Assessment of Lipolytic and Oxidative Changes in Fish Oil Stored under Refrigeration and Freezing

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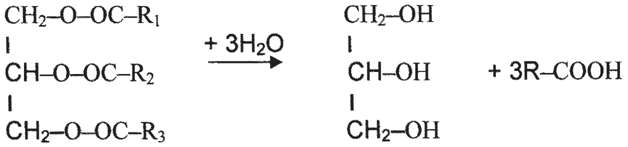
The purpose of this work was to monitor the storage stability of fish oil extracted from liver of farmed carp (Cyprinus Carpio) during refrigeration (2...4°C) and freezing (-15...-18°C) storage in order to establish its shelf-life and to ensure consumer protection. Appreciation of lipolytic changes was performed by fatty acids profile determinations and of oxidative changes by following quality parameters determination: iodine value (IV), peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and the presence of epyhidrinic aldehyde (Kreis reaction). 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). For oxidized fish oil was found a SFA : MUFA : PUFA = 1 : 2.09 : 1.60 report, and essential fatty acids : nonessential fatty acids = 1 : 1.30. The quality parameters, PV, TBARS gradually increased, while IV decreased during both temperatures storage. The decreasing of IV indicates a reduction of oil unsaturation that was considerably lower at -18°C than at +2°C during storage. Storage temperature had a very significant effect ($Pd \leq 0.001$), and storage time a significant one ($P \leq 0.05$), on the installation of advanced lipolysis and oxidation processes, the shelf life under frozen storage was almost twice from that under refrigerated storage.

Keywords: fish oil; lipolysis; oxidation; fatty acids profile; storage

Storage of animal fats even in refrigerated and frozen conditions affects in time the organoleptic, physico-chemical and nutritional properties following oxidative or lipolytic rancidity and may result in reduction of their shelf life. Oxidation of food is responsible for degradation of the sensory characteristics and nutritional value and even the formation of toxic substances such as peroxides, which requires intimate knowledge of these processes and taking appropriate measures to avoid losses that can be recorded. Lipids, heterogeneous class of natural compounds, esters of alcohols with higher fatty acids, characterized by hydrophobic apolar structure which conferr solubility in organic solvents and insolubility in water, are strictly indispensable components in human nutrition, performing many functions in the body [1].

Lipolytic degradation occurs due to hydrolytic degradation of lipids in fat composition. The first factor that condition hydrolysis is the water content of fat, the other factor being specific hydrolytic enzymes. After hydrolysis are released fatty acids, so fat acidity increases with decomposition process [2-4].



$$\begin{array}{l} CH_2\text{–}O\text{–}OC\text{–}R_1 \\ | \\ CH\text{–}O\text{–}OC\text{–}R_2 \\ | \\ CH_2\text{–}O\text{–}OC\text{–}R_3 \end{array} \xrightarrow{+\,3H_2O} \begin{array}{l} CH_2\text{–}OH \\ | \\ CH\text{–}OH \\ | \\ CH_2\text{–}OH \end{array} + 3R\text{–}COOH$$

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 [5]. 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 [6-8]. Animal fats contain polyunsaturated fatty acids (oleic, linoleic, linolenic) that are exposed to autooxidation during storage, so that the shelf life depends on polyunsaturated fatty acids content, temperature and storage time [9].

From oleic acid are formed a mixture of C_8, C_9, C_{10} and C_{11} hydroperoxides (Figure 1). Amount of C_8-hydroperoxide and C_{11}-hydroperoxide is greater than the C_9 and C_{10} isomers. At 25° C the amounts of *cis* and *trans* C_8 and C_9 hydroperoxides are similar but C_{10} and C_{11} isomers are mainly trans. Degradation of oleic acid monohydroperoxides can occur both by radical and enzyme pathway [10, 11]. The main products of oleic acid monohydroperoxides decomposition are decanal, nonanal, octane, octane-1-ol, heptane, heptanal, heptanoic acid, heptane-2-one and hept-2-enal. Relative proportions of volatile compounds that are formed depend on the duration and temperature oxidation [11-13].

A key feature of fish lipids are the polyunsaturated fatty acid content from triglyceride structure. Polyunsaturated fatty acids are generically called as PUFA (polyunsaturated fatty acids) (polyunsaturated fatty acids containing more than one double links), or HUFA (highly unsaturated fatty acids) (containing 5 or more double links) [14]. Eicosapentaenoic acid (EPA-C20:5) and docosahexaenoic acid (DHA-C22:6) exerts a positive influence on health, influencing development of neuronal activity and visual acuity [15, 16].

The purpose of this research was to track the lipolytic changes for carp fish oil, by determining the content of saturated, monounsaturated and polyunsaturated fatty acids, for fresh fish oil, and also at the time when advanced oxidation process was installed during frozen storage following the changes in fatty acids composition, which represents the original part of the work, and also to follow the stability in refrigerated (2...4°C) and frozen (-15...-18°C) storage by monitoring of quality parameters to determine its validity.

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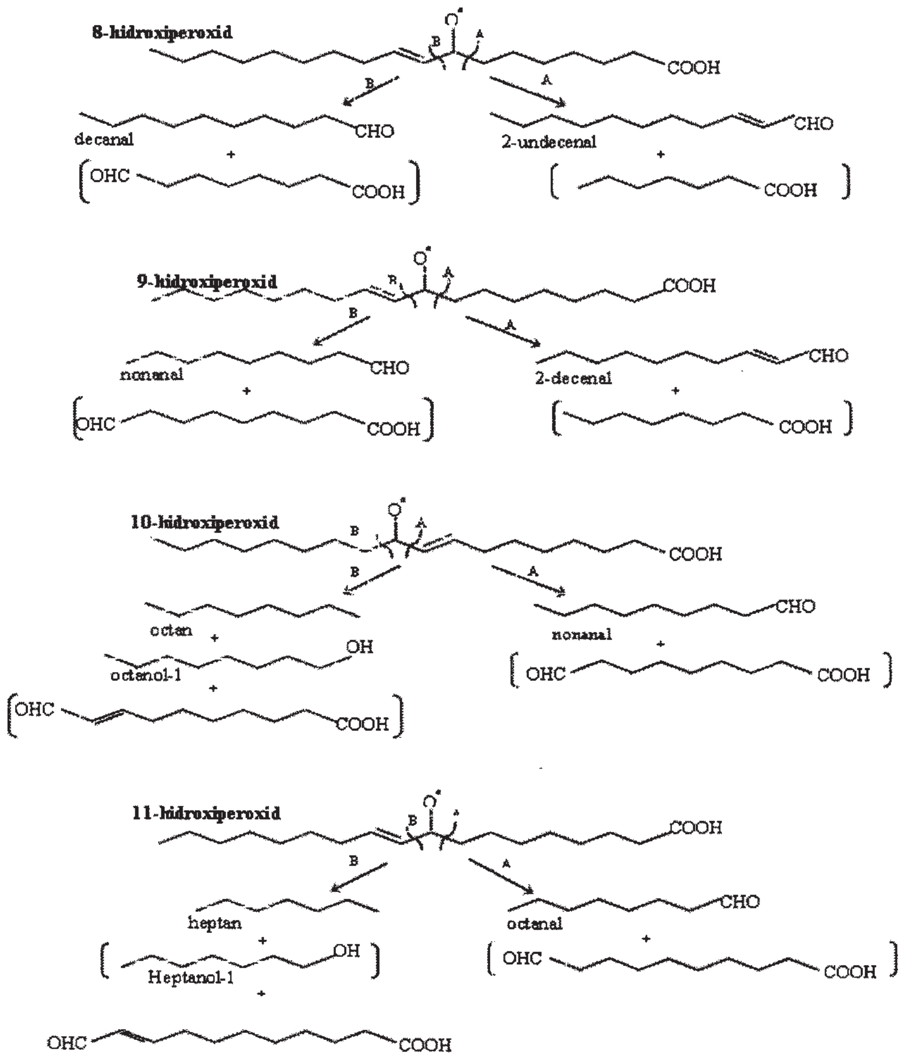

Fig. 1 Breakdown of hydroperoxides from oleic acid [12]

Experimental part

Materials and methods

Fish oil obtained by Soxhlet extraction from farmed carp liver, packaged in brown glass tubes, airtight, was stored under refrigeration (2...4°C) and freezing (-15...-18°C), and samples of 10 g were used at 5 days intervals for quality parameters analysis.

Physicochemical examination

Fatty acid composition was determined using gas chromatography (GC-FID) Shimadzu GC-17 A, coupled with flame ionisation detector. The method consists in transforming 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. Results were expressed as (%) total fatty acids [17] .

Iodine value (IV) 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 [17].

Peroxide value (PV) 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). 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 [18].

Thiobarbituric acid reactive substances (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 weighed into a glass-stoppered test tube and 5 mL of TBA reagent was added. 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 [18].

Epyhidrinic aldehyde, formed during advanced oxidation of fats, released in an acid environment, reacts with phluoroglucine, giving a colored compound. Color intensity is proportional to the quantity of epyhidrinic aldehyde, and so with the oxidation process [17].

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

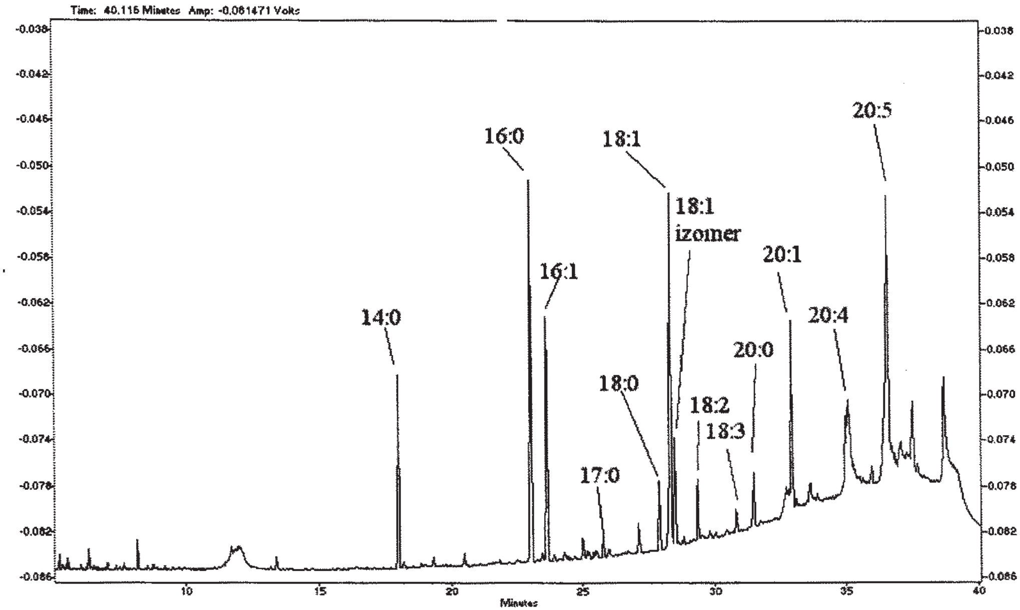

Fig. 2 Chromatogram of fresh fish oil

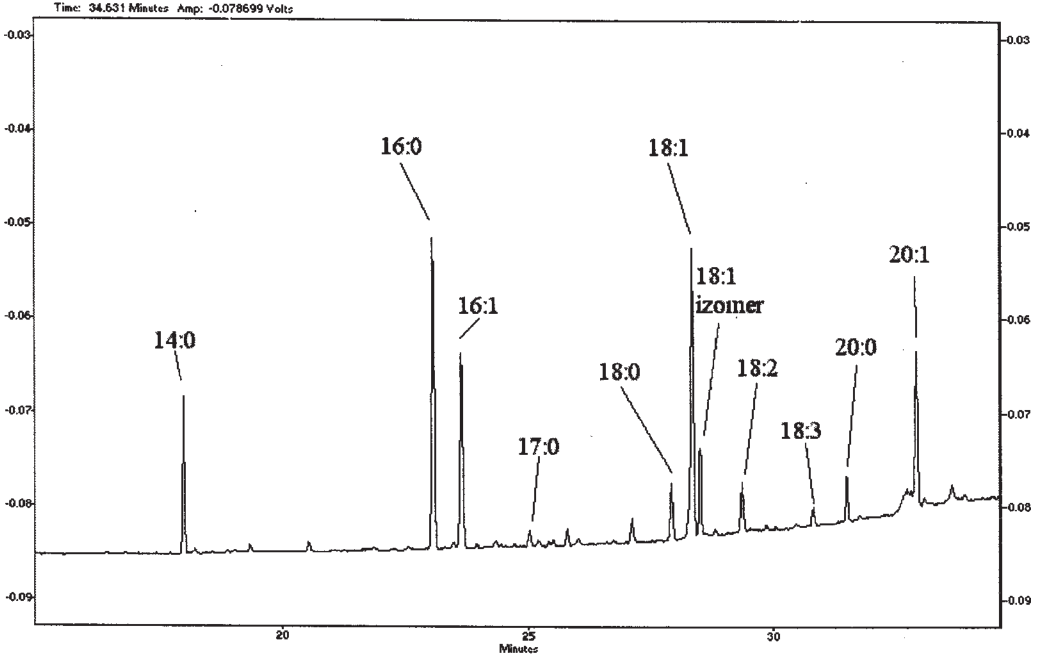

Fig. 3 Chromatogram of oxidised fish oil

differences between mean values were determined by using “Student” (“t”) distribution.

Results and discussions

Fresh fish oil presented the following chemical composition: 20.39% SFA, 44.56% MUFA and 35.05% PUFA. The high proportion of mono and polyunsaturated fatty acids gives it liquid consistency at room temperature, high values for refractive index and iodine index, but also small values for saponification index. The main determined fatty acids were: acid *cis*-5,8,11,14,17 eicosapentaenoic (17.05%), oleic (17.14%), palmitic (17.90%), palmitoleic (10.12%), *cis*-11-eicosenoic (10.81%) and arachidonic (9.81%) acids (fig. 2). DHA acid has not been detected probably due to its oxidation during the extraction process. ω-3 eicosapentaenoic acid (C20:5-EPA) is considered the most valuable fatty acid from fish lipids composition due to the positive influence on the health [19]. For fresh fish oil was determined a SFA : MUFA : PUFA = 1 : 2.17 : 1.71 report, and essential fatty acids : non-essential fatty acids = 1 : 1.27.

Michail et al., reported low levels of EPA in trout (5.9-7%), average values in herring and cod, 10.2% and 14.6% respectively, and a high level in carp of 20.03 %, while DHA has determined in the highest proportion in code (37.8%), but in herring and trout in moderate proportions ranging from 16.7 to 28.5%. This study also reported a decrease in the content of EPA and DHA during fish frying. The researhers determined a EPA+DHA content in salmon, mackerel and fried sardines of 1.7; 0.39 respectively 0.88 g/100 g sample, calculating as to get 1 g EPA+DHA/day it is necessary to consume ≈ 59 g salmon, 256 g mackerel or 114 g fried sardines. The results showed that fish oil was most susceptible to oxidation due to its higher proportion of unsaturated fatty acids [20].

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 (fig. 3). The content of palmitic, heptadecanoic, arachidic acids remained relatively constant, arachidonic and *cis*-5,8,11,14,17 eicosapentaenoic acids were not detected, which also highlights the early installation of oxidation in the case fish oil. For oxidized fish oil was found a SFA : MUFA : PUFA = 1 : 2.09 : 1.60 report, and essential fatty acids: nonessential fatty acids = 1 : 1.30.

Between storage time and PUFA content there was a strong negative correlation, the correlation coefficient (R) was -0.979. During storage there was a decrease in fatty acid content in order: PUFA>MUFA>SFA, but these

Table 1

PHYSICOCHEMICAL INDICES VALUES OF REFRIGERATED FISH OIL

Fat tipe	**Peroxide value (meq O_2 kg^{-1})**	**Iodine value (g I_2 0.01 g^{-1})**	**TBARS test (mg MDA kg^{-1})**	**Kreis reaction**
F0	1.3 ± 0.07	94.9 ± 0.14	1.30 ± 0.006	negative
Fr5	1.7 ± 0.14^{a}	94.4 ± 0.07^{a}	1.64 ± 0.009^{a}	negative
Fr10	2.1 ± 0.13^{a}	93.8 ± 0.12^{a}	2.09 ± 0.010^{a}	negative
Fr15	2.6 ± 0.09^{a}	92.9 ± 0.08^{b}	2.56 ± 0.013^{b}	negative
Fr20	3.2 ± 0.11^{b}	91.7 ± 0.11^{b}	2.85 ± 0.011^{b}	negative
Fr25	7.2 ± 0.10^{c}	87.1 ± 0.09^{c}	3.27 ± 0.014^{b}	negative
Fr30	7.8 ± 0.08^{c}	86.2 ± 0.13^{c}	3.69 ± 0.008^{c}	negative
Fr35	8.6 ± 0.06^{c}	85.4 ± 0.06^{c}	4.35 ± 0.007^{c}	negative
Fr40	7.1 ± 0.12^{c}	83.9 ± 0.10^{c}	5.30 ± 0.012^{c}	positive

Values are expressed as the mean ± standard deviation of three determinations, significant differences: $^{a}(1\%<P\leq5\%)$; $^{b}(0.1\%<P\leq1\%)$; $^{c}(P\leq0.1\%)$

F0–fresh fish oil, Fr5–fish oil to 5 days of refrigeration, Fr10–fish oil to 10 days of refrigeration, Fr15–fish oil to 15 days of refrigeration, Fr20–fish oil to 20 days of refrigeration, Fr25–fish oil to 25 days of refrigeration, Fr30–fish oil to 30 days of refrigeration, Fr35–fish oil to 35 days of refrigeration, Fr40–fish oil to 40 days of refrigeration

variations are quite small, and storage time did not significantly affect fatty acids profile.

Manat et al., observed changes in fatty acid profile during freezing storage of sardines. Fresh sardines had a content of 45.9% SFA, 16.7% MUFA and 35.7% PUFA, of PUFA, DHA was the most abundant followed by EPA. The authors found a reduction in PUFA content during storage, especially of EPA which decreased from 6.14% to 5.33% in the 6th day and to 4.96% in the 15th day, and a decrease in DHA from 19.7% to 18.6% in the 6th day and to 18.5% in the 15th day. The study showed a decrease in PUFA in the 15th day storage by 8.1%, in MUFA by 9.7% and a increase in SFA by 2.3% [21].

Isabelle Sioen et al., studied the effects of frying in margarine on the fatty acids composition of cod and salmon fillets. The authors found that both margarine and olive oil increased the proportion of SFA and MUFA in both species. The main fatty acids in cod fillet were palmitic (18.3%), oleic (12.2%), EPA (11.1%) and DHA (38.6%). After frying in margarine, the total content of fatty acids in cod increased from 551 to 4149 mg/100 g, the growth was significant for each fatty acid except arachidonic acid. If the ratio of PUFA and SFA in cod fillet was 56% and 25.9% respectively, after frying in margarine resulted a content of 16.7% PUFA and 50.7% SFA. If the content of EPA and DHA in cod fillet was 49.7%, after frying it decreased to 9.8%. Salmon is an oily fish, the major acids determined in raw fillets were palmitic (13.2%), oleic (14.6%), EPA (8.1%) and DHA (11.1%) acids, after frying in margarine the total content of fatty acids decreased from 13.914 to 10.571 mg/100 g, the decrease was recorded separately for each acid except lauric acid. After frying in olive oil, the EPA and DHA content decreased slightly from 74.7% to 66.5%, SFA content increased from 22.8% to 27.2%, and PUFA content increased from 11.43% to 16.54%. The study showed that the absorption of fatty acids from cooking fat in fried samples was inversely correlated with the total content of fatty acids for studied fish [22].

The quality parameters, PV, TBARS gradually increased, while IV decreased during both temperatures storage. The decreasing of IV indicates a reduction of fat unsaturation that was considerably lower at -18°C than at +2°C during storage (table 1).

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). Between TBARS values and storage time there was registered a positive correlation (R=0.994). In the 40th day of refrigerated storage was also identified the presence of epyhidrinic 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.

Between PV and storage time under freezing it 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) (table 2).

Table 2

PHYSICOCHEMICAL INDICES VALUES OF FROZEN FISH OIL

Fat tipe	**Peroxide value (meq O_2 kg^{-1})**	**Iodine value (g I_2 0.01 g^{-1})**	**TBARS test (mg MDA kg^{-1})**	**Kreis reaction**
F0	1.3 ± 0.07	94.9 ± 0.14	1.30 ± 0.006	negative
Ff15	2.6 ± 0.05^{a}	94.1 ± 0.08^{a}	1.90 ± 0.009^{a}	negative
Ff30	3.9 ± 0.11^{b}	93.8 ± 0.07^{b}	2.26 ± 0.013^{b}	negative
Ff45	5.8 ± 0.09^{b}	92.1 ± 0.12^{b}	2.89 ± 0.011^{b}	negative
Ff60	9.2 ± 0.06^{c}	90.3 ± 0.11^{b}	3.40 ± 0.014^{c}	negative
Ff75	8.7 ± 0.12^{c}	89.7 ± 0.07^{c}	4.65 ± 0.012^{c}	negative
Ff90	8.1 ± 0.14^{c}	89.1 ± 0.13^{c}	5.17 ± 0.007^{c}	positive

Values are expressed as the mean ± standard deviation of three determinations, significant differences: $^{a}(1\%<P\leq5\%)$; $^{b}(0.1\%<P\leq1\%)$; $^{c}(P\leq0.1\%)$

F0–fresh fish oil, Ff15 –fish oil to 15 days of freezing, Ff30 –fish oil to 30 days of freezing, Ff45 –fish oil to 45 days of freezing, Ff60 –fish oil to 60 days of freezing, Ff75 –fish oil to 75 days of freezing, Ff90 –fish oil to 90 days of freezing

Based on the obtained results it can be concluded that fish oil stored under freezing was fresh up to 30 days, up to 60 days was relatively fresh due to the slightly acidic taste and smell and the high value of PV, and after 60 days of storage oxidative alteration has been installed also highlighted by the defects of color (yellow-brown), taste (bitter) and odor (pungent).

Conclusions

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 lipolysis and oxidation processes, the shelf life under frozen storage was almost twice from that under refrigerated storage.

Between storage time and PUFA content there was a strong negative correlation. 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.

In the case of fish oil hydrolysis processes were installed earlier than oxidative ones both during refrigeration and freezing storage, oxidation was also prevented by limiting the contact with atmospheric oxygen and light intensity throught the method of packing, but fish oil was very susceptible to oxidation due to the high proportion of unsaturated fatty acids.

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