

Enzymatic Degumming of Ray Liver Oil Using Phospholipase A1: Efficiency, Yield and Effect on Physicochemical Parameters

Daniela Lorena Lamas^{1,2,*}, Agueda Elena Massa^{1,2}

¹National Council of Scientific and Technical Research, Mar del Plata, Buenos Aires, Argentina

²National Institute of Fisheries Research and Development, Mar del Plata, Buenos Aires, Argentina

Email address:

dlamas@inidep.edu.ar (D. L. Lamas)

*Corresponding author

To cite this article:

Daniela Lorena Lamas, Agueda Elena Massa. Enzymatic Degumming of Ray Liver Oil Using Phospholipase A1: Efficiency, Yield and Effect on Physicochemical Parameters. *International Journal of Bioorganic Chemistry*. Vol. 2, No. 3, 2017, pp. 87-93.

doi: 10.11648/j.ijbc.20170203.12

Received: February 15, 2017; **Accepted:** March 9, 2017; **Published:** March 29, 2017

Abstract: Crude oils have components such as metals, pigments, phospholipids and waxes, which alter its physicochemical and organoleptic characteristics. These components are commonly removed during the refining process by physicochemical methods. In recent decades, the oil industry has desired the development of biotechnological processes to replace traditional methods. In this paper the enzymatic degumming of ray liver oil is presented as an alternative innovative, sustainable and process to chemical degumming. The traditional process was performed using citric acid, while the enzymatic process was performed using the enzyme phospholipase A1 as degumming agent. Both treatments were performed in a batch thermostated reactor. The efficiency of the enzyme process, assessed by removing phosphorus percentage content, was significantly higher compared to the traditional treatment, (54.70% and 81.31% respectively). The quality characteristics of crude and degummed oils was determined by acidity, peroxide, p-anisidine and TOTOX indexes, beyond moisture, fatty acids contents, color and density. The fatty acid profile was rich in polyunsaturated omega-3 ratio in all the samples analyzed. Quality parameters evaluated were slightly modified by both degumming processes, highlighting a favorable change in the color and appearance of the oil obtained by the process carried out with enzymes. The obtained results suggest that the enzymatic process produces significant improved quality and nutritional values, with better attempt fish oil characteristics for use in the pharmaceutical and functional foods.

Keywords: Ray Liver Oil, Fatty Acids, Enzymatic Degumming, Quality, Stability

1. Introduction

The largest volumes of cartilaginous fish landed in Argentine ports are caught in the South-West Atlantic region between 34° and 41°S, at depths of less than 50m [1]. Currently, there is a great interest in the commercialization of these species. The fact is reflected in the sustained evolution of landings and the high commercial values acquired by their products as meat, fins, and cartilage, between others [1-3]. The commercialization of these products generates a high percentage of residues containing proteins of high biological value, lipids, vitamins, minerals and other molecules with functional properties [4, 5]. The liver of cartilaginous fish

contains significant amounts of lipids rich in polyunsaturated fatty acids (PUFAs). These fatty acids, particularly the eicosapentaenoic (C20: 5, EPA) and docosahexaenoic (C22: 6, DHA) acids are highly valued for their nutritional properties and their prophylactic and therapeutic actions in different diseases [6-10]. The crude oil has impurities and other compounds, such as free fatty acids, mono and diglycerides, phosphatides, steroids, vitamins, hydrocarbons, pigments, carbohydrates, proteins, and their degradation products, which can make it unsuitable for human consumption [11]. Therefore it is necessary to refine the oil before its use [12]. The method commonly used to improve the characteristics of the oil, from any nature, is the chemical refining process [12, 13]. The steps may include degumming,

neutralization, washing, clarification and deodorization [13]. Degumming is able to remove phosphatides. There are two types of phosphatides: hydratable (HPL) and nonhydratable (NHPL). Most of the phosphatides in crude fish oil can be removed by water degumming, but NHPL is not hydrated by water. Removing NHPL requires more complex process with the use of citric acid or other degumming substances [14].

The aim of the present work was to evaluate the efficiency and performance of the enzymatic degumming process of ray liver oil, and comparing it with the chemical degumming traditional process. In addition, the effect of the applied processes on the composition, quality and stability of the oil was assessed.

2. Materials and Methods

The ray liver oil was provided by a local company Omega Sur S. A (Mar del Plata, Province of Buenos Aires, Argentine). The sample was stored in a refrigerator, in a dark container and free of oxygen until its use. The Phospholipase A1, PLA1, Lecitase Ultra® of *Termomyces lanuginosus*, expressed in a strain of *Aspergillus oryzae* was obtained from Novozymes, (Denmark). This microbial enzyme has an optimal reaction pH in the range of 5 to 6.5 and an optimum temperature of 50°C according to manufacturer. The citrate buffer pH5 was used as the reaction medium; it was prepared from sodium citrate solution ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) and 0.5M sodium hydroxide (NaOH). All reagents used were of analytical grade.

2.1. Degumming Treatments

The degumming tests were conducted in jacketed batch-reactor (100 ml) coupled to a heating plate. The treatment was carried out with continuous stirring provided by magnetic stirrers. The reactor was connected to a thermostated bath with temperature control. Figure 1 shows a graphical scheme of the batch system reactor used for degumming assays. The enzymatic method was performed using a dose of phospholipase A1 about 200U/kg of oil. The process conditions were set according to optimum parameters (pH 5 and 50°C) of the enzyme. The reaction was run for 120 minutes. At the end of this period, the enzyme was inactivated (85°C, 5 minutes) and the oil was removed from the gums by centrifugation at 3500 rpm for 10 minutes. The chemical method was performed using citric acid as the degumming agent, at 2% buffer / substrate ratio. The test was carried out for 120 minutes at 60°C. After the processing time, the sample was cooled to room temperature and the degummed oil was separated from the chemical gums by centrifugation for 10 minutes at 3500 rpm.

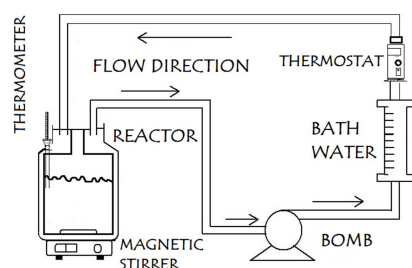


Figure 1. Batch system reactor used for degumming assays.

Efficiency: It was evaluated by spectrophotometric determination of the phosphorus content (AOCS, Ca 12a-02), [15] of crude oil and degummed oils. The result was expressed as the percent reduction of phosphorus content.

$$E = [(Pc-Pd) / Pc] \times 100 \quad (1)$$

E = efficiency

Pc = phosphorus of crude oil

Pd = phosphorus of degummed oil

The yield of oil recovered by the processes was determined as the percentage of the total crude oil used as a sample:

Yield (%) = (Volume of recovered oil/volume of total crude oil). The final loss was measured as crude gums obtained.

$$Y = (Md / Mc) \times 100 \quad (2)$$

Y = yield

Mc = mass of crude oil

Md = mass of degummed oil

2.2. Physicochemical Characterization of Oils

The fish oils thus obtained were characterized according to the American Oil Chemists' Society in terms of the peroxide index (IP) (AOCS, Cd 8-53, [15] and acidity index (AI) (AOCS, Ca 5a-40), [15]. The p-anisidine index IA was carried out by IUPAC Method 2.504 [16]. Total oxidation was determined by the TOTOX index, total oxidation ($\text{TOTOX} = 2\text{PV} + \text{IA}$).

Moisture and volatile material content were evaluated by the vacuum oven method (AOCS, Ca 2d-25), [15]. The relative density was determined using a pycnometer calibrated at 20°C. The color was measured using the Gardner color scale (Gardner-Delta Color Comparator, AOCS Td 1a-64), [15].

For determining the fatty acid profile, the crude and degummed oils were subjected to methylation and subsequent gas chromatography. Procedures for FA methylation were based on those of Dowd [17], with some modifications. Briefly, 60 mg of lipid sample were mixed with 2 ml hexane and 0.3 ml of KOH/MeOH reagent in a glass tube. Sample was mixed vigorously; then, 2 ml of NaCl and 2 ml of hexane were added and mixed again. The sample was allowed to stand for 5 minutes, and the upper hexane layer was separated and transferred to a clean tube. FAME were determined with a Shimadzu GC-2010 equipped with a flame-ionization detector (260°C) and capillary column (30 m x 0.32 mm; 0.25 µm film thickness; Omegawax 320). GC parameters were set as follows: split rate 50, injector temperature 250°C, column temperature 120°C and nitrogen as a carrier gas. The oven temperature was increased to 240°C at a rate of 5°C min⁻¹ and held for 5 min. A volume of 1 µL of sample was manually injected (in duplicate) and FA peaks were identified by comparison of their retention times with those of external reference standards (Supelco FAME Mix C4-C24 + PUFA N°1 Marine Source). Retention times and peak areas were processed by Shimadzu GC Solution software.

FA data were reported as percentage peak area made up of the total area of all FA in the sample.

2.3. Statistical Analysis

All analyzes were performed in triplicate, and expressed as mean \pm standard deviation. The difference between the mean values was assessed using the Duncan test, and was considered significant with a P value of 5%.

3. Results and Discussion

3.1. Efficiency and Yield of the Degumming Treatments

Fish oil contains some impurities, such as phospholipids, which causes many problems for the storage and processing of the crude oil and must be removed from oil during degumming process. Also, these compounds damage the appearance of the oil, darkening the final product and decreasing the yield by flocculation and emulsion [18]. The causes of these oil losses are due by the amphipathic characteristics of this type of molecules, which gives them the ability to carry oil inside, producing important losses [19].

There are two types of phospholipids: hydratable (HPL) and nonhydratable (NHPL), and they are removed from oil by degumming process. Most of the phospholipids in crude fish oil can be removed by the water degumming, but NHPL is not hydrated by water. Removing NHPL required more complex process at increased temperature with the utilization of citric acid or other degumming substances.

Citric acid is used not only for decomposition of metal salt but also as a chelating agent to keep the metals in water-soluble complex [14]. Commonly, the determination used in the industry for phospholipids estimation, is the total phosphorus content. So, the efficiency of the processes was expressed as the percent reduction of phosphorus content, while the yield was determined as the percentage of the oil recovery. The phosphorous content of crude oil was 123.21 mg/kg. The level reported for this element in crude fish oils varies from 5 to 100 mg/kg [20]. Degumming is not usually carried out with animal fats and fish oils because they are low in phosphatides, but values greater than 100 mg/kg require a degumming process, before the alkali-refining [21]. The efficiency and yield values of fish oil after degumming can be seen in Table 1. The results showed that the highest fish oil efficiency was obtained by the enzymatic treatment, while the lowest efficiency value was obtained by the treatment using citric acid. The yield showed the same trend. This behavior may be associated with the highest capacity in separating the phases during centrifugation after enzymatic treatment, due to the rigid consistency of the enzymatic byproducts obtained.

On the other hand, the enzymatic modification produced on the phospholipid molecule, eliminates the emulsifying properties of these compounds, avoiding losses of oils by entrainment [19]. These results are similar to those obtained

in the enzymatic degumming process of other types of oil using the same enzyme [22-26].

Table 1. Efficiency and yield of enzymatic and chemical degumming processes.

Parameters	Sample	
	Enzymatic Degummed	Chemical Degummed
Phosphorous (mg/kg)	23.02 \pm 2.20 a	55.81 \pm 4.47 b
Efficiency %	81.31 \pm 2.20 b	54.7 \pm 4.37 b
Yield %	94.18 \pm 1.86 b	89.03 \pm 2.17 a

Results are expressed as mean \pm standard deviation (n=3).

Different letters in the same row indicate significant differences (Duncan test P < 0.05).

3.2. Physicochemical and Quality Characterization of Oil Samples

The results of the physicochemical and quality parameters of crude and degummed oil using different treatments are presented in Table 2.

The acid value content in the crude oil depends on the fish species and season.

As can be seen in Table 2, the acidity index increased during degumming step for both treatments. This was expected due the addition of citric acid, which may not have completely reacted. On the other hand, the enzymatic reaction produces the hydrolysis and decomposition of the double bond during the treatment, and some fatty acids are released.

On the other hand, during the enzymatic treatment are produced the hydrolysis and decomposition of the double bond and some fatty acids are released. This result is in agreement with other studies from Morais et al. [11] and Crexi et al. [12] for other degummed fish oils.

It was previously reported increased in acidity index in studies from Morais et al. [11] and Crexi et al. [12] for other degummed fish oils. The release of free fatty acids is responsible for the development of the undesirable rancid flavor (hydrolytic rancidity) [26]. Therefore, any increase in the acidity of the oil must be avoided. In the present case, the obtained-acid values not represent drawback for its reduction during the next step of the refining process. A slightly higher value, although not significantly different, was found for the water degummed oil with respect to the crude oil.

Oxidation of the fish oil makes rancidity and flavors higher. An important stage in the oxidation is the addition of oxygen to the fatty acid molecules to form hydroperoxides, the amount of hydroperoxides can be used as measurement of the extent of oxidation in the early stages. The initial peroxide index for crude oils was 13.26 meq/kg. All treatments effectively reduced peroxide value, reaching a minimum of 7.66 meq/kg after enzymatic degumming. Nevertheless, fish oil from all treatments had peroxide value which was in accordance with the International Association of Fish Meal and Oil Manufacturers IFOMA [27] (3-20 meq/kg), but higher than the value required for the Codex Alimentarius Edible Fish Oil Standard CODEX [28] to fish liver oils (less than 5 meq/kg).

Citric acid is commonly used in vegetable oils as a metal chelator. Therefore, citric acid binds the metal ions that contribute to rancidity as they catalyze free radical oxidation of lipids

The results obtained showed the efficiency of the citric acid as an antioxidant at the temperature used in this study. The relative stability of the peroxide values at the lower temperatures is indicated of the efficiency of the citric acid when used as antioxidant for fish oil at low temperature [14]. Akaranta and Akaho [29], stated that citric acid and peanut skin extract that used as antioxidants at low temperature (60-80°C) caused the peroxide values in vegetable oil more stable than at high temperature (150-180°C).

P-anisidine test is a method commonly used as a measure of the level of secondary oxidation products (carbonyl compounds). The highest p-anisidine value was found in crude sample (26.05 meq/kg). This index showed a noticeably reduction during the degumming treatments. P-anisidine values of oils obtained from treatments were in the standard specified by IFOMA, ranged from 4-60 meq/kg [27], and the Eur. Ph. [30], up to 30meq/kg. Guillen and Cabo [31], stated that p-anisidine value is not always in line with the high value of peroxide, but the high value of peroxide can cause high value of p-anisidine if the process given allowing fish oil to go further degradation.

Table 2. Physicochemical parameter of crude and degummed oil sample.

Chemical indexes	Sample		
	Crude liver Oil	Enzymatic degummed Oil	Chemical degummed Oil
Acid value (mg KOH/g)	3.65±0.35 ^a	4.09±0.40 ^b	3.78±0.18 ^a
Peroxide index (meq/kg)	13.26±1.23 ^b	7.66±0.85 ^a	9.27±1.01 ^a
p-anisidine index (meq/kg)	26.05±1.42 ^c	21.74±1.65 ^b	16.68±1.63 ^a
TOTOX (meq/kg)	52.57	37.06	35.21
Physical indexes			
Color (Gardner scale)	42988	7-8	9-10
Moisture (g/100g)	0.129±0.020 ^a	0.16±0.070 ^b	0.155±0.060 ^b
Density (kg/m ³)	931.2±2.20 ^b	925.3±3.10 ^a	926.9±2.90 ^a

Results are expressed as mean ± standard deviation (n=3).

Different letters in the same row indicate significant differences (Duncan test P <0.05).

Peroxide index in conjunction with p-anisidine index can be used to map the past and future degradation profiles. TOTOX is a useful value for quantifying oxygen directed oil degradation [32]. Total oxidation value of crude oil was 52.52 meq/kg. The TOTOX values obtained in all samples were in accordance with the parameters analyzed to obtain it. Fish oil from all treatments had total oxidation value which is in accordance with the IFOMA standard. IFOMA standard assigned total oxidations for fish oil are at range of 10-60 meq/kg [27]. The physical aspect of the ray liver oil was improved by a significant reduction of the dark coloration mainly after enzymatic degumming. Lighter colour of enzymatic degummed oil samples has been attributed in the literature to the extraction of pigments [27]. Both degumming treatments improved the transparency of the crude oil. The color of oils is a determining factor in quality because dark-colored oils require high-cost processing to achieve an acceptable light-colored product.

The ray liver oil density was practically constants with the treatments (Table 2). It was observed a lower decreased after the both degumming treatments but with no significant difference (P > 0.05) between them. The values ranged from Nile tilapia oil range from 927.2 kg/m³ for crude oil to 928.4 kg/m³ for refined oil. The same behavior was observed by Menegazzo et al. [13] from hybrid sorubim oil, which the density varied from 935.0 kg/m³ for crude oil to 936.1 kg/m³ for the end product. The values found in this study were in agreement with values reported to *Dasyatis pastinaca* and *Dasyatis violacea* rays liver oil (916 and 922 kg/m³ respectively) [33], however, it underlines that these characteristics are oil-specific.

Both treatments improved the quality of the crude oil, but

the enzymatic degumming ray liver oil showed a brilliant and transparent light yellow color and an attempt appearance.

The moisture content of crude oil was 0.129%. This is in accordance with the moisture content reported (0.11%) of crude oil from Nile tilapia crude oil [13]. On the other hand, studies in others species found a minor value of moisture such as 0.57% from *Sardinops sagax caerulea* oil, 0.41% from crude hoki oil and 0.72% from tuna oil [34]. The percentage of moisture and volatile matter of degummed oil samples was higher than the crude oil as shown in Table 2. This increasing could be due to the addition of the buffer and aqueous solution necessary for the degummed treatments [25, 26].

3.3. Fatty Acid Profile of Oil Samples

Fatty acid profiles of ray liver oil samples were determined using GCMS. A total of 25 fatty acids were identified in this study; the fatty acid profiles (% of total fatty acids) are depicted in Table 3.

The percentage distribution of saturated, monosaturated and polyunsaturated fatty acids for all samples obtained in the present study is in accordance with data reported in previous studies for different liver oil from other cartilaginous species obtained in the same catch zone [35-37]. From the saturated fatty acids in all samples, palmitic acid (C16: 0) was obtained in the highest amount, accounting about 60% of the total saturated fatty acids.

The saturated fatty acids in all samples showed a predominance of palmitic acid (C16: 0), accounting about 60% of this fraction This result is in agreement with the percentage reported for several marine fish species, e.g., 70% of palmitic acid in the total saturated fatty acids [38].

The total monosaturated percentages of crude, enzymatic degummed and chemical degummed oil samples were similar to each other ($P > 0.05$) with values of 31.61%, 33.15% and 33.05%, respectively. Within the monoenoic fatty acids, the oleic (18:1n9) presented the higher concentration in all samples. These results agreed with the studies on fatty acid composition made by Navarro-Garcia et al. [6], for the liver oil of *Dasyatis brevis* and *Gymnuramarmorata* rays and those of Ozylmaz, and Oksuz [10], for liver oil from cartilaginous fish and Massa et al. [35], for liver oil from cartilaginous species. According to [10], n-9 fatty acids, found as oleic acids (C18:1 n-9), play a moderately important role in the human body. The palmitoleic acid (16:1) was also present in

substantial amounts. Among those unsaturated fatty acids, the n-3 PUFAs content was 33% with 26% of DHA and 1.5% of EPA, which is in the range with the marine liver oil cartilaginous species compositions already found [35-37]. Ozylmaz, and Oksuz [10], reported similar percentages for liver oil from Stingray, Eagle ray and Cownose ray fish species. In addition to these omega-3 compounds, omega-6 fatty acids were also found in all samples: arachidonic acid (20:4 n-6) represented about 1.7% and the level of linoleic acid (18:2 n-6) was approximately 1.5%. Our study confirms previous results [6, 10, 35-37] indicating that ray oil can also be an alternative source for omega-3.

Table 3. Fatty acid composition of liver oil crude and degummed samples.

Fatty acid (g/100g)	Crude Oil	Enzymatic degummed Oil	Chemical Degummed Oil
C14:0	1.84±0.098	2.089±0.045	2.016±0.089
C15:0	0.903±0.018	0.924±0.005	0.918±0.008
C16:0	14.303±0.737	15.602±0.211	15.484±0.239
C17:0	0.772±0.007	0.763±0.016	0.751±0.019
C18:0	2.907±0.074	3.044±0.025	3.037±0.007
C20:0	2.014±0.038	2.54±0.026	2.36±0.052
C21:0	0.306±0.069	0.308±0.010	0.325±0.000
C22:0	1.69±0.110	1.605±0.010	1.597±0.020
C23:0	0.871±0.043	0.993±0.111	0.839±0.018
C24:0	0.478±0.078	0.528±0.149	0.524±0.060
ΣSFA	26.084±0.706	28.396±0.194	27.851±0.264
C16:1 n7	7.7±0.279	8.263±0.086	8.125±0.121
C17:1	0.482±0.031	0.445±0.006	0.486±0.013
C18:1 n7	2.892±0.135	2.801±0.301	3.065±0.009
C18:1 n9	16.339±0.768	17.378±0.053	17.183±0.034
C18:1 n11	0.08±0.013	0.08±0.012	0.065±0.002
C20:1	1.071±0.005	1.207±0.006	1.066±0.001
C22:1 n9	2.362±0.045	2.231±0.230	2.283±0.031
C22:1 n11	0.684±0.005	0.747±0.086	0.776±0.027
ΣMUFA	31.61±1.254	33.152±0.572	33.049±0.120
C18:2 n6	1.444±0.006	1.516±0.030	1.466±0.007
C18:3 n3	2.757±0.013	2.866±0.330	2.748±0.003
C18:3 n6	0.779±0.013	0.801±0.047	0.773±0.006
C20:3 n6	0.57±0.156	0.763±0.055	0.46±0.069
C18:4 n3	2.208±0.107	2.147±0.116	2.195±0.012
C20:4 n6	1.695±0.057	1.727±0.113	1.674±0.026
C22:5 n3	1.581±0.096	1.597±0.145	1.531±0.007
C22:6 n3	26.871±1.201	26.169±0.483	26.446±0.160
Σ PUFA	37.904±1.098	37.585±0.427	37.295±0.239
Σn3	33.417±1.203	32.779±0.182	32.921±0.157
Σn6	4.487±0.105	4.806±0.245	4.374±0.082
EPA+DHA	28.452±1.297	27.766±0.628	27.977±0.167

Results are expressed as mean ± standard deviation (n =3).

4. Conclusions

In any refining step, a high-quality crude product is. The present study demonstrated that both degumming processes resulted in a significant improvement in quality characteristics and nutritional values of crude ray liver oil. The phosphorus content achieved during the degumming processes was in the level required by this type of oils. Some compounds that contributed with the oxidizing were eliminated during both treatments, so it is suggested implement the degumming step of less-stable oils. Color

pigments were better extracted by PLA1 than by citric acid. Therefore, it could be recommended the use of Lecitase® Ultra enzyme when the oil to be treated has dark color. Impurities that enhance the density were removed during all the processes analyzed. The fatty acids composition was not significantly affected in the concentrations after the degumming treatments. Indeed, such oil, which could be obtained in relatively high amounts, is an excellent source of EPA and DHA and after enzymatic degumming had exceptional nutritional characteristic for human food. So, the obtained results suggest that the enzymatic process produces a significant

improved quality and nutritional values, with better attempt fish oil characteristics for use in the pharmaceutical and functional foods.

Acknowledgements

We would like to thank Omega Sur for providing the samples of crude fish oils. Also, we thank to National Council of Scientific and Technical Research and National Fisheries Research and Development Institute for providing the resources for the financial support.

References

- [1] A. Massa, N. Hozbor, L. Lucifora and J. H. Colonello, "Sugerencias de manejo para el 2003 de gatuzo (*Mustelus schmitti*), peces ángel (*Squatina spp.*) y rayas costeras (familia Rajidae)", Informe Técnico Interno INIDEP, 47, 2003, pp. 13.
- [2] R. P. Sanchez, G. Navarro, E. Calvo. and F. Del Castillo, "La pesca y. comercialización de condrictios en la Argentina", Aporte de la Dirección Nacional de Planificación Pesquera para la elaboración del Plan de Acción Nacional. En Wohler O. C., Cedrola, O. and Cousseau M. B. (Eds.). "Contribuciones sobre Biología Pesca y comercialización de tiburones en la Argentina", 2011, pp. 151-184.
- [3] N. M. Hozbor, A. M. Massa, and O. Wohler, "Recomendaciones sobre tiburones y. rayas: Impacto de las medidas establecidas a través del Acta CFP N. 32/2009", Informe Técnico Interno INIDEP, 30, 2012, pp. 8.
- [4] K. Kelleher, "Discards in the world's marine fisheries. An update". FAO Fisheries Technical Paper N 470 Rome, 2005, pp. 131.
- [5] N. E. García Nuñez, "Tiburones: conservación pesca y comercio internacional. Ministerio de Medio Ambiente y Medio Rural y Marino. Ed bilingüe, Madrid, 2008, pp. 117.
- [6] G. Navarro-Garcia, R. Pacheco-Aguilar, L. Bringas- Alvarado, and J. Ortega-Garcia, "Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnuramarmorata rays*". Food Chem. 87, 2004, pp. 89-96.
- [7] S. Akabas, R. Deckelbaum, "N-3 fatty acids: recommendations for therapeutic and prevention", Am. J. Clin Nutr. 83, 2006, pp. 1451-1462.
- [8] M. B., MacLennan, S. E. Clarke, K. Perez, G. A. Wood, W. J. Muller, J. X. Kang, and D. W. Ma, "Mammary tumor development is directly inhibited by lifelong n-3 polyunsaturated fatty acids", J. Nutr. Biochem. 24 (1), 2013, pp. 388-395.
- [9] T. O. Eroldoğan, A. H. Yılmaz, G. M. Turchini, M. Arslan, N. A. Sirkecioğlu, K. Engin, I. Özşahinoğlu, and P. Mumoğullarında, "Fatty acid metabolism in European sea bass (*Dicentrarchus labrax*): effects of n-6 PUFA and MUFA in fish oil replaced diets", Fish Physiol. Biochem. 2013, pp. 941-955.
- [10] A. Ozyılmaz, and A. Oksuz, "Determination of the biochemical properties of liver oil from selected cartilaginous fish living in the northeastern Mediterranean", J. Anim. Plant Sci. 25, 2015, pp. 160- 167.
- [11] M. M. Morais, L. A. A. Pinto, S. C. A. Ortiz, V. T. Crexi, R. L. Silva, and J. D. Silva, "Study of fish oil refining process", Rev. Inst. Adolf Lutz. 60 (1), 2001, pp. 23-33.
- [12] V. T. Crexi, L. A. S. Soares, L. A. A. Pinto, "Carp (*Cyprinus carpio*) oils obtained by fish meal and ensilage processes: Characteristics and lipid profiles", Int. J. Food Sci. Technol. 44, 2009, pp. 1642-1648.
- [13] M. L. Menegazzo, M. E. Petenuci, and G. G. Fonseca, "Production and characterization of crude and refined oils obtained from the co-products of Nile tilapia and hybrid sorubim processing". Food Chem. 2014, pp. 100-104.
- [14] S. H Suseno, A. M. Jacob, S. Bija, N. Fitriana, N. Puspa Ruspatti, "The effect of citric acid and sodium chloride (NaCl) to quality of sardine oil (*Sardinella Sp.*)", Pak. J. Biotechnol. 13 (3), 2016, pp. 181 - 186.
- [15] American Oil Chemists' Society (AOCS), "Official Methods and Recommended Practices of the American Oil Chemists Society", AOCS Press, Champaign, US, 2009.
- [16] International union of pure and applied chemistry (IUPAC), "Standards methods for the analysis of oils, fats and derivatives", In C. Paquot, & A. Hautfenne (Eds.) Oxford: Blackwell Scientific Publications Inc, 2001.
- [17] M. K. Dowd, "Identification of the unsaturated heptadecyl fatty acids in the seed oils of *Thespesia populnea* and *Gossypium hirsutum*". JAOCS, 89, 2012, pp. 1599-1609.
- [18] H. C. Autino, "Temas Selectos en Aceites y Grasas" Vol. 1 e Procesamiento. In J. M. Block, & D. B. Arellano (Eds.), Desgomado, 2009, pp. 97-142.
- [19] F. Gallardo, C. Dayton, H. Autino, M. Odone, V. Imoda, V. and A. Lascano, "Desgomado enzimático de aceites vegetales. Comparación de diferentes alternativas de procesos". Aceites & Grasas, 79, 2010, pp. 204-212.
- [20] A. P. Bimbo, "Guidelines for characterizing food-grade fish oil", Inform, 9, 1998, pp. 473-483.
- [21] J. A. Noriega-Rodríguez, J. Ortega-García, O. Angulo-Guerrero, H. S. García, L. A. Medina-Juárez and N. Gamez-Mezac, "Oil production from sardine (*Sardinops sagax caerulea*) Producción de aceite a partir de sardina (*Sardinops sagax caerulea*)", CyTA J. Food 7, 2009, pp. 173-179.
- [22] K. Clausen, "Enzymatic oil-degumming by a novel microbial phospholipase". Eur. J. Lipid Sci. Technol. 103, 2001, pp. 333-340.
- [23] B. Yang, R. Zhou, J. G. Yang, Y. H. Wang, and W. F. Wang, "Insight into the enzymatic degumming process of soybean oil", JAOCS. 85, 2008, pp. 421-425.
- [24] M. Jahani, M. Alizadeh, M. Pirozifard, and A. Qudsevali, "Optimization of enzymatic degumming process for rice brain oil using response surface methodology", LTW Food Sci. Technol., 41, 2008, pp. 1892-1898.
- [25] D. Lamas, G. H. Crapiste, D. T. Constenla, "Changes in quality and composition of sunflower oil during enzymatic degumming process", LTW Food Sci. Technol., 58, 2014, pp. 71-76.
- [26] D. Lamas, D. T. Constenla, D. Raab, "Effect of degumming process on physicochemical properties of sunflower oil", BAB Journal., 2016, pp. 138-143.

- [27] International Association of Fish Meal and Oil Manufactures (IFOMA), "Method of analysis for chemistry value of fish oil", England 1981.
- [28] Codex Alimentarius Recommended International Code of Practice for the Storage and Transport of Edible Fats and Oils in Bulk, CAC/RCP 36 – 1987 Rome: FAO, http://www.codexalimentarius.net/download/standards/101/CXP_036e.pdd, 2011.
- [29] O. Akaranta, and A. A. Akaho, "Synergic effect of citric acid and red onion skin extract on the oxidative stability of vegetable oil", *J. Appl. Sci. Environ. Manage.* 16 (4), 2012, pp. 345-351.
- [30] Eur. Ph. European Pharmacopoeia, "Fish oil, rich in omega-3 acids". In: *European Pharmacopoeia (7th edn)*. Strasbourg, France: European Directorate for the Quality of Medicines; 1912. EP 01, 2011. European Pharmacopoeia.
- [31] M. D. Guillen, and N. Cabo, "Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils", *Food Chem.* 77, 2002, pp. 503-510.
- [32] A. Miyagi, H. Tani, and M. Kajima, "Reduction in Oxidation Index Value of Fish Oils Using Hydrophobic Nonporous Denser Membrane Process". *Food Sci. Technol. Res.*, 15 (3), 2009, pp. 245 – 248.
- [33] M. Sellami, F. B. Rebah, Y. Gargouri, N. Miled, "Lipid composition and antioxidant activity of liver oils from ray species living in Tunisian coasts", *Arabian J. Chem.* 2014, <http://dx.doi.org/10.1016/j.arabjc.2014.07.010>.
- [34] M. Tengku, R. Tengku, and E. J. Birch, "Physicochemical characterisation and oxidative stability of refined hoki oil, unrefined hoki oil and unrefined tuna oil", *Int. J. food Sci. Technol.*, 48 (11), 2013, 2331–2339.
- [35] A. Massa, A. Fernandez Compas, P. Casagrande, "Determinación de la composición química y perfil de ácidos grasos de especies cartilaginosas presentes en el atlántico sudoccidental", *Informe Técnico Interno INIDEP*, 70, 2011, pp. 7.
- [36] A. Massa, A. Fernandez Compas, P. Casagrande, "Caracterización bioquímica de distintos tejidos del pez angel *Squatina gggeinhem*". *Informe Técnico Interno INIDEP*, 71, 2011, pp. 8.
- [37] A. Massa, M. Vittone, A. Fernandez Compas, P. Casagrande, "Caracterización bioquímica de distintos tejidos gatuzo *Mustelus schmitti*". *Informe Técnico Interno INIDEP*, 39, 2014, pp. 11.
- [38] Y. Ozogul, F. Ozogul, E. Çiçek, A. Polat, E. Kuley, "Fat content and fatty acid compositions of 34 marine water fish species from the Mediterranean Sea" *Int. J. Food Sci. Nutr.*, 60 (6), 2009, pp. 464-475.