JAP&T
TPA-Timisoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2018, *24* (2), 69-74

Journal of Agroalimentary Processes and Technologies

Sensory and physicochemical evaluation of crude fish oil under different storage conditions

Flavia Pop[1*], Anca Dumuța[1], Zorica Voşgan[1], Lucia Mihalescu[1]

[1] *Technical University of Cluj-Napoca, North University Center of Baia Mare, Chemistry and Biology Department, 76A Victoriei Str., 430122, Baia Mare, Romania*

Abstract

The content of saturated, monounsaturated and polyunsaturated fatty acids, for fresh fish oil, and also at the installation of advanced oxidation process was determined. To assess the state of freshness during refrigerated and frozen storage the following parameters were determined: titrable acidity, peroxide index, iodine index, thiobarbituric acid reactive substances and the presence of epyhidrinic aldehyde.
Malondialdehyde was detected since the beginning of the storage period and registered a very significant increase ($P \leq 0.001$) from the 30th day of storage at 2...4°C, in the 40th day riched the value of 5.30±0.014 mg/kg, five times higher from the first day. Iodine value determined for fresh fish oil was 94.9±0.07 g I_2/100 g, during refrigerated storage the value gradually decreased, a very significant decrease ($P \leq 0.001$) was registered in the 25th day of srorage. There was observed an inverse correlation between iodine value and peroxide value (R=-0.879), due to the reduction of unsaturation degree by unsaturated fatty acids oxidation. Based on this results it can be concluded that fish oil stored under refrigeration was fresh about 20 days, up to the 35th day was relatively fresh with slightly acidic taste and smell and a high value of peroxide index, and from the 40th day the advanced alteration was installed due to the formation of secondary oxidation compounds that modified the organoleptic properties in rancid taste and smell. Storage temperature had a very significant effect ($P \leq 0.001$), and storage time a significant one ($P \leq 0.05$), on the installation of advanced hydrolysis and oxidation processes, the shelf life under frozen storage was almost twice from that under refrigerated storage.

Keywords: fish oil, fatty acid profile, oxidation, refrigeration, freezing

1. Introduction

The fact that marine products have become increasingly popular is due to largely and valuable fat contribution that these products bring to the human diet. Until the last decade, fish oil was considered in the diet only for the content of vitamins, whereas there was little information on nutritional and biological importance of polyunsaturated fatty acids present in marine creatures [1, 2].

A reference work which highlighted the beneficial effect of fish lipids was that of Bang and Dyreberg, who attributed the low incidence of atherosclerosis in Greenland eskimos to the high consumption of animal fats [3]. The investigations were continued later, and some works showed a high consumption of fish correlate with the prevention and treatment of coronary heart disease [4-6].

As, was proven the ability of fish lipids to reduce the concentration of blood cholesterol and triglycerides and the risk of atherosclerosis. These fats can reduce adhesion of blood platelets, preventing the clotting process, the risk of thrombosis and heart attacks, and also may reduce red cell rigidity [7]. A key feature of fish and marine invertebrates lipids are the polyunsaturated fatty acid content from triglyceride structure. Polyunsaturated fatty acids are generically called as polyunsaturated fatty acids (PUFA) (polyunsaturated fatty acids containing more than one double bond), or highly unsaturated fatty acids (HUFA) (containing 5 or more double bonds) [8, 9]. Eicosapentaenoic acid (EPA) and docosahexaenoic

Corresponding author: *e-mail:* flavia_maries@yahoo.com

acid (DHA) exerts a positive influence on health, influencing development of neuronal activity and visual acuity [10-12].

Fish fillets are a source of fish oils for the everyday diet, unlike to extract fish oils from fish liver to prepare material for supplements. The liver and liver products (such as liver oil) of fish contain omega-3, and also the active form of vitamin A, but at high levels, this form of the vitamin can be dangerous (hypervitaminosis A) [13]. Because the liver is the major filtering and detoxifying organ, toxic contaminants levels are much higher in liver-based oils than in fish oil produced from the processing of tissues [14].

The ways of fish oil spoilage are oxidative and hydrolytic spoilage. Due to polyunsaturated fatty acids high content, including EPA and DHA, fish oil is highly susceptible to oxidative deterioration, and the oxidation rate of fish oil is different from that of other oils. The course of oxidation is often quite different between fish oils extracted by different methods and fish tissues lipids. The most important cause of quality deterioration is the autoxidation of fish oils. There are developing undesirable odours and flavours at small peroxide values at an early oxidation stage, even during the induction period [15]. Oxidative rancidity takes place under oxygen action and consists in the formation of free radicals and hydroperoxides, the transformation of unstable hydroperoxides in stable peroxides, and finally, the split into aldehydes and acids with characteristic odor. Involves the oxidation of unsaturated fatty acids, especially polyunsaturated fatty acids (PUFA) and generates compounds that affect food quality by altering of color, flavor, texture, nutritional value and food safety [14, 15].

The purpose of this study was to follow the acceptability tolerance under refrigerated (2 ... 4ºC) and frozen (-15 ...- 18ºC) storage of crude fish oil by monitoring the quality and freshness parameters. For evaluation of oil stability and monitoring of deterioration during storage, commonly methods include iodine value (IV), peroxide value (PV), thiobarbituric acid reactive substances (TBARS), acid value (AV) and fatty acid composition were used.

2. Materials and Methods

2.1. Samples

Fish oil was obtained by Soxhlet extraction from liver of farmed carp, packaged in brown glass tubes, airtight, was stored under refrigeration (2...4ºC) and freezing (-15...-18ºC), research aim was to study organoleptic and physicochemical changes, and the installation time of alterative processes (hydrolysis and oxidation).

2.2. Physicochemical examination

2.2.1. Fatty acid composition: Fatty acid composition was determined using gas chromatography (GC) Shimadzu GC-17 A coupled with flame ionisation detector FID. Gas chromatography column was Alltech AT-Wax, 0.25 mm I.D., 0.25 μm thick stationary phase (polyethylene), used helium as carrier gas at a pressure of 147 kPa, temperature of the injector and detector was set to 260ºC, the oven programm was the following: 70ºC for 2 min., then the temperature was raised up to 150ºC with a gradient of 10ºC/min., a level of 3 min. and the temperature was raised up to 235ºC with a gradient of 4ºC/min. The method consists in transforming of fatty acids in methyl esters in the sample under analysis, followed by separation of components on a chromatography column, their identification by comparison with standard chromatograms and quantitative determination of fatty acids. By comparing the distances of each peak from analyzed sample chromatogram with peaks distances from standard chromatograms, we identify each fatty acid present in the analyzed sample [16].

*2.2.2. Peroxide value (PV):*Peroxide value was determined using UV-VIS spectrophotometer, this protocol was based on the spectrophotometric determination of ferric ions (Fe^{3+}) derived from the oxidation of ferrous ions (Fe^{2+}) by hydroperoxides, in the presence of ammonium thiocyanate (NH_4SCN). Thiocyanate ions (SCN^-) react with Fe^{3+} ions to give a red-violet chromogen that can be determined spectrophotometrically, the absorbance of each solution was read at 500 nm. To quantify PV, a calibration curve (absorbance at 500 nm vs. Fe^{3+} expressed in μg) was constructed and peroxide value was expressed as meq O_2/kg sample [17].

2.2.3. Iodine value (IV): Iodine value was determined using Hanus method. Approximately, 0.5 g sample (dissolved in 15 mL CCl_4) was mixed with 25 mL Hanus solution (IBr). After storing the mixture in dark for 30 min., excess IBr was reduced to free I_2 in the presence of 20 mL of KI (100 g/L) and 100 mL distilled water. Free I_2 was measured by titration with 24.9 g/L $Na_2S_2O_3 \cdot 5H_2O$ using starch (1.0 g/100 mL) as an indicator. IV was calculated as g I_2/100 g sample [16].

2.2.4. Thiobarbituric acid reactive substances (TBARS): TBARS determination was carried out as follows: TBA Reagent (0.02 M 2-thiobarbituric acid in 90% glacial acetic acid) was prepared, then 1 g of oil sample was weight into a glass-stoppered test tube and 5 mL of TBA reagent was added. Then, the tube was immersed in a boiling water bath for 35 min. A distilled water-TBA reagent blank was also prepared and treated like the sample. After heating, the sample was cooled in tap water for 10 min. a portion was transferred to a cuvette and the optical density of the sample was read against the blank at a wavelength of 538 nm in a UV-VIS spectrophotometer. The optical density value was converted to the moles of malondialdehyde per gram of fat sample by using a standard curve [17].

2.2.5. Acid value (AV): Determination of acidity is the basic criterion for assessing the installation and intensity of hydrolysis.

The method consists in neutralizing acidity with sodium hydroxide 0.1 N, using phenolphthaleine, as an indicator. Acidity was expressed as oleic acid grams to 100 grams sample [27].

2.2.6. Statistical analysis: All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation (X ± SD). Significant differences between mean were determined by using "Student" ("t") distribution.

3. Results and Discussions

The fresh fish oil presented the following chemical composition: 20.39% saturated fatty acids (SFA), 44.56% monounsaturated fatty acids (MUFA) and 35.05% polyunsaturated fatty acids (PUFA). In the case of oxidized fish oil SFA content increased from 20.39% to 21.58%, MUFA content decreased from 44.56% to 43.80%, and PUFA content decreased from 35.05% to 33.37%, PUFA showed the significant variations. The fatty acid content for fresh and oxidized fish oil is presented in Table1. Between storage time and PUFA content there was a strong negative correlation (R=-0.979). During storage there was a decrease in fatty acid content in order: PUFA>MUFA>SFA, but these variations are quite small, and storage time did not significantly affect fatty acids profile.

Table 1. Fatty acid composition for fresh and oxidised fish oil

Fatty acid name	**Abbreviation**	**Fresh fish oil**	**Oxidized fish oil**
		%	**%**
Myristic	14:0	5.76	5.80
Palmitic	16:0	17.90	17.90
Palmitoleic	16:1	10.12	10.01
Heptadecanoic	17:0	1.07	1.08
Stearic	18:0	2.75	2.79
Oleic	18:1	17.14	16.84
Vaccenic	18:1 isomer	6.49	6.34
Linoleic	18:2	3.22	12.81
Alfalinolenic	18:3	2.97	20.26
Arachidic	20:0	2.51	2.51
Cis-11-Eicosenoic	20:1	10.81	10.47
Arachidonic	20:4	9.81	traces
Cis-5,8,11,14,17 Eicosapentaenoic	20:5	17.05	traces

The determined acidity for fresh fish oil was 0.34±0.014% (g oleic acid), and during refrigerated storage presented an upward trend, hydrolysis was carried out at a fast pace, registered a very significantly increase (P≤0.001) of acidity from the 20th day and exceeded the maximum permitted level of 1% (g oleic acid) for fresh oil in the 25th day of storage. At this time there were no found essential color changes, only slightly acidic taste and smell, between acidity values and storage time there was a strong positive correlation (R=0.996).

Peroxide index determined for fresh fish oil was 1.3±0.07 meq O_2/kg, absorption of oxygen can also occur during the melting process. The first 20 days of storage at 2...4°C correspond to the initiation phase of oxidation, PV registered a statistically significance (P≤0.01) up to 3.2±0.14 meq O_2/kg, in the 25th day the increase was very significant (P≤0.001) when the propagation phase was installed, in the 30th and 35th days the increase was relatively low from the 25th day, probably due to the balance formed between peroxides and secondary compounds, in the 40th day the value decrease to 7.1±0.14 meq O_2/kg because of hydroperoxides decomposition. It can be concluded that from the 40th day of storage the oxidative status passed from primary to secondary status (Figure 1).

Iodine index determined for fresh fish oil was 94.9±0.07 g I_2/100 g, during refrigerated storage the value gradually decreased, a very significant decrease (P≤0.001) was registered in the 25th day of storage (Figure 2). There was observed an inverse correlation between IV and PV (R=-0.879), due to the reduction of unsaturation degree by unsaturated fatty acids oxidation. Between PV and storage time there was a positive correlation up to the 35th day of storage, correlation coefficient was R=0.965, and from the 40th day of storage there was an inverse correlation (R=-0.946).

Malondialdehyde was detected since the beginning of the storage period with a value of 1.30±0.006 mg/kg and registered a very significant increase (P≤0.001) from the 30th day of storage at 2...4°C, in the 40th day riched the value of 5.30±0.014 mg/kg, 5 times higher from the first day. It was observed an inverse correlation between MDA and PV in the 40th day (R=-0.879), when PV registered a decline, MDA recorded the most significant increase, indicating the presence of secondary oxidation compounds with toxic effects on the body. Between TBARS values and storage time there was registered a positive correlation (R=0.994). It can be set a maximum value of TBARS of about 4.5 mg MDA/kg, value to which the chilled fish oil presented a relative freshness, and above this value a rancid taste and odor was installed. In the 40th day was also identified the presence of epihidrinic aldehyde, whose amount is proportional with the intensity of the developed color, and regardless of the intensity of the reaction (weak positive or positive), fish oil should be excluded from the food chain.

Based on the obtained results it can be concluded that fish oil stored under refrigeration was fresh 20 days, up to the 35th day was relatively fresh with slightly acidic taste and smell and a high value of PV, and from the 40th day the advanced alteration was installed due to the formation of secondary oxidation compounds that modified the organoleptic properties in rancid taste and smell.

Manat et al. [18], have studied the lipids changes from sardines muscle tissue during 15 days of freezing, and found an increase in PV by day 6, a steady evolution until day 9, followed by a decrease. The authors also reported a initial TBARS value of 17.2 mg/kg, a slow growth in next 3 days, followed by a sharp increase on day 6, and in the following days the value gradually increased. The researchers explained accelerated lipolysis as due to lipase release from internal organs in muscle tissue where fats are located, about 20% of the lipids are hydrolyzed during storage in freezing conditions. *Kolakowska et al.* [19], found intestinal lipases and phospholipases in sardines oil. The authors reported a decrease in triglycerides and phospholipids content and a increase of free fatty acids, mono and diglycerides content with increasing storage time (P<0.05) in muscle tissue of sardines.

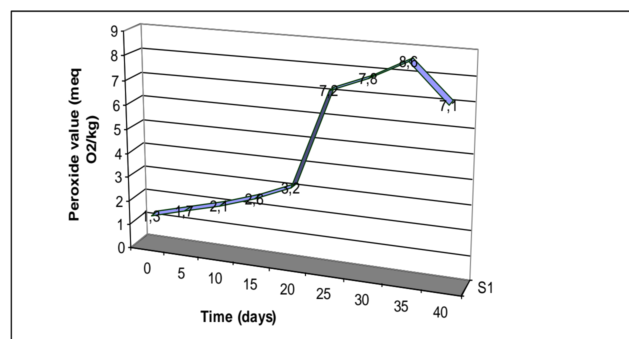

Figure 1. Peroxide index variation of fish oil during refrigerated storage

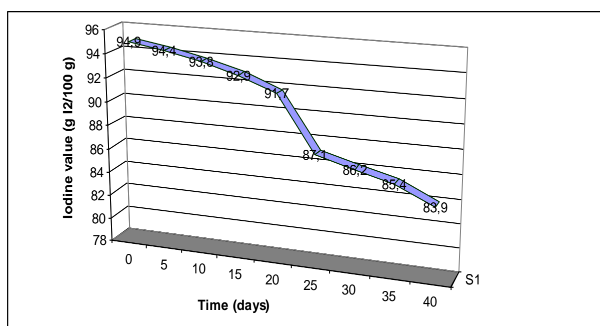

Figure 2. Iodine index variation of fish oil during refrigerated storage

During frozen storage the acidity evolved upward, and registered a very significant increase (P≤0.001) in the 45th day of storage when riched the value of 1.07±0.007% (g oleic acid), and exceeded the maximum permitted level of 1% (g oleic acid) for fresh oil, between acidity values and storage time there was a positive correlation (R=0.986). From this moment was installed a slightly acidic taste and smell.

Peroxide index registered a increase during storage at -15...-18ºC, a very significant increase (P≤0.001) was observed up to 60 days of storage when riched the value of 9.2±0.06 meq O_2/kg due to the formation of large quantities of peroxides, up to the 90th day there was observed a decrease up to the value of 8.1±0.14 meq O_2/kg which corresponds to disruption phase and to the splitt in secondary compounds. It can be concluded that the induction period in the case of frozen fish oil was about 30 days, propagation period was also about 30 days, and the decline period started after 60 days when secondary oxidation compounds were formed, ie after 60 days of storage oxidative status passed from primary to secondary state.

Iodine value decreased during frozen storage, a statistical significance (P≤0.01) was observed at 60 days, there was an inverse correlation between IV and PV (R=-0.859) due to the splitting of unsaturated bounds from fatty acids structure. Between PV and storage time there was observed a positive correlation up to 60 days of storage, correlation coefficient was R=0.965, after 60 days an inverse correlation (R=-0.916), and between IV and storage time there was an inverse correlation (R=-0.984).

To assess the state of freshness we used the descriptive sensory analysis, which is an ideal technique to identify flavors in a product and to distinguish the products between them, using tasters familiar with methods of scoring and sensory language. Sensory analysis was performed on a sample of 10 people, aged between 20 and 55 years, according to *Pop et al.* [17].

Selected attributes were: smell, taste, color, appearance and texture.

For sensory analysis the descriptive scale was the next: 1 = very little normal, 2 = less than normal, 3 = moderately normal, 4 = almost normal, 5 = normal.

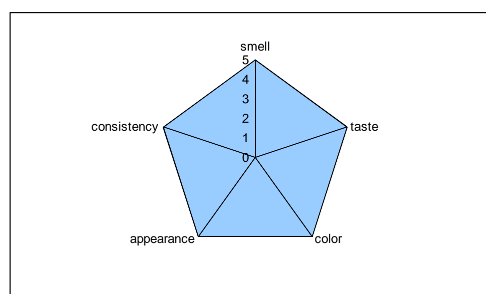

Figure 3. Sensory analysis of fresh fish oil

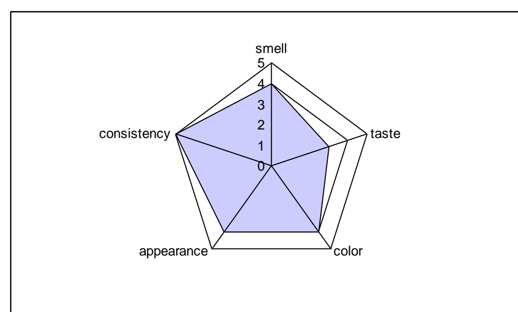

Figure 4. Sensory analysis of relatively fresh fish oil

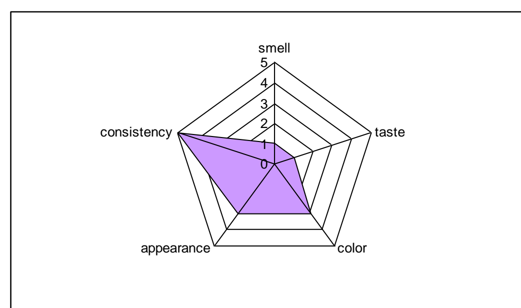

Figure 5. Sensory analysis of altered fish oil

4.Conclusion

Between storage time and PUFA content there was a strong negative correlation (R=-0.979). During storage there was a decrease in fatty acid content in order: PUFA>MUFA>SFA, but these variations are quite small, and storage time did not significantly affect fatty acid profile.

Fish oil stored under refrigeration was fresh 20 days, up to the 35th day was relatively fresh with slightly acidic taste and smell and a high value of PV, and from the 40th day the advanced alteration was installed due to the formation of secondary oxidation compounds that modified the organoleptic properties in rancid taste and smell. The content of unsaturated fatty acids had a very significant effect on the stability towards oxidative processes. Storage time had no significant effect on the entire profile of fatty acids, although were recorded small variations in fatty acids profile.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

References

1. Hearn, T.L.; Sgoutas, S.A.; Sgoutas, D.S; Hearn, J.A., Stability of polyunsaturated fatty acids after microwave cooking of fish, *Journal of Food Science,* **1987**, *52(2)*, 1430-1431.
2. Hogan, S.A.; O'Riordan, E.D.; O'Sullivan, M., Microencapsulation and oxidative stability of spray-dried fish oil emulsions, *Journal of Microencapsulation* **2,003**, *20(5)*, 675-688.
3. Moraru Carmen; Carmen-Mirela Tudorică, *Protein and fish fat with functional value*, In: Functional foods, **1999**, Academica Press, Galaţi.
4. Harris, P.; Tall, J., *Rancidity in fish*. In J.C. Allen & R.J. Hamilton (Eds.), Rancidity in foods, **1994**, London, UK.
5. Huhges, D.A., Fish oil and the immune system, *Nutrition & Food Science,* **1995**, *95(2)*, 12-16.
6. Huss, H.H., *Fresh fish quality and quality changes,* **1988**, Rome, Italy: FAO.
7. Frank, B.; Joann, E.; Manson, W.C.; Willett, C., Types of Dietary Fat and Risk of Coronary Hearth Disease, *Journal of the American College of Nutrition,* **2001**, *20*, 15-19.
8. Gladyshev, M.I.; Sushchik, N.N.; Gubanenko, G.A.; Demirchieva, S.M.; Kalachova, G.S., Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*), *Food Chemistry,* **2006**, *96(3)*, 446-451.
9. Gladyshev, M.I.; Sushchik, N.N.; Gubanenko, G.A.; Demirchieva, S.M.; Kalachova, G.S., Effect of boiling and frying on the content of essential polyunsaturated fatty acids in muscle tissue of four fish species, *Food Chemistry,* **2007**, *101(4)*, 1694-1700.
10. Cháves, J.; Ana Castellote; Martin, M.; Chifré, R.; Carmen López-Sabater, Stability during storage of LC-PUFA supplemented infant formula containing single cell oil or egg yolk, *Food Chemistr,y* **2009**, *113*, 484-492.
11. Connor, W.E., Importance of n-3 fatty acids in health and disease, *American Journal of Clinical Nutrition ,* **2000**, *71*, 171-175.
12. Couet, C.; Delarue, J.; Ritz, P.; Antoine, J.M.; Lamisse, F., Effects of dietary fish oil on body fat mass and basal fat oxidation in healthy adults, *Journal Home,* **1997**, *21(8)*, 637-64.
13. Immanuel, G.; Menenthira, V.; Palavesam, A.; Peter Marian, M., Physicochemical properties and fatty acid profile of *Odonus niger* liver oil, *Indian Journal of Fishery,* **2002**, *49(2)*, 147-153.
14. Kinsella, J.E., *Sea foods and fish oils in human health and disease,* **2001**, Marcel Dekker, New York (USA).
15. URL: http://www.oilofpisces.com/fishnews.html, Fish Oil and Angina/Heart Attack, Health Benefits of Fish Oils, **2008**.
16. Pop, F.; Semeniuc, C.A.; Mihalescu, L., Oxidative and hydrolytic stability of alimentary poultry fats under refrigeration and freezing, *European Journal of Lipid Science and Technology*, **2016**, *118(11)*, 1795-1798.
17. Pop, F.; Boltea, D., Evaluation of oxidation and hydrolysis in milk fat during freezing storage, *Journal of Agroalimentary Processes and Technologies*, **2014**, *20(1)*, 39-45.
18. Manat, C.; Soottawat, B.; Visessanguan,W.; Faustman, C., Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage, *Food Chemistry,* **2006**, *99*, 83-91.
19. Kolakowska, A., *Lipid oxidation in food systems* **2002**. In Z.E. Sikorski, A. Kolakowska (Eds.), Chemical and functional properties of food lipids, FL, USA: CRC Press.