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O Impacto na Estabilidade dos Triacilgliceróis em Peixes de Elevado Percentual de Ácidos Graxos Poli-insaturados Estocados em Freezer

The Impact on the Stability of Triacylglycerols in Fish with a High Percentage of Polyunsaturated Fatty Acids Stored in a Freezer

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The present work evaluated the fatty acid composition and triacylglycerol (TAG) profile of three fish species stored at -18 °C for 60 days through gas chromatography with flame ionization detection (GC-FID) and direct infusion electrospray ionization mass spectrometry (ESI-MS). It was found that oleic, linoleic, arachidonic, eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids were highly susceptible to lipid oxidation over a period of 60 days. Additionally, the decrease in the relative percentage of triacylglycerols (TAGs) containing unsaturated fatty acids such as EPA, DHA, and oleic acid occurred mainly when they were in the *sn*-3 or *sn*-1 positions of the TAG molecule. Therefore, it was proved that in fish meat even a low storage temperature does not prevent for the oxidation of the main unsaturated fatty acids to take place.

Keywords: Temperature; food; lipid oxidation

1. Introduction

Fish consumption is fundamental to human health because it represents a source of important nutrients, such as long chain polyunsaturated fatty acids (LC-PUFAs), including α-linolenic acid (Ln, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Among the benefits of consuming LC-PUFA it is worth listing primary and secondary prevention of cardiovascular diseases,[1] reduced risk of sudden death,[2] contribution to the neurodevelopment of the brain tissue in unborn children and improvement of visual acuity. Although α-linolenic acid can also be found in vegetable oils and flaxseeds, EPA and DHA are mainly found in marine-based sources, microalgae and food supplements.[3,4]

Among the fish species with significant PUFA concentrations, salmon, tuna and sardines are the most commonly consumed by the population. However, the high level of LC-PUFA in the tissues of these fish makes them susceptible to lipid oxidation reducing their quality.[1] In this sense, preserving the quality of lipids in fish tissue is the major challenge in fish storage.

Freezing is currently the best method to contrast lipid oxidation.[5] According to Zaritzky (2009)[6] and FAO[7], the minimum temperature of -18°C is recommended for fish storage and is also the most widely used since it is found in home freezers and supermarkets. However, even during freezing, the enzymatic lipolysis occurs negatively affecting the acceptance of fish.[1,8]

Rudy *et al.* (2016)[9], Boran *et al.* (2006)[10] and Tenyang *et al.* (2017)[11] reported in their work degradation of LC-PUFAS in fish stored at -18 °C. On the other hand, it should be noted that to date no studies have been presented on triacylglycerols (TAGs) that are degraded during the fish storage process. In this way, the present study may provide a basis for novel methods to improve fish conservation over storage time. Therefore, the aim of our work is evaluate the stability of TAGs in fish with a high percentage of LC-PUFAS (salmon, tuna, and sardine) at a temperature of -18 °C for 60 days.

2. Materials and Methods

2.1. Samples

Ten fresh fillets of Atlantic salmon (*Salmo salar*), tuna (*Thunnus*), and sardines (*Sardinella brasiliensis*) were purchased from fish markets in the Brazilian cities of Maringá-PR

(23° 25" 31' S. 51° 57" 19' W), and Itajaí-SC (26° 54' 30" S. 48° 39' 45" O). Each fillet was vacuum packed and stored in a freezer at -18 °C in the food analysis laboratory (APLE-A) of the State University of Maringá (UEM). The analyzes were performed on the following storage periods 0, 15, 30, 45, and 60 days. Over the predetermined period, the fillets were thawed, crushed in a multiprocessor (Unique, São Paulo, Brazil) to ensure homogenization of the sample, and evaluated according to predetermined methodologies.

2.2. Preparation of fatty acid methyl esters

The fatty acid methyl esters were obtained by the methodology of Figueiredo *et al.* (2016)[12], wherein 100 mg of the sample from each fillet was weighed in a test tube, and the samples prepared were analyzed in triplicate. Then, 2.0 mL of $NaOH/CH_4O$ was added. After, the test tubes were placed in an ultrasonic bath for 5 min at 25 °C. Next, 2.0 mL of H_2SO_4/CH_3OH was added and the tubes were again placed in the ultrasonic bath for 5 min. Lastly, 1.0 mL of *n*-heptane was added for phase separation, and then the tubes were shaken and centrifuged at 1046 xg for 1 min. After that, the upper phase was collected to determine the fatty acid methyl esters by gas chromatography with flame ionization detection (GC-FID). The identification of the compounds was carried out by comparing the retention times with the relative analytical standards (FAME Mix, C4-C24, St. Louis, MO, USA), and the results were automatically processed in the ChromQuest™ 5.0 software.

2.3. Fatty acids determination by gas chromatography with flame ionization detector (GC-FID)

Chromatographic analysis was performed in order to obtain the fatty acid profile of fish samples allowing to compare the results with the literature, checking for possible inconsistencies. For this, the analyzes were performed in a Thermo Scientific gas chromatograph, Trace GC Ultra-Triplus model, equipped with flame ionization detector (FID), *slipt/splitless* injector, and fused silica capillary column CP-7420 (Select FAME, 100 m long, 0.25 mm internal diameter and 0.25 µm of a thin film of polysiloxane modified with cyanopropyl as stationary phase). The operating parameters were as follow: column temperature of 165 °C for 18 min; then heated to 235 °C (4 °C min^{-1}) for 20 min. Injector and detector temperatures were kept at 230 and 250 °C, respectively. Gas flows were 1.2 mL min^{-1} for carrier gas (H_2), 30 mL min^{-1} for make-up gas (N_2), and 30 and 300 mL min^{-1} for the FID gas H_2 and synthetic air, respectively. The samples were injected in *split* mode with a 40: 1 ratio. Injection volume was.1 µL.

2.4. Sample preparation for TAG analysis in direct infusion by ESI-MS

TAG determination was performed following the method of Silveira *et al.* (2017)[13], 50.0 µL of the lipids extracted from each fillet by the Bligh & Dyer (1959)[14] method were added to 950.0 µL of chloroform (HPLC grade, Riedel-de Haën, Germany). Then 5.0 µL of this solution was added to 1.0 mL of methanol/chloroform 9: 1 (v v^{-1}) (HPLC grade methanol, J.T.Baker®, USA). In the final solution, 20.0 µL of ammonium formate 0.10 mol L^{-1} in methanol (97% Sigma-Aldrich, Darmstadt, Germany) were added in order to obtain ammonium adducts, $[TAG + NH_4]^+$.

2.5. Mass spectrometer conditions for TAG analysis

For TAG analysis it was used a mass spectrometer Xevo TQ-D™ (Waters, Massachusetts, USA), equipped with Zspray™ electrospray ionization source, operating in positive mode (ESI+).

Samples prepared were analyzed in triplicate and were infused into a mass spectrometer at a flow rate of 50.0 µL min^{-1}, according to the following MS conditions: source temperature, 150 °C; desolvation temperature, 250 °C; desolvation gas flow (nitrogen), 450 L h^{-1}; capillary voltage, 3.0 kV; and cone voltage, 35.0 V. ESI(+)-MS comprises the mass/charge range (*m/z*) between 700-1050 *m/z*. The results were processed using MassLynx™ software.

2.6. TAGs assignment and estimate

The TAGs were assignment and estimated (%) via the LAMES Platform which is based on the mathematical algorithm that describes the distribution of the fatty acids in the TAG molecules[15] using the fatty acid percentage determined by GC-FID. With the LipidMaps® database, it was possible to find the molecular formula of TAGs.

2.7. Statistical analysis

Results for analysis of the fatty acid composition, characterization of identity, and quality of samples were analyzed for variance (ANOVA) and averages were compared employing the Tukey test, with 95% confidence, using the software PAST3.[16]

Principal component analysis (PCA) was performed to verify the possible trends of clustering and/or separation of the samples, assisting in the heat processing. The matrices were constructed using data obtained by the GC-FID and processed using the RStudio software.[17]

3. Results and Discussion

3.1. Fatty acid composition

GC-FID determination of fatty acids was used to better attribute TAG isomers using Plataforma LAMES[15] and LipidMaps®, based on the most probable fatty acids combination to form TAG species. Note that GC-FID

is perhaps the most commonly used technique for determining differences in lipid composition and is capable of differentiating fatty acid isomers. The use of fatty acid determination with software projection to estimate TAG composition has already proven to be equivalent to direct TAG determination via GC and has also been demonstrated to correlate with quantitative analysis of lipids using ESI-MS.[18] The fatty acids profile initially found in the tuna, salmon, and sardine fillets are exhibited in Table 1.

As can be seen in Table 1, fifteen fatty acids were quantified in each fish species analyzed. The majorities for both tuna and salmon were the hexadecanoic (16:0), octadecanoic (18:0), 9-octadecenoic (18:1n-9), and *cis*-4,7,10,13,16,19-docosahexaenoic (22:6n-3, DHA) acids. Regarding the sardine, besides those already mentioned for the tuna and salmon, the *cis*-7,10,13,16,19-docosapentaenoic (20:5n-3, EPA) also highlighted.

The results found are consistent with which has already been reported in the literature. Solaesa *et al.* (2014)[19]determined the same major fatty acids in their work of characterization of TAG by HPLC in tuna fish oil samples (hexadecanoic acid, 23.2%; 9-octadecenoic acid, 18.4%; EPA, 6.5%; and DHA, 21.4%). Maluly *et al.* (2019)[20] evaluated the composition of TAG in salmon and sardine by ESI-MS and found for sardine (hexadecanoic acid, 23.7%; 9-octadecenoic acid, 10.9%; EPA, 8.8%; and DHA, 14.7%) and for salmon (hexadecanoic acid, 12.3%; 9-octadecenoic acid, 14.9%; EPA, 7.7%; and DHA, 11.1%). Fatty acid levels in fish tissues can vary depending on their diet, time of year, reproductive period, and environmental factors, such as local temperature.[21] Figure 1 shows the effect of storage time at -18 ºC on the fatty acid composition of tuna, sardine, and salmon fillets.

Based on Figure 1, it is noted for the three evaluated fish species that the degradation of unsaturated fatty acids including 18:1n-9, 18:2n-6, 18:3n-6, 20:4n-6, 20:5n-3, and 22:6n-3 is significant (p<0.05) over time. The percentage of degradation over the 60 days of the evaluation was different for each fatty acid. Comparing the same fatty acid, it is observed that there were differences also between each species (p<0.05). As described in other studies, enzymatic lipolysis affects fish tissue and release fatty acids even when stored at -18ºC.[1,8] Therefore, the methodology of Figueiredo *et al.* (2016)[12] was chosen for methylating free fatty acids and the acyl group. Thus, any reduction in concentration would not be due to the enzymatic lipolysis but to oxidative processes.

Karlsdottir *et al.* (2014)[1] and Aubourg *et al.* (2007)[8] reported that the oxidative process in frozen fish occurs mainly through mechanisms of lipid autoxidation. Since the autoxidation process mainly depends on two factors, such as the amount of unsaturation of the fatty acid, as well as according to its (*sn*-1, *sn*-2, or *sn*-3) position in the TAG, this discussion will be performed after section 3.2 which present to the results concerning TAG determination in the samples.

3.2. TAG composition

The identification of the main TAGs detected in each sample was done using the LAMES platform and the

Table 1. Fatty acid composition of tuna, salmon and sardine

Fatty acid		(g/100g of sample [%])		
		ATM	SAL	SAR
14:0	Tetradecanoic acid	2.94±0.017	8.57±0.010	7.93±0.005
16:0	Hexadecanoic acid	23.68±0.015	20.89±0.015	31.28±0.007
16:1n-7	9-Hexadecenic acid	1.86±0.010	6.85±0.010	5.78±0.008
18:0	Octadecanoic acid	12.81±0.015	6.17±0.004	11.42±0.002
18:1n-9	9-Octadecenoic acid	17.19±0.006	20.54±0.031	6.70±0.003
18:1n-7	11-Octadecenoic acid	2.85±0.012	4.87±0.010	4.27±0.022
18:2n-6	9,12-Octadecadienoic acid	1.72±0.010	3.63±0.010	0.98±0.009
18:3n-3	6,9,12-Octadecatrienoic acid	3.96±0.026	0.96±0.017	0.68±0.004
20:1n-11	*cis*-11-Eicosenoic acid	3.54±0.010	2.70±0.010	0.67±0.010
20:2n-6	*cis*-11,14-Eicosadienoic acid	5.02±0.004	1.03±0.010	0.96±0.002
20:3n-6	*cis*-8,11,14-Eicosatrienoic acid	0.87±0.010	0.40±0.004	1.48±0.005
20:4n-6	5,8,11,14-Eicosatetraenoic acid	1.05±0.020	1.96±0.026	0.43±0.001
20:5n-3	*cis*-5,8,11,14,17- Eicosapentaenoic acid (EPA)	2.13±0.032	3.74±0.010	11.16±0.009
22:5n-3	*cis*-7,10,13,16,19-Docosapentaenoic acid (DPA)	0.01±0.010	0.04±0.006	0.03±0.002
22:6n-3	*cis*-4,7,10,13,16,19-Docosahexaenoic acid (DHA)	20.37±0.010	17.65±0.010	16.23±0.003
SFA	Saturated fatty acid	39.43±0.12	35.63±0.10	50.63±0.05
MUFA	Monounsaturated fatty acid	25.44±0.08	34.96±0.07	17.42±0.08
PUFA	Polyunsaturated fatty acid	35.13±0.11	29.41±0.09	31.95±0.06

Results are expressed as means ± standard deviation of three replicates; ATM: tuna; SAL: salmon; SAR: sardine.

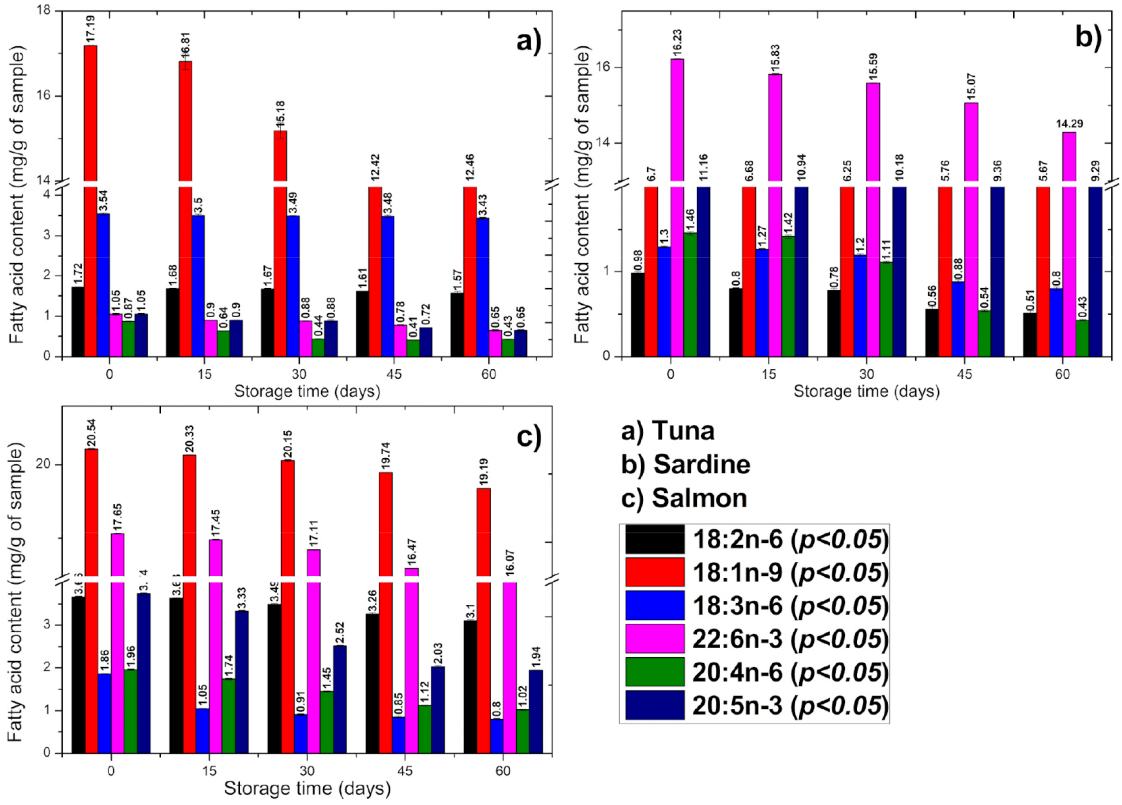

Figure 1. Percentage of the main unsaturated fatty acids present in a) tuna, b) sardine c) salmon. Data are given as the mean of 10 samples

LipidMaps® database.[15] Through the LAMES platform and the result of the relative percentage, the predicted concentration (%) and distribution of FAs in the major TAG molecules detected in each sample were obtained. Moreover, the position of each FA in the TAG molecule was considered.[22]

Table 2 shows the TAG profile of the tuna, salmon, and sardine analyzed at time zero. On account of the high concentration of palmitic (P), oleic (O), and docosahexaenoic (DHA) acids in tuna fillets (Table 1), the most abundant ion (Table2) found was the $[TAG+NH_4]^+$ PDHAO of *m/z* 922 as expected, with a percentage estimated by the LAMES platform of 11.0%, followed by the TAGs PDHADHA (*m/z* 968), PDHAP (*m/z* 896), SDHAP (*m/z* 924) and ODHADHA (*m/z* 994), with respective estimates of 7.3, 6.9, 6.2, and 5.8%. No data were found in the literature for the determination of TAGs in tuna for comparison with the profile determined in the present study. Thus, this study will provide important collaboration and a basis for further research on the lipid profile of this species. It should be mentioned that the lipid profile of fish may change depending on the diet received by the fish.

In the salmon and sardine (Table 1) the major fatty acids were also palmitic, oleic, and docosahexaenoic acids. Nevertheless, as the concentration of them differed between the two species the main TAGs also differed. In the salmon (Table 2) the most abundant ion was the $[TAG+NH_4]$ + PDHAO of *m/z* 922 with a percentage estimated by the LAMES platform of 14.5%. Similarly, for the sardine the most abundant ion was the $[TAG+NH_4]$ + PDHAP of *m/z* 896 with a percentage estimated by the LAMES platform of 10.3%. The results are befitting to those found by Maluly *et al.* (2019)[20] that analyzed the TAG profile in different salmon and sardine species wherein the majority ion for sardine was also the PDHAP, whereas for salmon the PDHAP was the second most intense.

Due to the high concentration of (LC-PUFA) in fish fillets that were analyzed, fatty acids in TAGs decreased during the 60 days storage period at -18 ºC. (Figure 1). These changes are attributed to the lipid oxidation which occurs in these fatty acids that correspond to the TAG molecule. However, the lipid oxidation rate of these fatty acids is variable and depends on the amount of unsaturation in the fatty acid as well as its position in the TAG molecule. Furthermore, the *sn*-1 and *sn*-3 positions of the TAG are more susceptible to a degradation reaction than *sn*-2.[10] Figure 2 reveals the TAGs that reduced in intensity over the 60 days of storage at -18 °C.

According to Figure 2(a), for tuna the main TAGs that had a reduction of intensity over the 60 days of storage at -18 ºC were POP, PLnO, PDHAP, PDHAO, PDHALn, ODHALn, PDHADHA, ODHADHA. The results are consistent with the reduction in unsaturated fatty acids for the same sample (Figure 1). All reduced TAGs are comprised of fatty acids that are unsaturated in their structure. DHA was a fatty acid that reduced 38% over the 60 days since in the PDHADHA and ODHADHA it is in the *sn*-3 position susceptible to autoxidation, unlike of *sn*-2 position where

Table 2. The main TAGs identified from the direct infusion ESI (+)-MS analysis of the tuna, salmon, and sardine in relative percentage (%)

Molecular Formula	(CN:DB) [a]		*m/z*	TAG assignment	TAG estimate molar (%)		
					ATM	SAL	SAR
$C_{49}H_{94}O_6$	[46:0]	$[M+NH_4]^+$	796	MPP	-	-	4.2
$C_{51}H_{98}O_6$	[48:0]	$[M+NH_4]^+$	824	PPP	2.2	-	6.0
$C_{51}H_{98}O_6$	[48:0]	$[M+NH_4]^+$	824	MPS	-	-	2.7
$C_{51}H_{96}O_6$	[48:1]	$[M+NH_4]^+$	822	POM	-	5.3	-
$C_{51}H_{96}O_6$	[48:1]	$[M+NH_4]^+$	822	PPoP	-	-	3.6
$C_{52}H_{102}O_6$	[50:0]	$[M+NH_4]^+$	840	SPP	2.9	-	5.0
$C_{53}H_{100}O_6$	[50:1]	$[M+NH_4]^+$	850	POP	5.2	7.1	4.2
$C_{53}H_{100}O_6$	[50:1]	$[M+NH_4]^+$	850	PVP	-	-	2.7
$C_{53}H_{96}O_6$	[50:3]	$[M+NH_4]^+$	846	PLnP	2.4	-	-
$C_{53}H_{92}O_6$	[50:5]	$[M+NH_4]^+$	842	MEPAP	-	-	3.8
$C_{55}H_{104}O_6$	[52:1]	$[M+NH_4]^+$	878	SOP	4.7	-	-
$C_{55}H_{102}O_6$	[52:2]	$[M+NH_4]^+$	876	POO	4.2	7.7	-
$C_{55}H_{102}O_6$	[52:2]	$[M+NH_4]^+$	876	PVO	-	3.7	-
$C_{55}H_{98}O_6$	[52:4]	$[M+NH_4]^+$	872	PLnO	3.9	-	-
$C_{55}H_{96}O_6$	[52:5]	$[M+NH_4]^+$	870	PEPAP	-	-	7.0
$C_{55}H_{94}O_6$	[52:6]	$[M+NH_4]^+$	868	PDHAM	-	5.0	5.6
$C_{55}H_{94}O_6$	[52:6]	$[M+NH_4]^+$	868	PEPAPo	-	-	2.8
$C_{57}H_{108}O_6$	[54:1]	$[M+NH_4]^+$	906	AOP	2.4	-	
$C_{57}H_{100}O_6$	[54:5]	$[M+NH_4]^+$	898	SEPAP	-	-	3.9
$C_{57}H_{98}O_6$	[54:6]	$[M+NH_4]^+$	896	PDHAP	6.9	6.7	10.3
$C_{57}H_{98}O_6$	[54:6]	$[M+NH_4]^+$	896	PEPAO	-	-	3.2
$C_{57}H_{96}O_6$	[54:7]	$[M+NH_4]^+$	894	PDHAPo	-	4.8	4.1
$C_{57}H_{96}O_6$	[54:7]	$[M+NH_4]^+$	894	MDHAO	-	5.5	-
$C_{59}H_{102}O_6$	[56:6]	$[M+NH_4]^+$	924	SDHAP	6.2	-	5.6
$C_{59}H_{100}O_6$	[56:7]	$[M+NH_4]^+$	922	PDHAO	11.0	14.5	4.8
$C_{59}H_{100}O_6$	[56:7]	$[M+NH_4]^+$	922	PDHAV	-	3.5	3.1
$C_{59}H_{98}O_6$	[56:8]	$[M+NH_4]^+$	920	ODHAPo	-	5.2	-
$C_{59}H_{96}O_6$	[56:9]	$[M+NH_4]^+$	918	PDHALn	5.1	-	-
$C_{59}H_{94}O_6$	[56:10]	$[M+NH_4]^+$	916	PEPAEPA	-	-	2.7
$C_{61}H_{98}O_6$	[58:10]	$[M+NH_4]^+$	944	ODHALn	4.1	-	-
$C_{61}H_{94}O_6$	[58:11]	$[M+NH_4]^+$	942	PDHAEPA	-	-	8.0
$C_{61}H_{106}O_6$	[58:6]	$[M+NH_4]^+$	952	ADHAP	3.2	-	-
$C_{61}H_{104}O_6$	[58:7]	$[M+NH_4]^+$	950	SDHAO	5.0	-	-
$C_{61}H_{102}O_6$	[58:8]	$[M+NH_4]^+$	948	ODHAO	4.4	7.9	-
$C_{61}H_{102}O_6$	[58:8]	$[M+NH_4]^+$	948	ODHAV	-	3.8	-
$C_{61}H_{100}O_6$	[58:9]	$[M+NH_4]^+$	946	SDHALn	2.3	-	-
$C_{63}H_{98}O_6$	[60:12]	$[M+NH_4]^+$	968	PDHADHA	7.3	6.9	5.9
$C_{63}H_{108}O_6$	[60:7]	$[M+NH_4]^+$	978	ADHAO	2.6	-	-
$C_{65}H_{102}O_6$	[62:12]	$[M+NH_4]^+$	996	SDHADHA	3.2	-	-
$C_{65}H_{100}O_6$	[62:13]	$[M+NH_4]^+$	994	ODHADHA	5.8	7.5	-
$C_{65}H_{96}O_6$	[62:15]	$[M+NH_4]^+$	990	LnDHADHA	2.3	-	-
$C_{69}H_{98}O_6$	[66:18]	$[M+NH_4]^+$	1040	DHADHADHA	2.6	-	-

Results expressed as mean of three spectral replicates; ATM: tuna; SAL: salmon; SAR: sardine; fatty acid abbreviations: M: myristic acid (14:0); P: palmitic acid (16:0); Po: palmitoleic acid (16:1n-7); S: stearic acid (18:0); O: oleic acid (18:1n-9); V: vaccenic acid (18:1n-7); L: linoleic acid (18:2n-6); Ln: γ-linolenic acid (18:3n-6); A: arachidic acid; (20:0); EPA: eicosapentaenoic acid (20:5n-3); DHA: docosahexaenoic acid (22:6n-3).

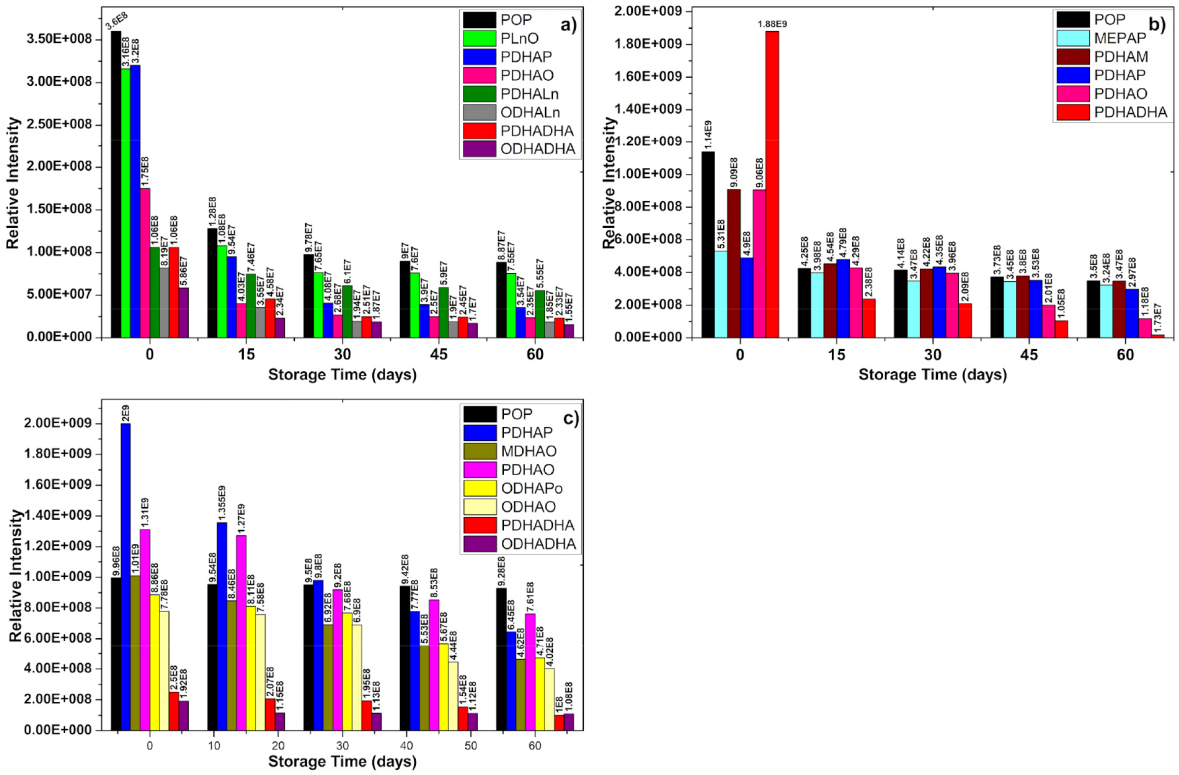

Figure 2. The relative intensity of the main triacylglycerols that undergone a reduction in the samples of a) tuna; b) sardine; c) salmon according to the storage period analyzed

the steric impediment difficult this reaction resulting in less impact on the hydroperoxides production. Oleic acid with a 27% reduction in its percentage is also present in the *sn*-3 position in PLnO and PDHAO, and in the *sn*-1 position in ODHALn. However, studies reporting which TAGs reduce during the storage period of tuna are not available in the literature, thus the present work will be useful as a basis for this proposes.

Figure 2(b) shows the main TAGs that decreased for the salmon fillet sample which were POP, PDHAP, MDHAO, PDHAO, ODHAPo, ODHAO, PDHADHA, ODHADHA. There was only a reduction of 5.75% in DHA during the storage period for the salmon because this fatty acid is mostly in the *sn*-2 position of the TAG making it difficult for the autoxidation process.

The main TAGs which decreased during the storage period for sardine are shown in Figure 2(c) and includes POP, MEPAP, PDHAM, PDHAP, PDHAO, PDHADHA. Despite the high level of DHA in sardine is located in the *sn*-2 position, the TAG PDHADHA is the majority. Therefore, the lipid oxidation of 11.95% of this fatty acid is mostly due to the DHA in the *sn*-3 position of this TAG.

4. Conclusion

Regarding the stability of fatty acids in samples fillets of tuna, sardine, and salmon stored in a commercial freezer at -18 °C, it was found that this temperature was not enough to prevent the lipid oxidation of some main unsaturated fatty acids such as EPA, DHA, oleic, and linoleic acids. Thus, the degradation of these compounds occurred during the sixty days of storage. Moreover, it was also verified that when the unsaturated fatty acid is in the *sn*-2 position of the TAG molecule it undergoes less effective oxidation than in the *sn*-1 and *sn*-3 positions. Hence, it would be viable for the consumer of these fish to quickly consume this food after purchase, avoiding lipolysis or product autoxidation.

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