times and peak areas were

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processed using the Agilent 7890B-MSD 5977A software. Fatty acids were identified by comparison of their retention time and peak areas with those of a mix of reference standards diluted in hexane (50 mg/l). Fatty acids were quantified as percentage ratio of peak areas with the total area.

The lipid quality indices were evaluated from the data on the fatty acid composition, lipid quality indices, that is, atherogenic index (AI) and thrombogenic index (TI) determined according to Ulbricht and Southgate (1991) [6].

Index of atherogenic (AI) is indicating the relationship between the sum of the main saturated fatty acids (considered pro-atherogenic, favouring the lipids adhesion to cells of the immunological and circulatory system) and that of the main classes of unsaturated, considered anti-atherogenic, inhibiting the aggregation of plaque and preventing the appearance of micro- and macro-coronary diseases [3].

To calculate the atherogenic index (AI) and thrombogenic index (TI), it was applied the following equations:

$$A.I. = \frac{12{:}0+(4\times 14{:}0)+16{:}0)}{\sum MUFA+PUFA\ n6+PUFA\ n3}, \text{ and}$$

$$T.I. = \frac{(14{:}0 + 16{:}0 + 18{:}0)}{0.5 \times \left(\sum MUFA + 0.5 \times PUFA\ n6 + 3 \times PUFA\ n3 + \frac{PUFA\ n3}{PUFA\ n6}\right)}$$

RESULTS AND DISCUSSIONS

Total lipids amount for fresh and frozen fish are showed in Table 1. The total fat content was bigger in frozen fish than in fresh fish.

Table 1 Lipid content of fresh and frozen fish

Sample	Dry weight, %	Total lipid content, %
Fresh fish	43.96	24.99
Frozen fish	-	27.23

Data regarding the total lipid content are following the literature, where the level of lipid content of breeding salmon is 26%. It can also be observed that the level of total lipids extracted from the frozen fish is higher than the fresh one; this can be explained by the fact that during thawing, the ice crystals could cut the fish tissue and facilitate the extraction of lipids.

The chromatogram analysis show the presence of 29 compounds in the oils extracted from fresh fish (Figure 1).

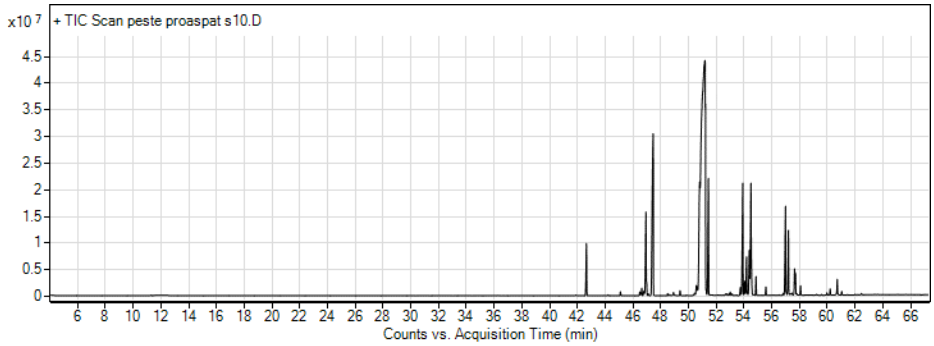

Fig. 1 The oil chromatogram in fresh fish

In the specific chromatografic data, the fatty acids start to separate at 42.64 minutes after starting the test and end after 60.71 minutes. The quality and quantity analysis of the chromatogram is followed in Table 2 and presenting the compound name, formula, code, class, retention time, score, area, % and algorithm.

Table 2 The oil chemical composition in fresh fish and chromatographic characteristics

Cod	File	Name	Formula	Code	Class	RT	Score	Area	%	Algo-rithm	Library
1	Fresh fish 10.D	Methyl tetradecanoate	C15H30O2	C14:0	SFA	42,64	95,11	31311063	1,88	Find by Integration	NIST14.L Famedb23.L
2		(Z)-Methyl hexadec-11-enoate	C17H32O2	C16:1	MUFA	46,93	96,93	55282039	3,33		
3		7,10-Hexadecadienoic acid, methyl ester	C17H30O2	C16:2	PUFA	46,97	92,83	4765024	0,29		
4		Hexadecanoic acid, methyl ester	C17H34O2	C16:0	SFA	47,45	95,87	171028789	10,29		
5		(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	C19H30O2	C18:4	PUFA	50,56	91,18	6979804	0,42		
6		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,79	95,91	78674191	4,73		
7		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,81	97,19	23931317	1,44		
8		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,83	97,08	16975285	1,02		
9		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	51,03	92,11	365044141	21,96		
10		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,11	97,28	214953675	12,93		
11		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,17	97,62	123243807	7,42		
12		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,18	97,5	89071198	5,36		
13		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,21	98,15	44035746	2,65		
14		Methyl stearate	C19H38O2	C18:0	SFA	51,43	96,12	68307215	4,11		
15		5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	C21H34O2	C20:4	PUFA	53,73	90,6	4250538	0,26		
16		5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	C20:5	PUFA	53,90	94,95	71824313	4,32		
17		C 20:3 (cis - 8,11,14) (omega 6)		C20:3	PUFA	54,04	90,13	7437392	0,45		
18		Methyl (Z)-5,11,14,17-eicosatetraenoate	C21H34O2	C20:4	PUFA	54,17	91,96	23154403	1,39		
19		cis-11,14-Eicosadienoic acid, methyl ester	C21H38O2	C20:2	PUFA	54,38	95,3	30394875	1,83		
20		cis-Methyl 11-eicosenoate	C21H40O2	C20:1	SFA	54,49	94,56	87384356	5,26		
21		Eicosanoic acid, methyl ester	C21H42O2	C20:0	SFA	54,86	96,22	9658518	0,58		
22		(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	C19H30O2	C18:4	PUFA	55,57	90,11	4318081	0,26		
23		4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	C23H34O2	C22:6	PUFA	56,98	96,7	54467942	3,28		
24		5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	C20:5	PUFA	57,18	89,98	22671684	1,36		
25		5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	C20:5	PUFA	57,19	91,02	14734836	0,89		
26		13-Docosenoic acid, methyl ester, (Z)-	C23H44O2	C22:1	MUFA	57,63	92,69	14712623	0,89		
27		13-Docosenoic acid, methyl ester, (Z)-	C23H44O2	C22:1	MUFA	57,70	94,84	9979703	0,60		
28		Docosanoic acid, methyl ester	C23H46O2	C22:0	SFA	58,07	94,03	4773170	0,29		
29		15-Tetracosenoic acid, methyl ester, (Z)-	C25H48O2	C24:1	MUFA	60,71	93,12	8569928	0,52		

Thus, it can be observed that the fresh fish oil contains high quantities of linoleic acid (C18:2) - 29,15%, oleic acid (C18:1) - 28,36% and palmitic acid (C16:0) – 10,29%.

If is analyse the percentage distribution of fatty acids by class, we observe that PUFA (poli-unsaturated fatty acids) reach 43.9%, followed by MUFA (mono-unsaturated fatty acids) with 38.94% and on the third place, SFA (saturated fatty acids) with 17.15% of total fatty acid content (Figure 2).

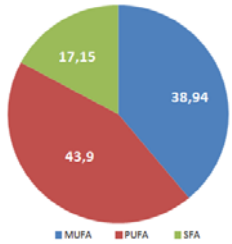

Fig. 2 The percentage distribution of fatty acids classes in fresh fish

For the frozen fish samples, the chromatogram has also shown 29 compounds (Figure 3).

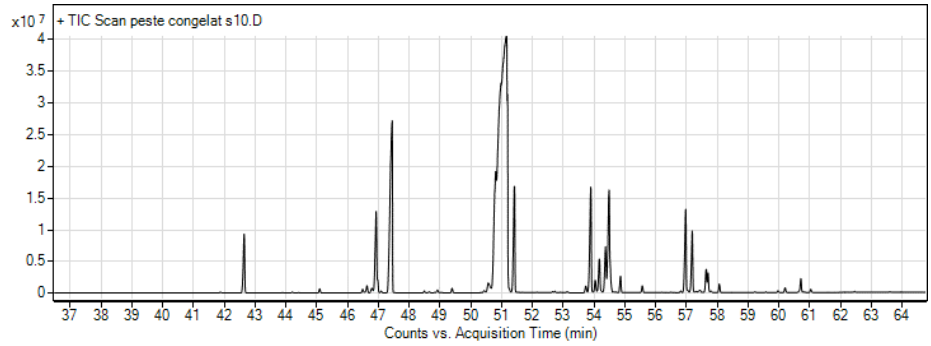

Fig. 3 The chromatogram of frozen fish oil

The quality and quantity analysis of the chromatogram is followed in Table 3 and presenting the compound name, formula, code, class, retention time, score, area, % and algorithm.

It can be shown that the frozen fish oil contains high quantities of linoleic acid (C18:2) – 24,84%, oleic acid (C18:1) – 34,13% and palmitic acid (C16:0) – 10,51%. Thus, unlike fresh fish oil, the level of oleic acid (MUFA) in frozen fish oil is higher despite the linoleic acid (PUFA), an acid susceptible to oxidation.

The same pattern was observed for the percentage distribution of fatty acids: the majority is represented by mono-unsaturated fatty acids, MUFA (43.99%), followed by poli-unsaturated fatty acids, PUFA (38.74%) and saturated fatty acids, SFA (19.56%) (Figure 4).

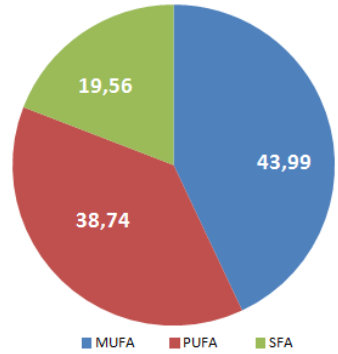

Fig. 4 The percentage distribution of fatty acids classes in frozen fish

This data shows that frozen fish, processed by low temperature for low time (5 days) register a change in the essential fatty acids content, especially poli-unsaturated ones.

Table 3 The chemical composition of oil in frozen fish and chromatographic characteristics

Cod	File	Name	Formula	Code	Class	RT	Score	Area	%	Algo-rithm	Li-bra-ry
1	Frozen fish 10.D	Methyl tetradecanoate	C15H30O2	C14:0	SFA	42,64	95,87	29510012	2,13	Find by Integration	NIST14.L
2		9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	C18:3	PUFA	46,63	90,78	2970362	0,21		
3		(Z)-Methyl hexadec-11-enoate	C17H32O2	C16:1	MUFA	46,92	97,02	44570668	3,22		
4		7,10-Hexadecadienoic acid, methyl ester	C17H30O2	C16:2	PUFA	46,97	92,85	3544064	0,26		
5		Hexadecanoic acid, methyl ester	C17H34O2	C16:0	SFA	47,44	95,8	145456602	10,51		
6		(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	C19H30O2	C18:4	PUFA	50,56	91,51	8561880	0,62		
7		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,80	95,93	87437836	6,32		
8		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,82	97,18	13545073	0,98		
9		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,83	96,92	7617114	0,55		
10		9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	C18:2	PUFA	50,97	93,58	235006542	16,99		
11		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	50,99	95,14	31656456	2,29		
12		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,06	97,1	138817417	10,03		
13		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,15	97,53	264051821	19,08		
14		9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	C18:1	MUFA	51,18	98,28	37830149	2,73		
15		Methyl stearate	C19H38O2	C18:0	SFA	51,41	96,3	53145941	3,84		
16		5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	C21H34O2	C20:4	PUFA	53,72	89,82	3155026	0,23		
17		5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	C20:5	PUFA	53,89	94,35	55521037	4,01		
18		9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	C18:3	PUFA	54,03	90,13	5470449	0,40		
19		Methyl (Z)-5,11,14,17-eicosatetraenoate	C21H34O2	C20:4	PUFA	54,17	91,39	17343623	1,25		
20		cis-11,14-Eicosadienoic acid, methyl ester	C21H38O2	C20:2	PUFA	54,37	94,48	23307557	1,68		
21		cis-Methyl 11-eicosenoate	C21H40O2	C20:1	MUFA	54,48	94,42	67042501	4,85		
22		Eicosanoic acid, methyl ester	C21H42O2	C20:0	SFA	54,85	95,78	7304572	0,53		
23		(6Z,9Z,12Z,15Z)-Methyl octadeca-6,9,12,15-tetraenoate	C19H30O2	C18:4	PUFA	55,56	91,31	3004440	0,22		
24		4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	C23H34O2	C22:6	PUFA	56,97	96,19	41247591	2,98		
25		5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	C21H32O2	C20:5	PUFA	57,18	89,66	28262259	2,04		
26		13-Docosenoic acid, methyl ester, (Z)-	C23H44O2	C22:1	MUFA	57,63	92,02	11095354	0,80		
27		13-Docosenoic acid, methyl ester, (Z)-	C23H44O2	C22:1	MUFA	57,69	93,9	7784024	0,56		
28		Docosanoic acid, methyl ester	C23H46O2	C22:0	SFA	58,06	94,05	3503529	0,25		
29		15-Tetracosenoic acid, methyl ester	C25H48O2	C24:1	MUFA	60,71	92,25	5814725	0,42		

The atherogenic index (AI) and thrombogenic index (TI) were also calculated with the formula described above (Table 4).

Table 4 The values of lipid quality indices in fresh and frozen fish

Sample	A.I.	T.I.
Fresh fish	0.232	0.385
Frozen fish	0.372	0.566

As it may be observed (Table 4), the atherogenic index AI is increasing during the storage at freezing temperature for 5 days. This might be due to the slight increase of the total saturated fatty acids (SFA) in frozen fish. The evolution of the thrombogenic index is quite similar; it increases in frozen fish compared to fresh fish. The results are similar to those found in the literature [5].

CONCLUSIONS

The salmon meat has a high economic value due to its benefits for human health, special taste and good colour. Thus, is a much appreciated fish species through the world and the market request for salmon will be always crescent. Fish needs special storage conditions in order to preserve its nutritional and safety characteristics. The best preservation technique is freezing right after harvesting especially because of the high level of poli-unsaturated fatty acids.

The present research has showed the following data:

- The raw material (fish meat from Norwegian provenance) is high quality one due to its high level of dry matter (43.96%).

- The amount of extracted lipids was 24.99% in fresh fish and 27.23% in frozen fish, which is in consistent with the literature (26% total lipids for breeding salmon).

- The fresh fish oil contains high quantities of linoleic acid (C18:2) - 29,15%, oleic acid (C18:1) - 28,36% and palmitic acid (C16:0) – 10,29%.

- The frozen fish oil contains high quantities of oleic acid (C18:1) – 34,13%, linoleic acid (C18:2) - 24,84% and palmitic acid (C16:0) – 10,51%.

- Percentage distribution of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poli-unsaturated fatty acids (PUFA) in fresh fish oil show a majority of PUFA (43.9%), followed by MUFA (38.94%) and only 17.15% of SFA.

- The oil extracted from frozen fish contains a majority of MUFA (43.99%), followed by PuFA (38.74%) and SFA 19.56%.

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