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Environmental and Economic Life Cycle Assessment of Enzymatic Hydrolysis-Based Fish Protein and Oil Extraction

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Abstract: As global fish consumption rises, a large amount of waste is generated that is generally neglected. Considering the value embedded in these resources, sustainable methods become more important in extracting valuable ingredients from fish processing residues. Enzymatic hydrolysis is a fast and easily reproducible method for recovering protein ingredients and obtaining valuable by-products. To confirm its advantages, an environmental and economic impact assessment is essential. This study overviewed the sustainability and economic viability of extracting protein compounds and oil from Atlantic mackerel processing residues using enzymatic hydrolysis. Life cycle assessment (LCA) and life cycle cost analysis (LCCA) methods were employed. It was found that the climate change impact of the whole process was 0.073 kg CO_2-eq per 1 g of fish protein hydrolysate (FPH). As the process produces FPH as the main product and fish oil as the by-product, economic allocation was used to distribute the impacts of FPH and fish oil. The findings of the LCCA showed that producing 1 g of FPH costs EUR 3.68. The contribution analysis indicated the crucial role of electricity and fish in environmental impacts. To ensure the accuracy of the calculation, the results of an LCA study published previously were recalculated. The sensitivity analysis showed that the results were susceptible to the region and source of electricity production. This research provides valuable insights into the sustainability and economic aspects of using enzymatic hydrolysis for extracting protein ingredients and oils from Atlantic mackerel. This can inform future investigations of environmentally friendly and economically viable solutions for extracting fish ingredients.

Keywords: enzymatic hydrolysis; LCA; LCCA; fish protein hydrolysate; fish oil; atlantic mackerel fish; sustainability

Citation: Bashiri, B.; Cropotova, J.; Kvangarsnes, K.; Gavrilova, O.; Vilu, R. Environmental and Economic Life Cycle Assessment of Enzymatic Hydrolysis-Based Fish Protein and Oil Extraction. *Resources* **2024**, *13*, 61. https://doi.org/10.3390/resources13050061

Academic Editors: Carlo Ingrao, Alberto Bezama, Annarita Paiano, Jakob Hildebrandt and Claudia Arcidiacono

Received: 8 February 2024
Revised: 16 April 2024
Accepted: 19 April 2024
Published: 24 April 2024

1. Introduction

Over the past few decades, global fish consumption has experienced a steady annual increase. In the 1960s, the worldwide per capita consumption stood at approximately 9.0 kg, nearly doubling by 2016 to exceed 20 kg per person [1,2]. The European Union shows an increase in per capita consumption from 22 to 24 kg among its members from 1998 to 2030. Notably, countries such as Spain and Portugal in the Iberian Peninsula are projected to have 39 and 57 kg per capita consumption, respectively, by 2030 [2]. The increasing trend in global fish consumption leads to generating a large amount of waste and by-products from the fish industry. This waste has generally been neglected, causing a loss in value embedded in this resource. In addition, the massive expansion of the aquaculture industry is causing significant environmental consequences [3].

With the increase in global fish consumption and consequential increase in the generated waste and processing residues, the demand for sustainable extraction methods to valorize the fish side streams and waste and isolate valuable compounds like protein and oil also rises [4,5]. This could make the aquaculture sector and fish industry more sustainable by developing a circular economy concept [6,7] but may also potentially put more pressure on the environment. This is because environmental impacts increase as the number of stages in a process increases [8,9]. Researchers have investigated alternative extraction methods, such as enzymatic extraction, that balance environmental considerations with product quality, recognizing the importance of addressing these concerns [5]. These sustainable methods enable us to meet the growing demand for fish products without putting more pressure on the environment. This change of attention is in line with the growing consumption of fish and is indicative of a positive move towards more sustainable and responsible use of marine resources.

Different methods have been utilized to extract protein and oil from fish products, such as heat application, chemical processes, subcritical water hydrolysis technique, and enzymatic procedures [10,11]. Researchers are investigating environmentally friendly extraction methods due to concerns about the environmental impact of solvent extraction [12]. On the other hand, heat extraction methods are time-consuming and may compromise the integrity of health-beneficial compounds. Subcritical water hydrolysis is environmentally friendly due to the absence of enzymes. However, it has some disadvantages such as high working temperatures (100–374 °C), which could lead to the thermal degradation of some heat-sensitive compounds. Also, subcritical water is more reactive and corrosive which makes the process optimization more difficult [11]. In recent times, enzymatic extraction has become popular for its environmentally friendly approach and adaptability. Enzymatic processes enjoy advantages over traditional methods, resulting in protein ingredients that have better biological value and are more digestible and functional [13].

Enzymatic hydrolysis is a fast and replicable method for isolating protein and extracting oils from insoluble solids [4,14–16]. Moreover, it has better control, which reduces the chance of undesired reactions that may damage high-value components like proteins [17]. Enzymatic hydrolysis does not leave residual toxic chemicals; therefore, it is preferred in food processing [4]. A potential drawback is the high cost of enzymes [18]. Nevertheless, enzymatic hydrolysis is a reasonable alternative for recovering proteins and creating products with potential applications in various areas [2]. Evaluating the sustainability performance of enzymatic hydrolysis becomes crucial in understanding its environmental impact. Sustainability considerations can also include economic aspects.

Life cycle assessment (LCA) is a very popular tool for the investigation of sustainability in various industrial, agricultural, and aquaculture sectors [19,20]. So far, the LCA studies in the aquaculture sector have been focused on fish capture technologies across different regions [21], but the sustainability of fish by-product extraction processes has been underinvestigated. As the aquaculture industry develops, there is a growing recognition of the need to conduct LCA studies to gain a more comprehensive understanding of the environmental impacts throughout the entire value chain, including by-products. A recent review by Ruiz-Salmon and colleagues discusses the LCA of fish and seafood products, and their main focus is on different fish species and associated industries [22]. A recently published paper by Garofalo et al. about oil extraction from tuna fish viscera using enzymatic hydrolysis incorporates an LCA study [23]. Another LCA, conducted by Coelho et al., investigates the production of meatballs from herring (*Clupea harengus*) and lingonberry (*Vaccinium vitis-idaea*) pomace, products rich in protein. These studies demonstrate the varying applications of LCA in assessing the environmental impact of fish-related processes [24].

This study aimed to perform LCA and life cycle cost analysis (LCCA) of FPH and oil extraction from Atlantic mackerel processing residues using enzymatic hydrolysis to shed light on its environmental performance. The fish processing residues used here are by-products and side streams of the fish processing facilities.

2. Materials and Methods

LCA is a well-established scientific tool for analyzing environmental footprints (e.g., global warming, eutrophication, and human toxicity) of products or processes [25]. According to the definition provided by the International Organization for Standardization (ISO), the framework of LCA has the following four stages: (a) goal and scope definition of the study, (b) inventory analysis, (c) impact assessment, and (d) interpretation for conclusions and recommendations [26–30].

2.1. Goal and Scope Definition

The definition of the goal is an essential step in conducting an LCA. It includes identifying the reasons for the assessment, determining the target audience, and specifying the product that will be studied. The LCA scope is defined in this stage, which involves establishing system boundaries, determining the functional unit (FU), deciding on an allocation method, and considering relevant assumptions.

This research project has three main goals:

- The first goal is to gain an overview of the environmental impacts of producing FPH and fish oil from Atlantic mackerel processing residues for human consumption through the enzymatic hydrolysis process. This enables us to recognize the hotspots and most important contributing flows to the environmental impacts.
- The second goal is to compare the environmental performance of FPH and fish oil.
- The third goal is to investigate the sensitivity of the LCA results to the geographical location of production.

Due to the small scale of the production in this study, the chosen FU is one gram (1 g) of FPH. All the environmental impacts of FPH and fish oil are evaluated per 1 FU (per 1 g of FPH).

2.2. System Boundary and Process Description

Figure 1 presents the process flow diagram and system boundary of the experiment. In September 2022, the Atlantic mackerel processing residues without viscera were obtained at a local fish company “Fosnavåg” and then taken to the Department of Biological Sciences Ålesund of NTNU (Ålesund, Norway) laboratory for processing. The transportation distance covered was approximately 68 km. The fish processing residues were generally without viscera, but some minor viscera were found that were separated in the preparation stage. The Atlantic mackerel processing residues were minced using a 4.5 mm hole size mincer (Hobart A 200 N). After mincing, the resulting fish mince was divided into 1 kg batches for further experiments. The batches were stored in a cold room at 5 °C for one week. Hydrolysis experiments were conducted in 4 L closed glass vessels in a water bath set at 52 °C. Warm (50 °C) distilled water was added to the fish mince in a 1:1 ratio, and the mixture was stirred at 150 rpm using an overhead stirrer. Enzymes (0.1% Alkalase from Novozymes) were added when the mixture reached a temperature of 50 °C.

Following 60 min of hydrolysis, bones were removed by filtering the hydrolysate through a sieve. The enzymes were then inactivated by heating the mixture at 90 °C for 10 min in a microwave oven. After cooling, the mixture was transferred to one-liter centrifugation bottles and centrifuged at $4100\times g$ at 4 °C for 30 min. Three phases were created: lipids, soluble proteins, and a sludge. The liquid phase (lipids and proteins) was separated from the solid phase (sludge) and further separated in a separative funnel. The resulting soluble water phase containing proteins was collected, frozen at −80 °C for 24 h and dried in a freeze drier for 72 h. The oil fraction was collected and frozen at −80 °C for further processing. The protein obtained in this process is soluble protein. The insoluble protein was separated into the sludge fraction during the enzymatic hydrolysis process. It is worth mentioning that the working temperature in the current experiment is below the temperature achieved in subcritical water hydrolysis and avoids damage to proteins.

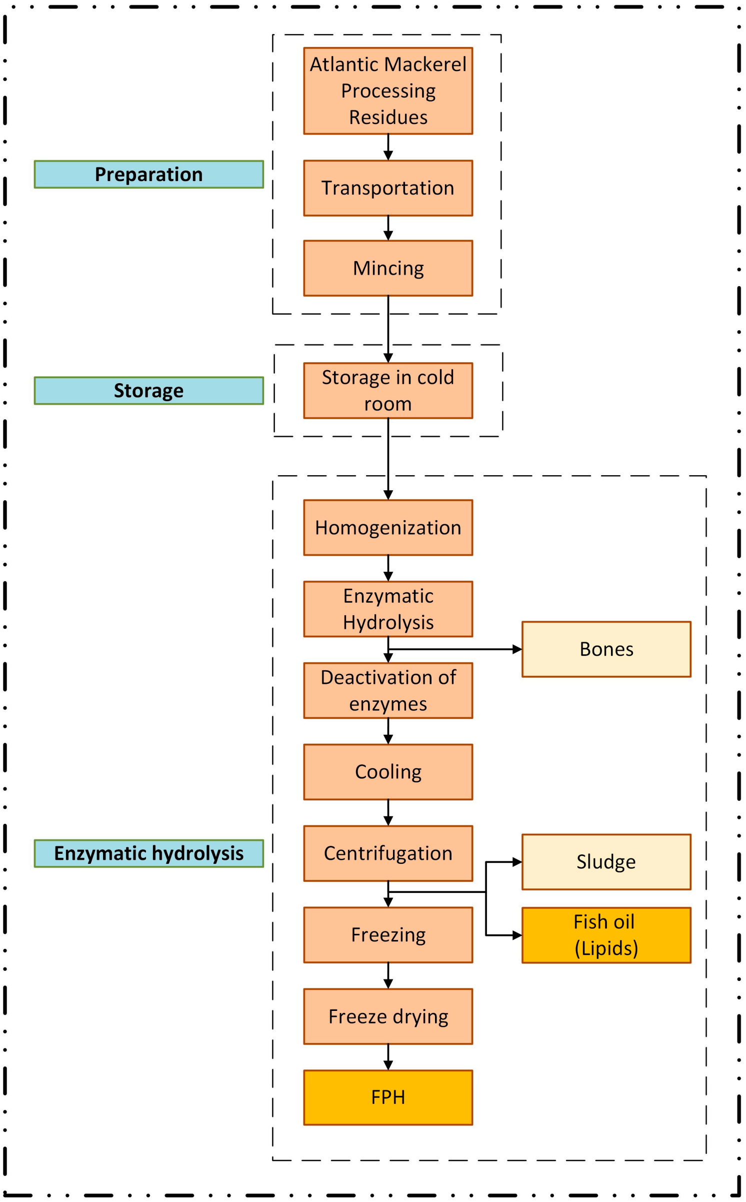

Figure 1. The process flow and system boundary of the FPH and fish oil extraction using enzymatic hydrolysis.

Table 1 shows the amounts of input and output flows at each stage of the production process per 1 g of FPH. The original amounts were measured during experiments, and after that, the amounts were adjusted per 1 g of FPH. To calculate the electricity consumption of the equipment, the technical manual was consulted for information on power consumption. The following formula was used to calculate the electricity demand:

$$\text{Electricity demand (kWh)} = \text{power (kw)} \times \text{operational time (h)}, \tag{1}$$

The OpenLCA software (v 1.11.0 by GreenDelta, Berlin, Germany) was employed in this study, utilizing the Ecoinvent v3.8 database (Ecoinvent organization, Zurich, Switzerland). As the experiments were carried out in Norway, the electricity provider considered was: Market for Electricity, High Voltage | Electricity, High Voltage | APOS, U, NO.

Table 1. Life Cycle Inventory table of the FPH and fish oil extraction using enzymatic hydrolysis.

Stages		Flows (Unit)	Values
Preparation	Input	Fish processing residue (g)	17.015
		Transportation (km)	68
		Electricity (Wh)	0.197
	Output	Fish processing residue (transported) (g)	15.951
		Viscera (g)	1.064
Storage	Input	Fish processing residue (g)	15.951
		Electricity (Wh)	0.1248
	Output	Minced fish (cold) (g)	15.951
Enzymatic hydrolysis	Input	Minced fish (cold) (g)	15.951
		Enzymes (g)	0.0159
		Electricity (Wh)	2481.496
		Distilled water (g)	15.951
		Water for the bath (g)	65.072
		Ice (g)	146.413
	Output	Bones (g)	1.301
		Sludge (g)	7.923
		Stick water (g)	231.014
		Fish oil (Lipids) (g)	2.165
		FPH (g)	1

2.3. Allocation

In LCA, allocating environmental impacts is crucial when multiple by-products exist [31]. The allocation of the environmental impacts is a critical aspect of the LCA methodology and can influence the overall sustainability assessment [32]. The main product in the current process under study is FPH, while fish oil is treated as a by-product. One method for distributing the environmental impacts between the main product and the by-product is through the economic value. This method is called economic allocation. Economic allocation is the most popular method for LCA of food products [8]. This study employs economic allocation to assess the environmental impacts of FPH and fish oil.

2.4. Validation

To validate the accuracy and functionality of the software and database, the results of the LCA study conducted by Garofalo et al. [23] were regenerated. One of their key processes was chosen for the validation study. The input–output values that were provided in their article were utilized to cross-check the results of this study.

2.5. Life Cycle Cost Analysis (LCCA)

LCCA is a strategic financial planning tool that helps decision making [33]. As sustainability assessments gain more attention, LCCA emerges as a pivotal method for evaluating the total cost implications of a product or process throughout its entire lifecycle. This analytical framework goes beyond the conventional emphasis on initial expenses, considering operation, maintenance, and end-of-life costs. By performing an LCCA, stakeholders can make informed decisions that align with economic and sustainable objectives. The cost groups are as follows [34]:

- Initial investment includes the cost of essential equipment, suitable building acquisition, and establishing connections to an energy source.
- Operating costs refer to the regular expenses incurred to keep a project or asset operational. This category includes the day-to-day costs associated with energy consumption, labor, and other operational necessities essential for the efficient functioning of the equipment.

- Maintenance costs involves routine checkups to ensure optimal performance.
- The end-of-life cost considers the possibility of generating profit after the useful life of an asset by selling the plant, its components, or materials, which contributes to the overall financial strategy.

As the research process is conducted at a lab scale using specialized equipment, the LCCA will primarily focus on operating costs. In this context, the typical considerations related to initial investment, maintenance costs, and end-of-life costs are omitted, as they may not be as pertinent to the scale and nature of the laboratory setting.

2.6. Sensitivity Analysis

To evaluate the sensitivity of the LCA results, an examination is conducted regarding the effect of changing the impact assessment methods. This investigation compares four impact assessment methods: Recipe Midpoint (H), IMPACT 2002+, CML v4.8 2016, and IPCC 2013. Only the global warming potential is presented in the results since the midpoint impact categories differ between these methods, and the values are expressed in different units. Furthermore, the sensitivity of LCA results are evaluated when the source of electricity production is changed to three different regions: Norway, Average EU, and Estonia.

3. Results

3.1. Allocation

Table 2 provides the breakdown of the estimation of the economic allocation ratios. To estimate the output price, first, the mass-based ratios of the two products are calculated and the unit price (market price) is used to obtain economic allocation rations. Market prices were averaged over different available online markets [35–38]. According to Table 2, 60% of the environmental impacts are allocated to FPH and 40% to fish oil.

Table 2. Physical and economic allocation metrics.

Products	Output (kg)	Physical Allocation (%)	Unit Price (EUR/kg) (Market Price)	Output Price (€)	Economic Allocation (%)
FPH	0.001	31.5	7.70	0.0077	60
Fish oil	0.00216	68.4	2.37	0.00513	40
Sum of products	0.00316			0.012	
Oil-to-protein ratio	2.165				

3.2. Results of Validation Study

The recalculated LCA results are reported in Table 3, demonstrating the good performance of used software and database. The values in Table 3 illustrate a close alignment between the recalculated results and the original findings by Garofalo et al. [23]. It shows the reliability and accuracy of the software and database.

Table 3. Recalculation of values reported by Garofalo et al. [23] to validate the accuracy of software and database.

	Climate Change kg CO_2 eq/FU	Ozone Depletion kg CFC/FU	Freshwater Eutrophication kg P/FU
Reported by Garofalo et al. [23]	0.8	8×10^{-8}	0.00004
Recalculated in the current study	0.87	9.95×10^{-8}	0.00006

3.3. Midpoint Results

Table 4 summarizes the LCA midpoint impact assessment results for FPH and oil, measured explicitly per 1 g of FPH using the Recipe Midpoint (H) impact assessment method across nine impact categories. In the context of this assessment, the environmental impacts are allocated based on the economic allocation method elucidated in Section 2.3. A distribution strategy is established for each impact category, with 60% of the environmental impact attributed to FPH and the remaining 40% allocated to oil. As the FU is 1 g FPH, the environmental impacts of fish oil are assessed based on this FU. However, the environmental impacts could be reported per 1 g of fish oil as well. This could be carried out using the yield ratio of 2.165 g of fish oil per 1 g of FPH (see Table 1).

Table 4. Midpoint impact results for fish protein and fish oil.

Impact Category	FPH (per 1 g FPH)	Fish Oil (per 1 g FPH)	Fish Oil (per 1 g Fish Oil)	Unit
climate change	0.044	0.029	0.0133	kg CO_2-eq
ozone depletion	4.341×10^{-9}	2.893×10^{-9}	1.337×10^{-9}	kg CFC-11-eq
particulate matter formation	0.00017	0.00011	5.080×10^{-5}	kg PM10-eq
freshwater eutrophication	1.280×10^{-5}	8.533×10^{-6}	3.941×10^{-6}	kg P-eq
fossil depletion	0.011	0.007	0.003	kg oil-eq
photochemical oxidant formation	0.0004	0.0002	9.237×10^{-5}	kg NMVOC-eq
water depletion	0.041	0.027	0.012	m^3 water-eq
terrestrial acidification	0.00045	0.00029	1.339×10^{-4}	kg SO_2-eq
freshwater ecotoxicity	0.0014	0.0009	4.157×10^{-4}	kg 1,4-DCB-eq

Figure 2A,B illustrate how inventory flows and production stages contribute to different midpoint impact categories. Electricity and fish are the major contributors to environmental impacts, as highlighted in Figure 2A. Regarding climate change, electricity accounts for a significant share of 57%, while fish makes up 38.6%. The impact of electricity is particularly substantial in freshwater eutrophication (89.1%) and water depletion (99.9%). Fish is the primary contributor to impact categories like freshwater ecotoxicity, photochemical oxidant formation, ozone depletion, and particulate matter formation, with contributions ranging between 70% and 80%. Solid waste is identified as a noteworthy flow in terrestrial acidification contributing 29.6%, while it remains insignificant in other impact categories. Figure 2B highlights the significant roles of the preparation and hydrolyzation stages, whereas the storage stage has either zero or negligible impact. In the context of climate change, the hydrolysis and preparation stages account for 57.8% and 42.2%, respectively. In freshwater ecotoxicity, each of these stages contributes equally. Hydrolysis primarily contributes to water depletion and freshwater eutrophication, while preparation significantly affects other impact categories.

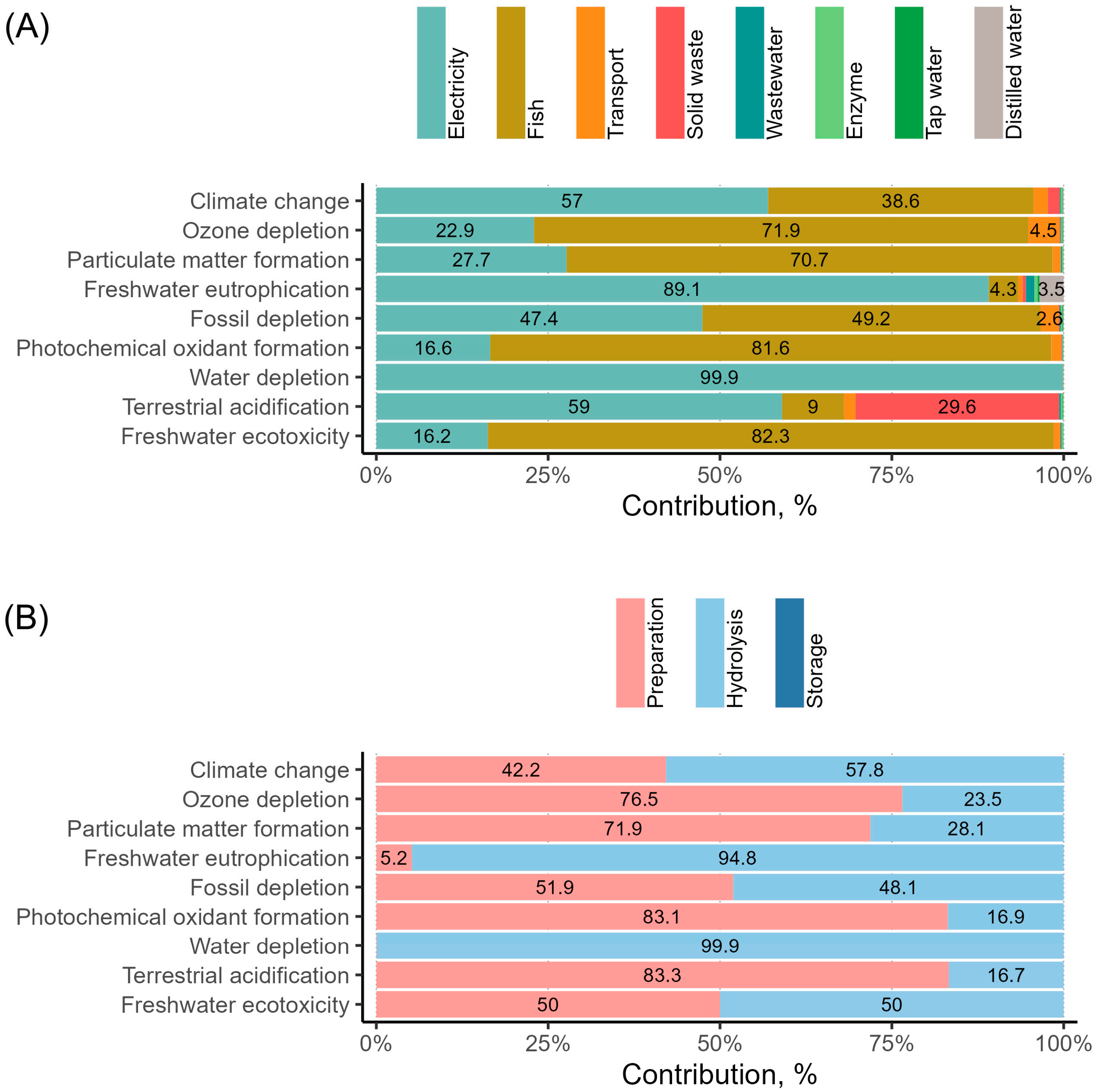

Figure 2. Contributions of inventory flows (**A**) and production stages (**B**) across various midpoint impact categories.

3.4. Life Cycle Cost Analysis (LCCA) Results

The breakdown of the cost of input materials to produce 1 g of FPH is provided in Table 5. The unit price and the price per 1 g of FPH are aggregated based on the geographical location of production, which is Norway. The unit price is the price of 1 unit of input flow on the market. For example, for electricity, the unit price is EUR 0.1177 per kwh. To evaluate the price per 1 g of FPH, the value per 1 g protein is multiplied by the unit price.

Table 5. Breakdown of costs associated with the production of 1 g of FPH.

Flow	Value per 1 g Protein	Unit	Unit Price (EUR/Unit)	Price per 1 g FPH (EUR)
Electricity	2.481	kWh	0.1177 [39]	0.2920
Tap water (for the bath)	0.065	kg	0.00551 [40]	0.00035
Distilled water	0.015	kg	12.61	0.1891
Ice	0.146	kg	2.5 [41]	0.365
Labor	0.131	hours	20.96 [42]	2.7457
Cleaning and sanitation	4.09	mL	0.00774 [43]	0.0316
Transportation	68	km	0.000442 [44]	0.0300
Enzyme (Alcalase)	1.595×10^{-5}	kg	177.6 [45]	0.0028
Fish processing residues	0.017	kg	1.59 [46]	0.0271

To estimate the labor costs, we assume that the production of 61 g of FPH takes 8 h. In the original experiment, 61 g of FPH were isolated in one run. The labor cost is thus calculated within this context.

To calculate the sanitation costs, it is assumed that 250 mL of detergent is required to clean the equipment after producing 61 g of FPH. The pricing structure is based on the cost of a 5 L detergent container. The unit price is provided per 1 mL of detergent. It is worth mentioning that cleaning and sanitation were omitted from the LCA study due to the lack of data in the databases.

A specific scenario is assumed for the estimation of the transportation cost. In this scenario, a 6-seat Bolt Drive van is used for a 68 km trip in Oslo. The pricing model for the Bolt Drive incorporates a base charge, a per km fee, and an additional charge per km. The transportation expense is calculated based on the average speed of 80 km/h and a load of 420 kg (equivalent to 6 persons), with km used as the unit of measurement.

According to the LCCA analysis, the cost of producing 1 g of FPH is EUR 3.68. The main cost drivers are labor and electricity. Inefficiency in labor and electricity usage is apparent in lab-scale production. However, as production scales up, resources are used more efficiently which leads to lower costs.

3.5. Sensitivity Analysis

Table 6 shows that the change in the impact assessment method has a limited effect on the results [47]. This insight provides confidence in the reliability of the results, indicating that the choice of impact assessment method may not significantly affect the conclusions drawn from this study.

Table 6. Sensitivity analysis of results to changes in the impact assessment method.

Impact Category	Unit	FPH Recipe Midpoint (H)	FPH IMPACT 2002+	FPH CML v4.8 2016	FPH IPCC 2013
Global warming	kg CO_2-eq	0.044	0.041	0.044	0.044

This study evaluated the sensitivity of LCA to variations in electricity production (Table 7). The results show a significant response. It has been observed that Norway produces electricity with the lowest global warming potential. This outcome is because 88% of Norway's electricity comes from hydropower [48], which is recognized as one of the cleanest sources of electricity. In contrast, the global warming potential increases to 0.58 kg CO_2-eq when considering the average EU electricity mix. Moreover, when generating electricity in Estonia, where approximately 65% of the electricity mix is sourced from oil shale [49], it rises substantially to 1.26 kg CO_2-eq, imposing a significant burden on environmental resources [50].

Table 7. Sensitivity analysis of results to changes in the region of electricity production.

Impact Category	Unit	FPH Norway	FPH Average EU	FPH Estonia
Global warming	kg CO_2-eq	0.044	0.58	1.26

4. Discussion and Comparison

In the investigation conducted by Garofalo et al. concerning the extraction of oil from tuna fish, an estimated global warming potential of 0.6–1.3 kg CO_2-eq per 1 g of fish oil was reported [23]. The study highlights the significant impact of hydrolysis and electricity consumption on the overall environmental sustainability of the extraction process. A thorough analysis of the inventory table in their research reveals that they used 258 g (approximately the weight of a large grapefruit) of tuna viscera to produce 1 g of fish oil. In contrast, the process in this study achieved an equivalent output using only 7.38 g of Atlantic mackerel, indicating higher yield efficiency. Moreover, the difference in the use of enzymes is noticeable in the study conducted by Garofalo et al. [23] This study used only 0.0073 g of enzymes per 1 g of fish oil compared to their 2.9 g of enzymes for the same amount of oil produced, as shown in Figure 3.

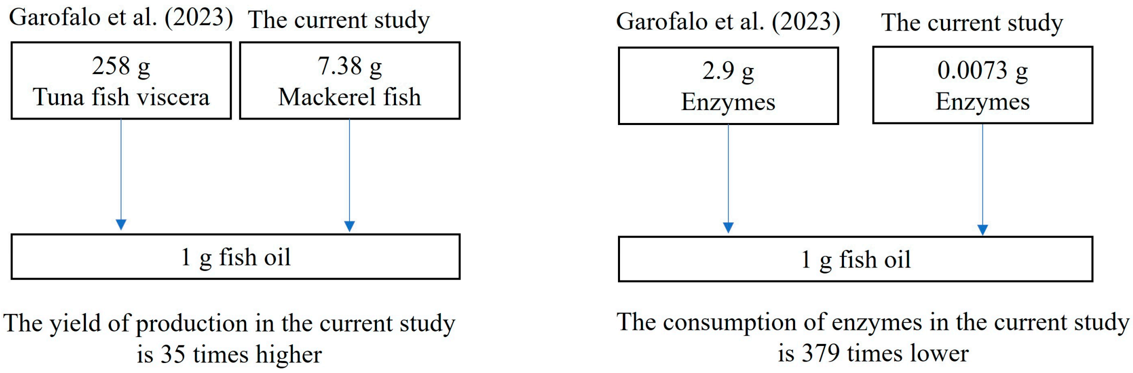

Figure 3. Comparison of fish and enzyme input in this study against the study by Garofalo et al. [23].

Table 8 provides the chemical composition of tuna viscera and Atlantic mackerel. The comparison of the compositions supports the findings. It reveals that Atlantic mackerel contains higher oil and protein content levels than tuna fish, which is one of the potential reasons behind the higher efficiency observed in this experiment. This highlights the potential advantages and sustainability gains in selecting appropriate raw materials and enzymatic hydrolysis approach for FPH and oil extraction, as it uses less raw material input and chemicals.

Table 8. Comparison of chemical composition of tuna fish viscera and Atlantic mackerel fish.

	Tuna Fish Viscera [51]	Atlantic Mackerel [52]
Protein	10.91%	18–19%
Oil	4.42%	4–12%
Dry matter	26.83%	24%
Salt	2.18%	1.3%

5. Conclusions

This study focused on assessing the environmental and economic impact of FPH and fish oil production via enzymatic hydrolysis. It highlighted a promising path towards sustainable practices in response to the increasing global demand for fish products. The use of enzymatic hydrolysis for extracting FPH and fish oil from Atlantic mackerel processing residues is a sustainable alternative production method despite potential drawbacks such as enzyme costs.

Economic allocation shows that 60% of environmental impacts could be attributed to FPH and 40% to fish oil. The LCCA shows that the production cost of 1 g of FPH is EUR 3.70, with labor and electricity identified as the primary drivers of costs. The inefficiencies observed in these areas on the lab-scale production level suggest that scaling up could enhance resource utilization and reduce overall costs and help industry stakeholders decide towards this more environmentally friendly practice. It is important to recognize that the source of electricity plays a crucial role in determining the sustainability of the overall process. Given that Norway boasts one of the cleanest sources of electricity, changes in the electricity production region can significantly impact the environmental sustainability of the process. Drawing parallels with previous studies, the enzymatic hydrolysis process in the current study shows distinct advantages for Atlantic mackerel FPH and fish oil extraction. The experiment reveals a higher yield coupled with significantly reduced amounts of raw materials and enzymes.

In conclusion, this research provides a foundation for further exploration and optimization of enzymatic hydrolysis as a sustainable solution for FPH and oil extraction. Integrating environmental and economic assessments contributes valuable insights for aquaculture and seafood processing industry stakeholders, guiding decisions towards more environmentally friendly and economically viable practices. As the demand for fish products surges, adopting such sustainable methodologies becomes pivotal for a responsible and resilient future.

Author Contributions: Conceptualization, B.B., O.G. and R.V.; methodology, B.B. and O.G.; software, B.B.; validation, B.B. and R.V.; formal analysis, B.B. and R.V.; investigation, J.C. and K.K.; resources, B.B., J.C. and K.K.; data curation, B.B., O.G., J.C. and K.K.; writing—original draft preparation, B.B.; writing—review and editing, J.C. and R.V.; visualization, B.B.; supervision, R.V., O.G. and, J.C.; project administration, R.V. and J.C.; funding acquisition, R.V. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was conducted as a part of the ERA-NET BlueBio project "IMPRESSIVE-Improved Processing to Enhance Seafood Sidestream Valorization and Exploration," grant number 341732 of RCN.

Data Availability Statement: All data utilized for the assessment have been appropriately cited and are accessible online.

Acknowledgments: The first author expresses gratitude for the assistance provided by Aleksei Kaleda in the generation of graphs, proofreading, and revising the manuscript. We would like to thank three anonymous reviewers for their valuable comments that improved the quality of this paper.

Conflicts of Interest: The authors declare that they have no known competing financial and non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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