Food Chemistry Advances 4 (2024) 100623

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

# Narrownose smoothhound (*Mustelus schmitti*) shark liver: From a residue to a high added value biocompounds

Daniela Lorena Lamas [a,b,*], Agueda Elena Massa [a,b]

[a] Instituto de Investigaciones Marinas y Costeras, IIMyC - Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET – Universidad Nacional de Mar del Plata, UNMdP. B7602GSD, Juan B Justo 2550, B7602GSD, Mar del Plata, Provincia de Buenos Aires, Argentina
[b] Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP Paseo Victoria Ocampo N°1, Escollera Norte B7602HSA, Mar del Plata, Provincia de Buenos Aires, Argentina

ARTICLE INFO

*Keywords:*
Shark
Fish waste
Livers
Oil
Omega-3
Encapsulation
Alginate
Breads

ABSTRACT

The exploration of fishery wastes for the extraction of value-added biocompounds has become a topic of current interest. The aim of this work was to promote a circular economy model by obtaining oil by enzymatic hydrolysis from *Mustelus schmitti* livers and incorporating it into bread. The oil extraction reaction was carried out using Alcalase® 2.4 L at pH 8.0, 55 °C of temperature for 60 min. Fatty acid analysis showed that the oil was rich in ω-3 long chain fatty acids (LCω3PUFAs), with the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) being the main constituents (13 and 70 % of total PUFAs, respectively). Then, an external gelation method was developed to produce alginate capsulates loaded with the extracted oil. The encapsulation efficiency (EE) was 93.41 %. Finally, capsulated and unencapsulated fish oil were used to develop breads and more than 95 % of EPA and DHA were recovered after baking. Thus, 20 g of baked product can provide about 45 % of the recommended minimum daily intake of EPA plus DHA. The results suggest that it is possible to use the liver from the narrownose smoothhound (*Mustelus schmitti*) shark fishery to produce value-added products based on a circular economy.

## 1. Introduction

Nowadays, the responsible use of natural resources is leading to the consideration of alternative and new approaches that allow sustainable development based on the integral use of raw materials. This global transition towards a circular economy affects all socio-productive sectors and fishing activity is one of them. The fish processing industry poses problems in the areas of environmental social and economy, due to the great amount of waste generated by the fish processing. These wastes are considered as important sources of compounds with high added value, such as hydroxyapatite, collagen, gelatin, lipids, enzymes, hydrolysates and bioactive peptides (Ideia et al., 2020). The narrownose smoothhound (*Mustelus schmitti*) is a shark that is commercially exploited by industrial and artisanal fleets in Argentina and Uruguay. It is generally marketed fresh for consumption, peeled, headless and gutted, generating a high percentage of waste and by-products.

Previous reports indicate that cartilaginous fish livers contain large amounts of oil rich in ω−3 long-chain fatty acids (LCω3PUFAs, which are important in the human diet and are of commercial and scientific interest (Özyılmaz & Öksüz, 2015; Sellami et al., 2018; Lamas & Massa, 2019). They are essential for growth, development, optimal functioning, and maintenance of lifelong health and well-being (Calder, 2014). At the functional level, n-3 eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) appear to be the most important due to their essential roles as structural components, signaling messengers, and precursors of metabolic processes (Morais et al., 2015). In addition, many studies have demonstrated the beneficial effects of these fatty acids in the prevention and treatment of some cardiovascular diseases, inflammatory processes, cancer, and hypertension (Calder, 2014). In recent years, the relationship between the consumption of these fatty acids and the reduction of the symptoms of COVID19 has been studied (Weill, 2020). As a result, the use of fish oil has gained increasing attention in the pharmaceutical and food industries over the past few decades.

The conventional method for fish oil extraction is wet reduction, which involves drastic temperature and pressure conditions for protein

* Corresponding author at: Instituto de Investigaciones Marinas y Costeras, IIMyC - Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET – Universidad Nacional de Mar del Plata, UNMdP. B7602GSD, Juan B Justo 2550, B7602GSD, Mar del Plata, Provincia de Buenos Aires, Argentina.
*E-mail address:* dlamas@inidep.edu.ar (D.L. Lamas).

https://doi.org/10.1016/j.focha.2024.100623
Received 30 June 2023; Received in revised form 18 October 2023; Accepted 17 January 2024
Available online 18 January 2024

coagulation and the use of organic solvents in the process (Bonilla Mendez & Concha, 2018). Due to the high degree of unsaturation, fish oil is extremely susceptible to oxidation, whithigh temperatures inducing oxidation and degradation of thermolabile substances while the use of solvents poses a health and environmental risk (Aitta et al., 2021). In line with the circular economy guidelines, the trend is to develop environmentally friendly methods for fish oil extraction based on processes with low energy consumption, allowing the use of alternative solvents, renewable natural substances and, in turn, providing a safe extract/product of high quality (Ivanovs & Blumberga, 2017). In this sense, several methods have been reported such as supercritical fluid extraction, microwave assisted extraction, ultrasound assisted extraction and enzymatic extraction (Ivanovs & Blumberga, 2017). Enzymatic hydrolysis is an emerging technology that offers advantages such as the use of moderate temperatures and pH, where a protease hydrolyzes peptide bonds and releases oil from soluble components in aqueous phases and sediments (Głowacz-Rozynska et al., 2016; Lamas & Massa, 2019; Lamas, 2022).

The importance of the extraction method lies in the need to obtain a constant supply of fresh fish oil with a high content of LCω3PUFAs and with physicochemical and organoleptic properties that are suitable throughout the period of manufacture and storage of fish oil-based products. Oxidation of LCω3PUFAs present in matrix foods can easily degrade their quality. Therefore, it is important to evaluate the chemical and physical factors that promote oxidation during processing, storage and distribution. Exposure to oxygen and light and heating are factors that accelerate the oxidation of fish oil lipids, reducing the stability and shelf life of fish oil-containing products (Kolanowski et al., 2006). Encapsulation technology is widely recognized as an effective tool to deliver and protect oil-based actives from the external environment, overcoming organoleptic limitations of their use in food systems (Bannikova et al., 2018). In this technique, a matrix or polymeric coating protects the fish oil from the effects of pH, temperature, moisture, and oxygen (Khoshnoudi-Nia et al., 2022). Sodium alginate, a water-soluble linear polysaccharide, is successfully used as an encapsulation material of bioactive compounds as its bioaccessibility and bioactivity are preserved due to its chemically inert interior (Wu et al., 2017).

In this context, this work aimed to promote a circular economy model by obtaining fish oil by enzymatic hydrolysis from the livers of narrownose smoothhound (*Mustelus schmitti)*. The oil obtained was characterized by its physicochemical parameters and fatty acid content. This study also reports the preparation of fish oil-loaded capsules using sodium alginate as shell material by the external gelation method. The encapsulation efficiency and the fatty acid content of the prepared capsulates were determined. Finally capsulated and un-encapsulated fish oil were used to develop an omega-3 enriched bread and the EPA and DHA recoveries were calculated. This will allow to valorize the liver waste from the narrownose smoothhound for application in the food industry for human consumption.

## 2. Materials and methods

### 2.1. Biological samples

Specimens of *Mustelus schmitti* gatuzo were caught off during the research cruises performed by the National Institute for Fisheries Research and Development (INIDEP) during 2022. These were received at the "Laboratory of Fishery Products Technology" and immediately gutted. The liver of each specimen was separated, packed in polyethylene bags, and frozen at −80 °C until use. For oil extraction, 2 batches of $n = 17$ livers were used and extraction treatments were applied to each batch.

### 2.2. Enzyme-assisted oil extraction procedure

An enzyme-assisted procedure was used for lipid extraction. The enzyme used was Alcalase® 2.4 L, an endopeptidase from *Bacillus licheniformis*, which is considered GRAS (Generally Recognized as Safe) for protein modification and pet food production, among other applications. Approximately 100 g of minced liver was mixed with water (1:1 w/v) and homogenized at 50 °C. The mixture was then heated to a temperature of 55 °C ± 2.5 °C and enzymatic hydrolysis was initiated by the addition of protease (enzyme/raw material ratio: 2 %). The pH was maintained at 8.0 ± 0.3 and controlled by the addition of 1 M NaOH. The reaction was allowed to proceed for 60 min, after which the enzyme was inactivated by heating at 85 °C for 10 min. The hydrolysate was then centrifuged at 20,000 g for 30 min at 4 °C. Finally, the tubes were placed upright in a freezer (at −20 °C) and all fractions were separated by cutting the frozen contents of the tubes and pooling the oil top layers.

### 2.3. Physicochemical characterization of oils

The nutritional value of the oils extracted by solvent extraction and the oils extracted by the methods studied was evaluated by quantifying the fatty acids. First, the oils were subjected to methylation to convert FAs to fatty acid methyl esters (FAMEs) using hexane and KOH/MeOH (ISO, 2011).The sample was mixed vigorously and then 2 mL of NaCl and 2 mL of hexane were added. The sample was left for 5 min, and the FAMEs dissolved in hexane were analyzed using a gas chromatograph (Shimadzu GC-2010 equipped with a flame ionization detector, Shimadzu Corporation, Kyoto, Japan) and a capillary column (30 m x 0.32 mm; 0.25 μm film thickness; Omegawax 320, Darmstadt, Germany). A split injector (50:1) was used at a temperature of 250 °C. The following temperatures were programmed into the column: 120 °C for 20 min, heated to 200 °C at 1 °C/min, held at 200 °C for 1 min, heated again to 220 °C at 5 °C/min, and finally held at 220 °C for 20 min. The carrier gas used was nitrogen. The temperature of the oven was increased to 240 °C at a rate of 5 °C/min and held for 5 min. A volume of 1 μL of sample was injected and FA peaks were identified using external standards (Supelco FAME Mix C4-C24 + PUFA N°1 Marine Source, Pennsylvania, USA). The results were processed using Shimadzu GC Solution software.

The quality was characterized according to the guidelines of the American Oil Chemists' Society (AOCS) by the acidity index (AI) (AOCS, Ca 5a-40, 2009). In addition, the Peroxide Value (PV) (AOCS, Cd 8-53, 2009) was measured to evaluate the primary oxidation level. Color was measured using the Gardner color scale (Gardner-Delta Color Comparator, Florida, USA, AOCS Td 1a-64, 2009). Relative density was determined using a pycnometer calibrated at 20 °C.

### 2.4. Preparation of fish oil rich in LCω3PUFAs beads

Fish oil obtained by enzymatic reaction was encapsulated using sodium alginate (Sigma Aldrich) by external gelation according to Alvarez and Lamas (2021). Sodium alginate was dispersed in preheated distilled water with stirring until homogenized (15 g/L). This solution was allowed to stand overnight at room temperature with constant stirring. The discontinuous emulsion was then prepared by adding the oil to the alginate solution. A 1:3 ratio of dispersed oil phase to continuous phase was used. The mixture was then homogenized for 2 min at medium speed using an Ultra-Turrax (Omni Mixer, Germany) to obtain the emulsion. The emulsion was then extruded through a tip-ended syringe and dropped into 0.1 M calcium chloride (CaCl2) gel to form oil-loaded calcium alginate beads. The tip was fixed 5 cm above the surface of the gelling bath. The solution was gently stirred with a magnetic stirrer to prevent the beads from sticking, and left in the gelling bath for approximately 30 min to harden. Finally, the beads were stored in a desiccator for 7 days and then oven dried at 30 °C for 24 h.

### 2.5. Encapsulation efficiency

To evaluate the encapsulation efficiency (EE), the unencapsulated oil was measured according to Morales et al. (2020) with modifications.

The lipid residues constituting the unencapsulated oil were weighted. First, a filter paper was used to absorb the excess oil from the wet beads. The filter paper was then dried in an oven until a constant weight was reached, followed by solid-liquid extraction with hexane for 5 h. Hexane was also used to extract the oil present in the calcium chloride solution, which was also quantified. The amount of encapsulated oil (Wo3) was calculated as the difference between the initial amount of oil used to prepare the emulsion (Wo1) and the total unencapsulated oil (Wo2).

$$Wo3 = Wo1 - -Wo2.$$

The EE was expressed as the percentage of encapsulated oil regarding the amount of oil weighted firstly.

$$EE\,(\%) = 100 \times (Wo3\,/\,Wo1)$$

To determine the free fatty acid profile of the encapsulated fish oil, lipids were extracted by the method of Bligh and Dyer (1959) and analyzed by methylation followed by gas chromatography according to ISO (2011).

### 2.6. Shape and sphericity of beads

Size and shape were determined by analyzing a photographic sequence using a Canon PowerShot G9 camera attached to a Zeiss Stemi 2000-C binocular loupe with Axiovision measurement software. The maximum and minimum diameter (dmax, dmin) were determined, and the sphericity was estimated as follows:

$$\Phi = 2\,\mathrm{dmin}/(\mathrm{d\,min} + \mathrm{d\,max}).$$

The average size and shape of the beads were calculated from at least 25 randomly selected beads from each batch. For shape, if the value obtained by the calculation of $\Phi > 0.95$, they were considered spherical and slightly oval; if $0.95 > \Phi > 0.90$, they were considered oval and pear-shaped, and for values below 90, the beads are deformed, i.e. unacceptable (Davarcı et al., 2017).

### 2.7. Breads formulations, developed and recoveries of EPA and DHA after baking

The experimental formulation of the developed breads contained the typically used ingredients such as wheat flour, whole wheat flour, salt, dehydrated yeast and water. Encapsulated and unencapsulated fish oil was added directly to develop a bakery product with high LCω3PUFA content as described in Table 1. These ingredients were mixed, homogenized, and kneaded. Kneading was performed using a Peabody brand stand mixer (PE-BM101 220v) until the desired texture was achieved, and then the bread dough was held for 30 min to leaven. The loaves were then baked at 120 °C for 25 min in pans (3 cm high; 6 cm diameter). Finally, the loaves were removed from the hot molds and allowed to cool to room temperature.

To determine the nutritional composition of the breads, moisture content, ash, protein, carbohydrates and fiber were measured (AOAC, 2005 methods). To assess the effect of baking on target fatty acids, recoveries of EPA and DHA were determined according to Henna and Norziah (2011). First, all bread samples were ground using a domestic mill (Philips) at high speed for 3 cycles of 30 s. Then, lipid extraction was performed using cloroformo: metanol, according to the method of Bligh and Dyer (1959). Then, the extracted lipid was subjected to methylation followed by gas chromatography as previously described (ISO, 2011) to evaluate the fatty acid profile. The following calculations expressed the recoveries of EPA (EPA R) and DHA (DHA R):

$$EPAR\,(\%) = 100 \times (\text{EPA Content GC analysis} \,/\, \text{EPA content added in the bread})$$

$$DHA\,R\,(\%) = 100 \times (\text{DHA Content GC analysis} \,/\, \text{DHA content added in the bread})$$

The encapsulation efficiency obtained was considered to calculte the final content of EPA and DHA added.

**Table 1**
Formulation of breads.

| Ingredient g% | Control | FEO | FFO |
|---|---|---|---|
| Wheat flour | 28 | 28 | 28 |
| Whole wheat flour | 28 | 28 | 28 |
| Fish Oil | – | 3 | 3 |
| Yeast | 0.4 | 0.4 | 0.4 |
| Salt | 0.6 | 0.6 | 0,6 |
| Water | 43 | 40 | 40 |
| Total | 100 | 100 | 100 |

FEO Encapsulated fish oil, FFO Free fish oil.

### 2.8. Statistical analysis

The experiments were performed in duplicate, and the results expressed as a mean value ± standard deviation. The coefficient of variation was assed to evaluate data dispersion, and taking as an acceptable value less than or equal to 10 %, indicative that the arithmetic mean was representative of the data set. To carry out the analysis, Duncan's test was conducted using the InfoStat (2017) Software.

## 3. Results and discuss

### 3.1. Extraction yield and physicochemical characterization of the obtained oils

According to Bligh & Dyer's determination, *Mustelus schmitti* livers showed a fat content of 19.90 %. The solvent extraction is assumed to result in the recovery of the total lipids present in the raw material. Enzymatic hydrolysis is a suitable method for the extraction of lipids from fishery by-products, which is more efficient than traditional thermal oil extraction in terms of oil recovery (Ivanovs & Blumberga, Zhang et al., 2021). The protease used, its activity, concentration, pH, and temperature affect oil yield and recovery (Ivanovs & Blumberga, 2017). In this study, the percentage yield of the oil obtained by enzymatic hydrolysis with respect to the initial fat content was around 80 %. Previous results using Alcalase® 2.4 L to extract oil from viscera of *Trachurus lathami* were lower (Lamas & Massa, 2022). Using the same enzyme, Gbogouri et al. (2006) achieved about 90 % oil yield from salmon heads. In contrast, Głowacz-Rozynska et al. (2016) obtained lower yields from the same feedstock (70 % oil from salmon heads) by hydrolysis with other proteases. Liu and Dave (2022) reported that the highest oil recoveries obtained with the use of Alcalase® 2.4 L were 96.3 % from the heads and frames of farmed Atlantic salmon and 67.1 % from the viscera of farmed Atlantic salmon. This suggests that the raw material, mainly the tissue type and lipid content, influences the final yield of extracted oil (Lamas & Massa, 2019).

The quality and oxidative stability of the extracted oil samples were evaluated (Table 2). The acid value was found to be 3.69 mg KOH/g oil and 3.27 mg KOH/g oil for solvent and enzyme extracted oil, respectively. Acidity is considered an important quality parameter because it is associated with the freshness of the raw material. In addition, the extraction methods used and the composition of the oil affect the acidity levels (Lamas & Massa, 2019). The PV indicates the degree of rancidity at the time of testing (De Greyt & Kellens, 2005). The oils showed an acceptable PV for crude fish liver oil of good quality for human consumption, which was less than 5 mEq /kg oil in both cases (Codex Alimentarius, 2017). The optimal values obtained for the oil enzyme extracted suggest that the procedure was carried out with care, keeping it free from the effects of oxygen, heat and light. According to Masson (1994), several limits and quality standards have been established for

**Table 2**
Physicochemical characteristics of Narrownose smoothhound liver oil.

| Parameter | Oil sample B&D | Alcalase® 2.4L |
|---|---|---|
| Acidity (mg KOH/g) | 3.69a±0,17 | 3.27a±0.13 |
| Peroxide value (meq$O_2$/kg) | 4.36b±0.11 | 3.52a±0.173 |
| Color (Gardner scale) | 11–12 | 7–8 |
| Density (mg/mL) | 0.938a±0.003 | 0.931a±0.003 |

Values are expressed by mean value ± standard deviation ($n = 2$).
Different lowercase letters in the row denote significant differences among the extraction methods ($p < 0.05$). "a" means the lowest value, "b" means the highest value.
B&D Bligh & Dyer extraction method.
Alcalase® 2.4 L extraction method.

fish oil for fish feed, with fresh oil having peroxide levels between 3.9 and 5 meq $O_2$/kg.

Regarding the color, shark liver oil enzyme extracted showed a dark yellow, translucent and bright hue. The Gardner scale showed values from 7 to 8 being optimun regarding the data of quality standards for crude fish oils (Gardner scale of 14) (Lamas & Massa, 2019). The solvent-extracted oil was darker and more opaque, with a Gardner scale value between 11 and 12. The density values obtained were in accordande with other values reported for cartilaginosus fish liver oils (Sellami et al., 2018; Lamas & Massa, 2019; Lamas, 2022).

### 3.2. Fatty acids profile of oils

The knowledge of the nutritional properties of the oils extracted from the Narrownose smoothhound liver was the first step towards their valorization in order to evaluate the formulation of bakery products and to be able to disseminate their quality. The fatty acid composition of shark liver oils obtained by solvent and enzymatic extraction are shown in Table 3. Also, the fatty acids profile obtained from the capsulates are described there. The profile of all samples is distributed as follows: PUFAs, saturatef fatty acids (SFAs), and monounsaturated fatty acid (MUFAs).

The most dominant SFA was palmitic acid (C16:0), followed by stearic acid (C18:0) and myristic acid (C14:0). Higher amounts of palmitic acid have been reported in liver oils from other cartilaginous species (Ould El Kebir et al., 2007; Sellami et al., 2018; Lamas & Massa, 2019; Lamas, 2022). Furthermore, the major MUFA was oleic acid (C18:1) followed by palmitoleic acid (C16:1). These results are consistent with data previously reported for other cartilaginous species caught in the same region, such as *Zearaja flavirostris* and *Atlantoraja castelnaui* (Lamas & Massa, 2019). In addition, Sellami et al. (2018) found a predominance of the same fatty acids in the MUFAs fraction in oils obtained from different ray species from the Tunisian coasts. Interestingly, the main LCω3PUFAs were EPA (C20:5) and DHA (C22:6), with the content of both in the oil obtained with enzymes standing out with respect to the oil obtained by extraction with solvents. As can be seen, EPA and DHA represent around the 8 and 77 % of the total PUFAs, respectively, in the oil obtained by solvent extraction. The content of EPA and DHA were found to be 13 and 70 %, respectively, in the enzyme-assisted obtained shark oil. The results were similar to those reported in studies from livers of other cartilaginous species using the same extraction methods (Lamas & Massa, 2019). Based on the fatty acid composition, oils from the liver of the shark Narrownose Smoothhound (*Mustelus schmitti*) could be considered an important source of LCω3PUFAs.

Thus, the fatty acid content obtained in this work and the physicochemical characteristics of the oil allow its encapsulation without the need for a refining treatment. In this way, the oil obtained by enzymatic hydrolysis was subjected to encapsulation and its efficiency and subsequent use in the preparation of bread were analyzed.

**Table 3**
Fatty acid composition (g fatty acid/100 g of oil) of Narrownose smoothhound (*Mustelus schmitti*): Oil extracted by solvents and enzymatic treatment, and encapsulated.

| Fatty acid | Oil sample B&D | Alcalase ® 2.4L | FEO |
|---|---|---|---|
| 14:0 | 1.97a±0.91 | 2.61a±0.13 | 2.16a±0.18 |
| 14:1 | 0.06a±0.01 | 0.04a±0.01 | 0.03a±0.01 |
| 15:0 | 0.36a±0.02 | 0.52a±0.08 | 0.33a±0.04 |
| 15:1 | 0.19a±0.02 | 0.23b±0.02 | 0.15a±0.01 |
| 16:0 | 13.20a±0.92 | 14.56a±1,23 | 12.55a±0.88 |
| 16:1 | 1.55a±0.05 | 2.10a±0.54 | 1.74a±0.62 |
| 17:0 | 0.51a±0.08 | 1.01a±0.07 | 0.53a±0.05 |
| 17:1 | 0.20a±0.02 | 0.23a±0.02 | 0.23a±0.03 |
| 18:0 | 4.18a±0.29 | 5.85b±0.22 | 4.05a±0.17 |
| 18:1n9 c y t | 10.43a±0.55 | 13.41a±1.24 | 11.49a±0.87 |
| 18:1n7 | 2.07a±0.64 | 2.50a±0.20 | 2.03a±0.33 |
| 18:2n6c | 0.42a±0.02 | 0.87a±0.04 | 0.49a±0.09 |
| 18:2n6t | 0.10a±0.03 | 0.11a±0.01 | 0.10a±0.01 |
| 18:3n6 | 0.14a±0.01 | 0.22b±0.01 | 0.10a±0.02 |
| 18:3n3 | 0.20a±0.01 | 0.46b±0.07 | 0.23a±0.03 |
| 20:0 | 0.19a±0.02 | 0.21a±0.01 | 0.15a±0.01 |
| 20:1 | 0.26a±0.01 | 0.40b±0.03 | 0.42b±0.02 |
| 20:2 | 1.39a±0.15 | 2.11b±0.33 | 1.25a±0.05 |
| 20:3n6 | 0.12a±0.02 | 0.13a±0.03 | 0.07a±0.02 |
| 21:0 | 0.02a±0.00 | 0.03a±0.01 | 0.04a±0.00 |
| 20:4 n-6 ARA | 3.18ab±0.18 | 4.28b±0.44 | 2.45a±0.22 |
| 20:3n3 | 0.09a±0.01 | 0.18a±0.04 | 0.09a±0.01 |
| 20:5 n-3 EPA | 4.07a±0.22[a] | 7.42b±1.11 | 5.86ab±1,83[a] |
| 22.0 | 0.08a±0.01 | 0.11±0.09 | 0.12a±0.03 |
| 22:1n9 | 0.28a±0.03 | 0.43±0.04 | 0.34a±0.01 |
| 24:0 | 0.07a±0.02 | 0.02a±0.00 | 0.04a±0.00 |
| 22:6 n-3 DHA | 36.17ab±2.06 | 38.03a±2.75 | 33.09a±1.17 |
| 24:1 | 0.28a±0.03 | 0.25a±0.01 | 0.40a±0.04 |
| SFA | 20.57a | 24.94b | 19.96a |
| MUFA | 16.32a | 20.58b | 17.83a |
| PUFA | 46.87a | 53.80b | 44.71a |

Values are expressed by mean value ($n = 2$) ±standard deviation. The same letter in the line means values of each snacks are not different by the Duncan test ($p < 0.05$). a it means the minor value, b it means the major value.
B&D Bligh& Dyer extraction method.
Alcalase® 2.4 L extraction method.
FEO Fish encapsulated oil.

### 3.3. Oil encapsulation, efficiency and fatty acid profile

The external gelation method to produce alginate capsules is widely used due to its simplicity, making it a versatile technique in various biological and pharmaceutical applications. Alginate hydrogel are usually used as a carrier matrix to encapsulate molecules with biological importance (Chan et al., 2011). The capsules are obtained due to the matrix formed by the cross-linking of the alginate chains present in the emulsion and the $Ca^{2+}$ ions of the bath solution where the mixture is dripped (Martins et al., 2017). The efficiency of the encapsulation is related to the degree of cross-linking at the polymer surface of the extruded droplet of emulsion (Bannikova et al., 2018). The encapsulation efficiency obtained in the present work was 93.41 %. Reports of previous work on encapsulation using the same method, using similar concentrations of alginate and carrier oil, showed an EE of 70 % (Bannikova et al., 2018) and 90 % Chan (2011). Morales et al. (2020) reported an EE between 83 and 97 % using alginate and *Prosopis nigra* biopolymer by the same method.

Thus, the efficiency obtained was satisfactory to evaluate its possible use in the fortification of bakery products. However, to decide on its suitability, the fatty acid profile was also analyzed. As expected, the fatty acid profile of the encapsulated oil showed the same distribution as that of the hydrolyzed oil used for encapsulation. The EPA content obtained in the capsule was 78.94 % with respect to the encapsulated free oil, while that of DHA was 87.02 %.

On the basis of these values, the required oil and capsule content was designed to prepare bakery products that provide a significant amount

of LCω3PUFAs.

### 3.4. Size and shape of wet beads

The size and shape of the beads obtained are shown in Fig. 1. The average bead size was calculated by measuring at least 25 beads from 19 randomly selected batches. The average size of the beads produced was 3 mm in diameter. Regarding the shape of the beads, a sphericity factor described by Davarcı et al. (2017) was used to determine it quantitatively. The sphericity index obtained from the measurement of the 19 batches was 0.97. Therefore, the beads were found to be spherical. Using the same concentration of alginate and similar oil loading (between 30 and 40 %), Chan (2011) reported that beads obtained from palm oil using the same encapsulation technique measured approximately 2 mm and were spherical. Similarly, Davarci et al. (2017) obtained beads with a sphericity index of 0.95 for beads produced with the same alginate concentration as in the present study. According to Chan (2011), the minimum concentration of alginate required to form spherical beads was 15 g/L, which is associated with a solution viscosity of 130 mPa s, and is the same as that used in this work. It is likely that by using this minimum concentration, viscous surface tension forces and impact drag forces compete to maintain the shape of the droplet as the alginate liquid droplet enters the gelling bath. In contrast, the viscosity of lower concentration alginate solutions is not sufficient to counteract the effects of impact and drag. As a result, the droplets deform (Chan, 2011).

### 3.5. Breads nutritional properties

In order to add value to the extracted oil and to evaluate the possible benefits of its encapsulation, bread was formulated as the final link in the circular economy model. Bread is a universally consumed food, which makes it a suitable product to be enriched with LCω3PUFAs. As shown in Table 4, the nutritional composition of the breads analyzed had no significant differences in ash and showed good protein content. Lipids were higher in the breads with encapsulated and unencapsulated oils than in the control. The fiber and carbohydrate contents correlated with the wheat flour used as raw material. The incorporation of LCω3PUFAs into bakery products presents the challenge of evaluating the effect of high cooking temperatures and exposure to oxygen during processing. In addition, in general, the long shelf life of different products requires that the fatty acids are stable over time, which is not an issue for bread, which usually has a short shelf life (Hernandez, 2013). In the present work, the focus was on the effect of processing and the selected cooking conditions on the content of fatty acids, which was acceptable for both encapsulated and free oil. As shown in Table 5 the concentration of EPA and DHA in breads containing encapsulated and free fish oil after baking was high. The recoveries of both fatty acids were more than 95 %, although the results obtained for DHA were slightly better. EPA recovery was higher in bread with encapsulated fish oil than in bread with free fish oil, but the difference was not significant. These results are similar to those found in previous work of our group (data not published). Regarding the minimum recommended daily intake for an optimal contribution of the benefits of these fatty acids mentioned above, 20 g of bread would contribute about 45 % of it (Simopoulos, 2002; Kris-Etherton et al., 2009).

In this sense, these results are acceptable and appropriate for the product developed as freshly baked. However, it will be necessary to study the shelf life of this product and to evaluate if there is a more noticeable difference between free and non-free fish oil in this case.

The choice of fortification of fresh bread was based on its universality, convenience and ease of consumption and its short shelf life. It is believed that the shelf life of bread is relatively short due to changes associated with aging such as starch retrogradation, loss of moisture content, distribution of water content between amorphous and crystalline zones, loss of flavor and crispness, increase in crumb and crust firmness, and reduction in specific volume (Gray & Bemiller, 2003). On

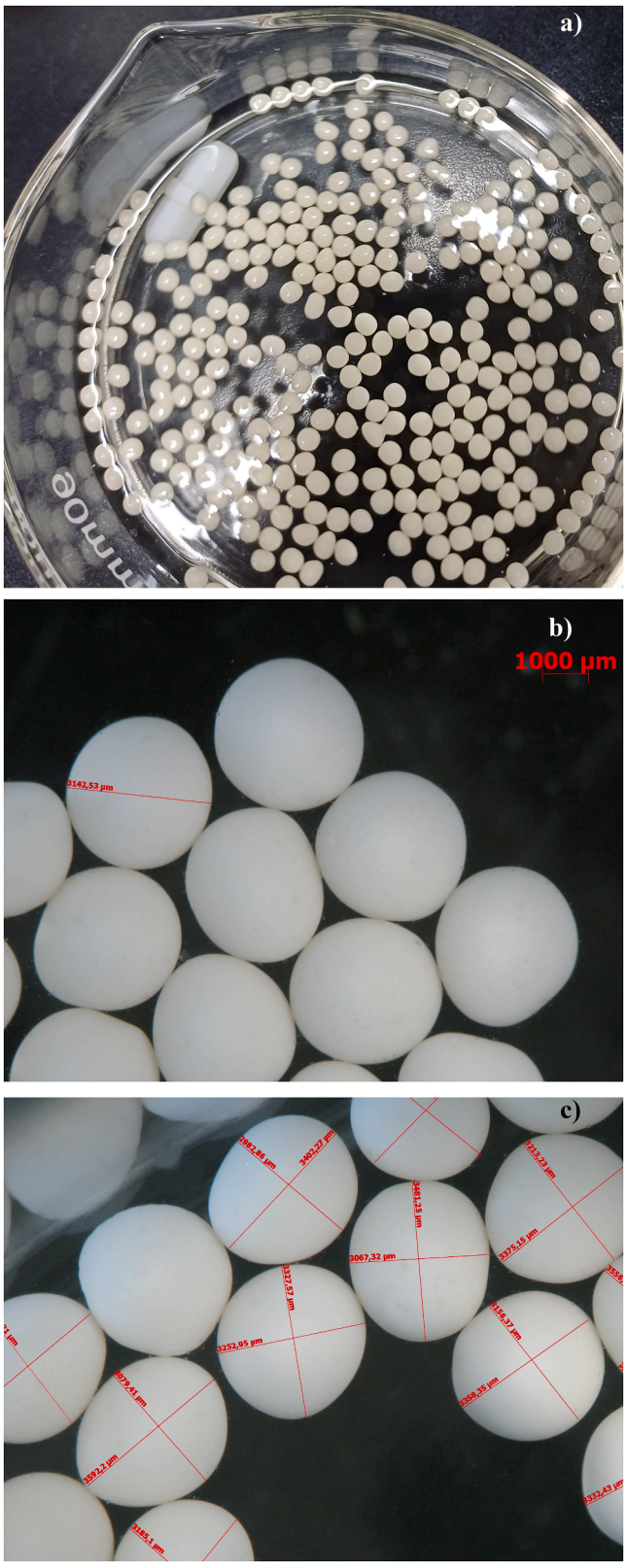


**Fig. 1.** Images of size and shape of oil-loaded calcium alginate wet beads a) in calcium chloride collecting bath b) in calcium chloride collecting bath with Canon PowerShot G9 camera attached to Zeiss Stemi 2000-C binocular loupe c) in calcium chloride collecting bath with measuring bars.

**Table 4**
Nutritional value of breads.

| Sample | % Moisture | % Ash | % Protein | % Lipids | %Carbohydrates | % Fiber |
|---|---|---|---|---|---|---|
| Control | 36.36±0.16a | 1.28±0.10a | 6.76±0.03a | 1.52±0.22b | 52.11±2.24a | 1.97±0.24b |
| FEO | 31.48±0.48a | 1.18±0.04a | 6.58±0.15a | 3.22±0.31b | 56.02±3.48b | 1.52±0.08a |
| FFO | 35.53±0.45b | 1.09±0.02a | 7.11±0.48ab | 2.41±0.24a | 52.30±2.72a | 1.56±0.03a |

Values are expressed by mean value ($n = 2$) ± standard deviation.
The same letter in the column means values of each snacks are not different by the Duncan test ($p < 0.05$).
a it means the minor value, d it means the major value.
FEO Encapsulated fish oil, FFO Free fish oil.

**Table 5**
Percentage recovery of EPA and DHA in bread added with encapsulated or free fish oil.

| Sample | EPA mg/ 100 g | EPA R % | DHA mg/ 100 g | DHA R % | EPA+DHA mg/100 g bread | EPA+DHA mg/20 g bread |
|---|---|---|---|---|---|---|
| FEO | 160.76 ±0.65[a] | 97.90 ±0.40[b] | 918.76 ±1.75[a] | 99.05 ±0.20[a] | 1079.52 ±2.78[a] | 215.90 ±3.22[a] |
| FFO | 168.09 ±1.07[a] | 95.18 ±0.61[a] | 981.05 ±9.82[a] | 98.79 ±1.00[a] | 1148.38 ±5.35[b] | 229.67 ±4.73[b] |

Values are expressed by mean value ($n = 2$) ±standard deviation. The same letter in the column means values of each snacks are not different by the Duncan test ($p < 0.05$).
a it means the minor value, c it means the major value.
FEO Encapsulated fish oil, FFO Free fish oil.

the other hand, it is known that spoilage is also induced by microorganisms that affect the shelf life of bread, especially in breads with high moisture content. Thus, it would be of interest for future studies to analyze both the effect of free and encapsulated fish oil on the physicochemical characteristics and microbiological quality of breads and the stability of fish oil added at the same time.

## 4. Conclusions

The fishing industry generates large amounts of waste worldwide throughout the year, highlighting the need to focus efforts on its reuse and promote the implementation of a circular economy in this sector. This study found that the liver of the shark narrownose smoothhound (*Mustelus schmitti*) is an important source of oil rich in long-chain ω−3 fatty acids. Its chemical and nutritional properties were shown to position it as a candidate for use as an encapsulated and non-encapsulated ingredient in bread formulation and potentially other functional foods for human consumption. Spherical capsules of 3 mm diameter were obtained with an encapsulation efficiency of more than 90 % using the method chosen to formulate the capsules. The breads produced had a good protein and fiber content compared with this type of product, while the enriched breads were able to retain concentrations of EPA and DHA of more than 95 %, even after baking. It should be noted that an enzymatic process was used for oil extraction, which gave good yield performance and represents an emerging technological alternative that does not have a negative impact on the environment. Finally, it is important to mention that this study is in line with several Sustainable Development Goals (SDGs) proposed by the United Nation.

## Ethical statement

The work not involves the use of live animals.

## Funding

This work was supported by the PIP 11220200101524CO National Council for Scientific and Technical Research, CONICET Argentine research grant.

## CRediT authorship contribution statement

**Daniela Lorena Lamas:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Agueda Elena Massa:** Funding acquisition.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This work was supported by the PIP 11220200101524CO National Council for Scientific and Technical Research, CONICET Argentine research grant.

## Data availability

Data will be made available on request.

## Acknowledgments

The authors would like to thank the Bromatologist Marina Vittone for her collaboration in the proximal analysis of the formulated breads. We would also like to thank the Biologist Paola Betti for her help in explaining the software and the use of the camera and magnifying glass for the characterization of the alginate capsules.

## References

Aitta, E., Marsol-Vall, A., Damerau, A., & Yang, B. (2021). .Enzyme-assisted extraction of fish oil from whole fish and by-products of baltic herring (*Clupea harengus membras*). *Foods, 10*(8), 1811 (Basel, Switzerland).

Alvarez, S. T., & &Lamas, D. L (2021). Chemical characteristics and sensory properties of novel snacks produced with okara fortified with omega-3 from fish oil. *International Journal of Food Studies, 10*, 182–194.

AOAC, Official Methods of Analysis. (2005). *Association of official analytical chemists* (16th Ed.). Washington DC: AOAC International.

AOCS, American Oil Chemists' Society. (2009). *Official methods and recommended practices of the American oil chemists society*. Champaign, US: AOCS Press.

Bannikova, A., Evteev, A., Pankin, K., Evdokimov, I., & Kasapis, S. (2018). Microencapsulation of fish oil with alginate: *In-vitro* evaluation and controlled release. *LWT Food Science and Technology, 90*, 310–315.

Bligh, E. L. G., & Dyer, W. J. A. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Bonilla-Méndez, J. R., & Hoyos-Concha, J. L. (2018). Métodos de extracción, refinación y concentración de aceite de pescado como fuente de ácidos grasos omega-3. *Ciencia y Tecnología Agropecuaria, 19*(3), 645–668.

Calder, P. C. (2014). Very long chain omega-3 (n-3) fatty acids and human health. *European Journal of Lipid Science and Technology, 116*, 1280–1300.

Chan, E. S. (2011). Preparation of Ca-alginate beads containing high oil content: Influence of process variables on encapsulation efficiency and bead properties. *Carbohydrate Polymers, 84*(4), 1267–1275.

Codex Alimentarius. (2017). Codex Standard for fish oils. Codex Alimentarius Commission FAO/OMS. *Stan, 329-2017*. https://www.iffo.com/system/files/downloads/Codex%20Standard%20for%20Fish%20Oils%20CXS_329e_Nov%202017.pdf.

Davarcı, F., Turan, D., Ozcelik, B., & Poncelet, D. (2017). The influence of solution viscosities and surface tension on calcium-alginate microbead formation using dripping technique. *Food Hydrocolloids, 62*, 119–127.

De Greyt, W., Kellens, M., & Shahidi, F. (2005). Deodorization. *Bailey'S industrial oil and fat products: Processing and technologies* (6th ed., pp. 352–357). New Jersey, NJ: Wiley Interscience. Vol. 5.

Gbogouri, G. A., Linder, M., Fanni, J., & Parmentier, M. (2006). Analysis of lipids extracted from salmon (*Salmo salar*) heads by commercial proteolytic enzymes. *European Journal of Lipid Science and Technology, 108*, 766–775.

Głowacz-Rozynska, A., Tynek, M., Malinowska-Panczyk, E., Martysiak Zurowska, D., Pawłowicz, R., & Kołodziejska, I. (2016). Comparison of oil yield and quality obtained by different extraction procedures from salmon (*Salmo salar*) processing byproducts. *European Journal of Lipid Science and Technology, 118*, 1759–1767.

Gray, J. A., & Bemiller, J. N. (2003). Bread staling: Molecular basis and control. *Comprehensive Reviews in Food Science and Food Safety, 2*, 1–21.

Hernandez, M. (2013). *Enrichment of baked goods with omega-3 fatty acids, in food enrichment with omega-3 fatty acids* (pp. 319–335). Woodhead Publishing Limited.

Ideia, P., Pinto, J., Ferreira, R., Figueiredo, L., Spínola, V., & Castilho, P. C. (2020). Fish processing industry residues: A review of valuable products extraction and characterization methods. *Waste and Biomass Valorization, 11*, 3223–3246.

InfoStat (2017). Software estadístico http://www.infostat.com.ar.

ISO (2011). 12966-2:2017. International organization for standardization. *Animal and vegetable fats and oils* — Gas chromatography of fatty acid methyl esters — Part 2: Preparation of methyl esters of fatty acids. https://www.iso.org/standard/72142.html.

Ivanovs, K., & Blumberga, D. (2017). Extraction of fish oil using green extraction methods: A short review. *Energy procedia, 128*, 477–483.

Khoshnoudi-Nia, S., Forghani, Z., & Jafari, S. M. (2022). A systematic review and metaanalysis of fish oil encapsulation within different micro/nanocarriers. *Critical Reviews in Food Science and Nutrition, 62*, 2061–2082.

Kolanowski, W., Jaworska, D., & Weißbrodt, J. (2006). Importance of instrumental and sensory analysis in the assessment of oxidative deterioration of omega-3 long-chain polyunsaturated fatty acid-rich foods. *Journal of the Science of Food and Agriculture, 87*, 181–191.

Kris-Etherton, P. M., Grieger, J. A., & Etherton, T. D. (2009). Dietary reference intakes for DHA and EPA. Prostaglandins, Leukotrienes and Essential Fatty. *Acids, 81*, 99–104.

Lamas, D., & Massa, A. (2019). Ray liver oils obtained by different methodologies: Characterization and refining. *Journal of Aquatic Food Product Technology, 28*(5), 555–569.

Lamas, D., & Massa, A. (2022). Enzymatic hydrolysis of residues from the rough scad Trachurus lathami processing: Characterization of the obtained fractions. *Revista Científica Universidad de San Carlos de Guatemala, 30*(2), 1–12.

Lamas, D. (2022). Effect of enzymatic degumming process on the physicochemical and nutritional properties of fish byproducts oil. *Applied Food Research, 2*, Article 100170.

Liu, Y., & Dave, D (2022). Beyond processing waste: Extraction of oil from Atlantic salmon (*Salmo salar*) by-products using immobilized Alcalase on chitosan-coated magnetic nanoparticles. *Aquaculture, 548*, Article 737546 (Amsterdam, Netherlands).

Henna Lu, F. S., & Norziah, M. H (2011). Contribution of microencapsulated n-3 pufa powder toward sensory and oxidative stability of bread. *Journal of Food Processing and Preservation, 35*(5), 596–604.

Martins, E., Poncelet, D., Rodrigues, R. C., & Renard, D. (2017). Oil encapsulation techniques using alginate as encapsulating agent: Applications and drawbacks. *Journal of Microencapsulation, 34*(8), 754–771.

Masson, L. S. (1994). Criterio de calidad para materias grasas utilizadas frecuentemente en la nutrición animal y de peces. In E. Campos (Ed.), *Control de calidad se insumos y dietas acuícola* (pp. 77–92). FAO. Documento de campo N° 16. Proyecto aquila II. Mexico.

Morais, S., Mourente, G., Martinez, A., Gras, N., & Tocher, D. R. (2015). Docosahexaenoic acid biosynthesis via fatty acyl elongase and Δ4-desaturase and its modulation by dietary lipid level and fatty acid composition in a marine vertebrate. *Biochimica et Biophysica Acta, 1851*, 588–597.

Morales, A. H., Alanís, A. F., Jaime, G. S., Lamas, D. L., Gómez, M. I., Martínez, M. A., et al. (2020). Blend of renewable bio-based polymers for oil encapsulation: Control of the emulsion stability and scaffolds of the microcapsule by the gummy exudate of *Prosopis nigra*. *European Polymer Journal, 140*, Article 109991.

Ould El Kebir, M. V., Barnathan, G., Gaydau, E. M., Siau, Y., & Miralle, S. J. (2007). Fatty acid in liver, muscle, and gonad of three tropical rays including non-methylene-interrupted dienoic fatty acids. *Lipids, 42*, 525–535.

Özyılmaz, A., & Öksüz, A. (2015). Determination of the biochemical properties of liver oil from selected cartilaginous fish living in the northeastern Mediterranean. *Journal of Animal and Plant Sciences, 25*, 160–167.

Sellami, M., Rebah, F., Gargouri, Y., & Miled, N. (2018). Lipid composition and antioxidant activity of liver oils from ray species living in Tunisian coasts. *Arabian Journal of Chemistry, 11*, 233–239.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy, 56*(8), 365–379.

Weill, P., Plissonneau, C., Legrand, P., Rioux, V., & Thibault, R. (2020). May omega-3 fatty acid dietary supplementation help reduce severe complications in Covid-19 patients? *Biochimie, 179*, 275–280.

Wu, Q., Zhang, T., Xue, Y., Xue, C., & Wang, Y. (2017). Preparation of alginate core–shell beads with different M/G ratios to improve the stability of fish oil. *LWT- Food Science and Technology, 80*, 304–310.

Zhang, Y., Sun, Q., Liu, S., Wei, S., Xia, S., Ji, H., et al. (2021). Extraction of fish oil from fish heads using ultra-high pressure pre-treatment prior to enzymatic hydrolysis. *Innovative Food Science and Emerging Technologies, 7*, Article 102670.