Proceedings of the 11th World Congress on New Technologies (NewTech'25)
Paris, France - August, 2025
Paper No. ICBB 116
DOI: 10.11159/icbb25.116

Development of Bioproducts from Fish Waste

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Abstract **-** Waste from the fish industry is rich in organic and inorganic compounds, raising concerns regarding improper disposal's potential environmental and ecological impacts. In Pinheiro, Maranhão-Brazil, fish waste generated by commercial activities is frequently disposed of improperly, particularly in the municipal market, where the high circulation of people and animals poses additional risks. Therefore, reusing this waste is essential to address environmental and socio-economic challenges. This study aimed to assess the residual fish biomass in the municipality by analyzing the waste generated by fish commercialization activities and the existing demand for bioproducts. The experiment was conducted in the Chemistry Laboratory at the Federal University of Maranhão (UFMA). Approximately two kilograms of fish waste, consisting of offal and fat, were initially collected from the street market for oil extraction procedures. Fatty acids were extracted using two thermal methods and one solvent-based method. Data analysis revealed that the samples extracted through oven and pressure methods showed a comparable relative area percentage for methyl elaidate, methyl 8,11-octadecadienoate, methyl oleate, and methyl stearate. In contrast, the Soxhlet extraction method predominantly yielded methyl oleate and methyl 11,14-eicosadienoate esters. The results demonstrated the potential for fish oil production, which can be applied in various contexts, alongside generating residual bran with promising properties for animal feed production. Furthermore, additional waste fractions can produce collagen, enzymes, and inorganic catalysts.

Keywords**:** waste, fish, extraction methods, fish oil, co-products.

1. Introduction

Seafood is a rich source of bioactive compounds, including micronutrients, minerals, essential fatty acids, and proteins [1,2]. Fish oil exhibits highly variable fatty acid profiles, typically resulting from the levels of fatty acids present within the trophic chain of a given ecosystem [2].

Fish oil generally comprises a range of fatty acids (saturated, monounsaturated, and polyunsaturated) and is particularly rich in vitamins, especially vitamin A [1]. The key constituents include long-chain polyunsaturated fatty acids, various lipid components, protein hydrolysates, peptides, essential minerals and vitamins, gelatin, and collagen. Among its numerous beneficial effects, fish co-products demonstrate therapeutic efficacy against cardiovascular diseases, hypertension, asthma, inflammatory bowel disease, rheumatoid arthritis, osteoporosis, and even neoplasms [3].

In the quest for less polluting alternatives and to minimize the environmental issues caused by fish waste, there is a growing need to establish waste utilization systems. These systems are both economically viable and conservation-oriented, particularly in terms of energy conservation. This can range from maximizing the use of raw materials to the development of new products utilizing waste [4,5]

Fish industry waste is rich in both organic and inorganic compounds, raising concerns about the potential environmental impacts associated with improper disposal [6,7].

In the municipality of Pinheiro, in northeastern Brazil, fish waste from commercial activities is frequently disposed of improperly, particularly at the municipal market, where there is a high volume of people and animals. Therefore, it is crucial to implement recycling strategies to mitigate potential environmental and socio-economic issues. The objective of this study was to obtain bioproducts from fish waste.

2. Methods

2.1. Obtaining Fatty Acids and Bioproducts from Fish Waste

Fatty acids were extracted using two thermal methods and one solvent-based method. Other potential low-cost extraction routes were also evaluated. Methodologies were developed to extract other valuable compounds from fish waste, including swim bladder.

2.2. Development of New Extraction and Separation Processes for Valuable Compounds

Methodologies for separating fatty acids and isolating valuable compounds were developed. Additionally, deodorization and removal of impurities from the extracted compounds were performed.

Liao [8] reported that lipids were determined by extraction using the Soxhlet method. A 150 mL volume of solvent (hexane) was used, which was placed in the flask and heated using a heating mantle to its boiling point (approximately 69 ºC). When the solute/solvent mixture filled the siphon, it was emptied and returned to the flask, where it was heated again. This reflux process was repeated until the end of the 8-hour extraction period.

Another method for oil production involves cooking 1 kg of waste in a pressure cooker at a high temperature (110ºC) for an average of 1 hour and 30 minutes. After cooking, the material is filtered, and the supernatant is removed. Then, it is placed in an oven at 60ºC for 24 hours to remove excess water.

The extraction methods also include thermomechanical processes, such as autoclaving at 150ºC and centrifugation. Afterward, the material is transferred to beakers and placed in an oven at 106ºC, where it is kept until it melts completely. While still hot, the oil is separated using a pipette and filtered through filter paper. The oil is stored in a glass container and refrigerated at 4ºC.

2.3. Physicochemical Analysis of Bioproducts

The protein, lipid, and moisture contents of the fish waste were analyzed. The fatty acids were evaluated for acidity index, iodine index, saponification index, oxidation stability, water content, viscosity at 40 ºC, and density at 20 ºC, in addition to the fatty acid profile.

To determine the oil's fatty acid profile, methyl esterification was performed following the methodology described by Hartman and Lago [9]. The quantification was obtained using a calibration curve with methyl ester standards (Supelco® 37 Component FAME Mix), and analysis was conducted using a GCMS-QP2010 (Shimadzu, Kyoto, Japan) equipped with a Durabond DB-23 column (30 m x 0.25 mm x 0.25 μm).

Analyses were conducted to produce bioproducts and raw materials for other commercial syntheses, such as cosmetics, pharmaceuticals, food, feed, and biofuels. These analyses aim to support the technological development of new bioproducts in the region.

2.4. Characterization of Residual Biomass

Studies have been conducted on the use of residual biomass from fish waste (after extracting the oil and ICBB compounds of interest). These studies aim to determine the physical and chemical characteristics of this material and later assess its potential use for feed and cosmetics. The following analyses were conducted: water content, relative density, porosity, elemental analysis, and calorific value.

The results obtained were compared with data from biomass used for bioproducts.

3. Results and Discussion

3.1 Obtaining Fatty Acids and Bioproducts from Fish Waste

The experiment was conducted in the Chemistry laboratory at the Federal University of Maranhão (UFMA). Initially, the waste (offal and fat) was collected at the free market in the municipality of Pinheiro, with approximately 2 kilograms of waste acquired for the oil extraction procedures. The fish guts were randomly collected without considering their respective species. The waste was ground and homogenized using a Philco All-in-One Citrus 800W multiprocessor.

Three processes were used to produce the oil. The first process followed the methodology proposed by Fujiwara et al. [10], in which 1 kg of waste was cooked in a domestic pressure cooker at a high temperature (120ºC) for an average of 40 minutes.

After cooking and cooling, the supernatant (oil) was removed. The second process was carried out according to Bery et al. [11], in which the material was heated in an oven for one hour at 110ºC, followed by separation of the composition by centrifugation. The Soxhlet method, according to IUPAC 1.122, was used for the last process, with hexane as the solvent. Three 2g samples were weighed into cartridges. The extraction lasted 6 hours, and the extracted oil was then placed in an

oven to dissipate any residual solvent.

Figure 1: Oil extraction equipment (A) Pressure cooker (B) Oven (C) soxhlet

After extraction, the lipids were packed in glass containers and stored in the refrigerator until the samples were submitted for chromatography. Chromatographic analyses were performed at the Fuels and Materials Laboratory of the Federal University of Paraíba and the Alternative Technologies Laboratory of the Federal University of Sergipe.

3.2 Development of New Extraction and Separation Processes for Valuable Compounds

The extraction processes were analyzed, and the oils obtained were characterized using gas chromatography. For the qualitative analysis of the fatty esters in the fish oils, solutions of approximately 1000 mg L^-1 were initially prepared from stock solutions of 5000 mg L^-1. The analyses were conducted using GC/MS equipment (Shimadzu QP2010 plus, Tokyo, Japan) equipped with an auto-injector (split/splitless).

To separate the compounds, a 5% phenyl—95% dimethylpolysiloxane ZB-5 column (53 m x 0.25 mm x 0.25 μm) was used, with ultra-pure helium as the carrier gas (White Martins S.A) at a flow rate of 1 mL min-1. The oven temperature program started at 90 °C (1.5 min), then increased to 190 °C at a heating rate of 5 °C min-1 (4 min), reaching the final temperature of 240 °C at a heating rate of 4 °C min-1 (5 min). The injector and interface temperatures were set to 200 °C and 250 °C, respectively. The injection mode was split (1:10), with electron impact ionization (EI) set at 70 eV. The total analysis time was 43 minutes. The system operated in SCAN mode, enabling qualitative identification of the compounds by comparing the spectra with the reference spectra from the NIST 107, 21, and Wiley 8 libraries. Peaks with similarity values above 80% and a relative area greater than 0.10% were considered.

Characterization was performed to determine the ester profile present in the oil; however, due to the coronavirus pandemic, the physicochemical properties (acidity index, iodine index, saponification index) to obtain by-products were not determined.

The chromatographic studies provided an overview of the probable esters in the fish waste oils obtained through extraction. Figure 2 presents the analyzed samples' Total Ion Current Chromatograms (TICCs).

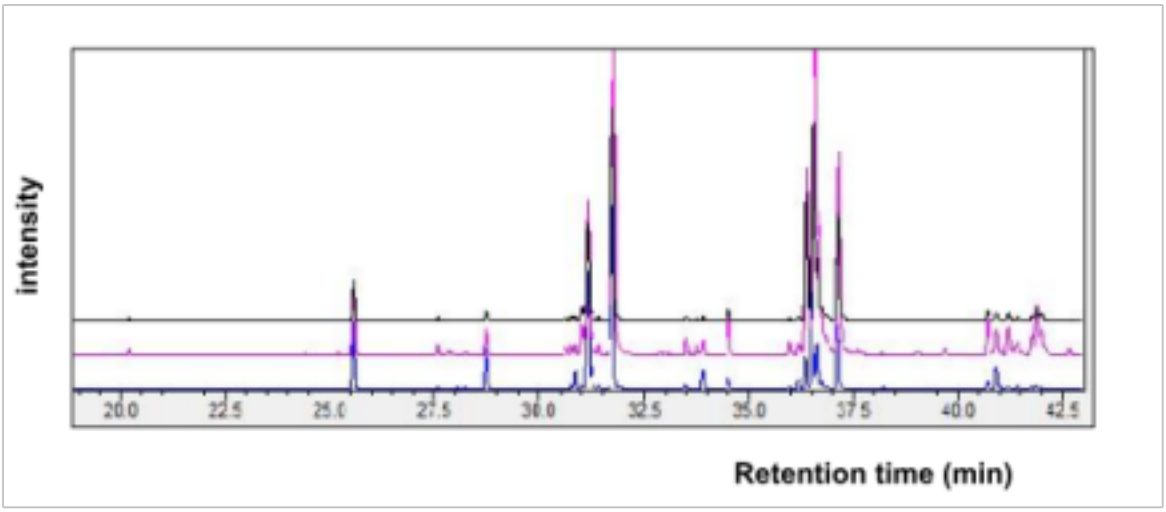

Figure 2: TICCs of the oils obtained from the waste in the different extraction methods.

The chromatograms showed a similarity in the profile and chemical composition of the esters identified across the different extraction methods used to obtain the oils; however, the relative percentage area of these compounds varied.

Several co-elutions were observed, indicating that the analysis could be further refined by modifying the chromatographic conditions. The qualitative results, as relative percentage area, are presented in Table 1.

Cn:Cni	**Relative area of esters (%)**			
	Name IUPAC	Samples		
		Greenhouse	**Pressure**	**Soxhlet**
C14:0	methyl tetradecanoate	3,1	2.86	7.40
C15:0	methyl pentadecanoate	0,72	0.88	4.17
C16:0	methyl hexadecanoate	0,2	0.31	0.25
C18:3	cis,cis,cis-9,12,15-methyl octadecatrienoate	0,26	0.31	1.93

C18:2	cis, cis-9,12- methyl octadecatrienoate	0,3	0.33	0.37
C18:1	cis-9-methyl octadecatrienoate	1,37	1.46	14.83
C18:1	trans-9-methyl octadecatrienoate	8,33	6.73	2.36
	trans-10,13-methyl octadecatrienoate	0,77	0.81	0.30
	nd	0,29	0.40	25.02
	nd	21,72	19.61	0.49
C18:0	methyl octadecatrienoate	0,31	0.57	2.32
C18:1	trans-9-l octadecatrienoate	0,31	0.57	1.11
C20:4	cis, cis, cis, cis-5,8,11,14-methyl eicosatetraenoate	0,23	0.50	0.94
C20:3	cis-11,14,17-methyl eicosatetraenoate	0,26	0.44	3.71
C20:2	11,14-methyl eicosatetraenoate	0,22	0.39	15.43
	8,11-	10,94	9.52	5.60
C18:1	cis-9-octadecadienoate	27,3	22.39	0.82
C18:1	trans-9-octadecadienoate	5,05	4.95	7.41
	nd	2,03	2.66	0.81
C18:1	methyl octadecanoate	10,03	11.80	3.05

C22:5	cis,cis,cis,cis,cis,cis-4,7,10,13,16,19- methyl docosahexaenoate	0,85	0.25	0.52
C20:3	cis-7,10,13-methyl eicosatrienoate	0,74	1.52	0.20
	nd	0,4	1.36	
	nd	1,16	1.08	
	nd	0,82	1.00	

nC:number of carbons; ni: number of double alloys nd: not detected

Data analysis revealed that the oven- and pressure-extracted samples exhibited similar relative percentages of area for the compounds methyl elaidate, 8,11-octadecadienoate, methyl oleate, and methyl stearate. In contrast, most of the esters in the sample obtained by Soxhlet extraction were methyl oleate and methyl 11,14-eicosadienoate. Notably, some peaks were not identified due to the presence of isomeric compounds that co-eluted, highlighting the necessity of using standards to better elucidate these compounds.

The results of this study demonstrated the production of fish oil, which can be utilized in various applications, and the output of residual bran with promising properties for animal feed. Additionally, other residues can be applied to produce collagen, enzymes, and inorganic catalysts. However, this study could not be fully concluded due to the need for further experiments.

4. Conclusions

The study highlighted the necessity of implementing hygienic and sanitary treatment and public policies to raise traders' awareness. It also observed that increasing the number of experimental tests, repeating extractions, using appropriate equipment to remove waste at the municipal market, and collecting waste at the optimal time (as material characteristics change and consequently affect the bioproducts extracted) are essential steps.

It was determined that repeating extractions leads to a higher yield of oil and bran, thus providing more material for further physicochemical tests, which can better inform potential applications. The COVID-19 pandemic hindered the progress of laboratory activities, ultimately affecting the achievement of more robust results for this work plan.

Despite these challenges, the project underscored the significance of the issue, both in obtaining new by-products and ensuring the proper disposal of waste to minimize environmental impacts. The study also highlighted the need for public policies focused on environmental and food safety concerns.

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