Proceedings of the
Estonian Academy of Sciences
2025, **74**, 1, 1–14

https://doi.org/10.3176/proc.2025.1.01

www.eap.ee/proceedings
Estonian Academy Publishers

SUSTAINABILITY
IN FOOD TECHNOLOGIES

RESEARCH ARTICLE

Received 13 May 2024
Accepted 22 August 2024
Available online 11 December 2024

Keywords:
high-pressure processing, life cycle assessment, chitosan, shrimp shell, circular economy, sustainability

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Citation:
Bashiri, B., Pinheiro, A. C. D. A. S., Tappi, S., Rocculi, P., Kaleda, A. and Vilu, R. 2025. Life cycle assessment of laboratory-scale chitosan production: comparison of high-pressure processing-assisted and conventional methods. *Proceedings of the Estonian Academy of Sciences*, **74**(1), 1–14. https://doi.org/10.3176/proc.2025.1.01

Life cycle assessment of laboratory-scale chitosan production: comparison of high-pressure processing-assisted and conventional methods

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ABSTRACT

Chitosan is in high demand due to its wide range of applications, resulting in a reliable market. Conventional chemical extraction methods of chitosan are harsh, require strong acids and bases, and produce toxic waste products. High-pressure processing (HPP)-assisted chemical extraction of chitosan has the potential to result in a higher production yield. It is crucial to evaluate the environmental performance of this method. This paper presents a comprehensive comparative analysis of chitosan production methods from an environmental perspective, focusing on HPP-assisted and conventional techniques. Employing life cycle assessment (LCA) methodologies, the study evaluates the environmental footprints of conventional and HPP-assisted chitosan production processes. Results reveal that HPP-assisted production exhibits superior environmental performance, particularly in reducing climate change impact by 64% compared to conventional methods. Sensitivity and scenario analyses confirm the robustness of findings, considering changes in electricity production regions and alternative characterization methods. Uncertainty analysis indicates moderate uncertainty levels, affirming data reliability. The study concludes that HPP-assisted chitosan production offers a more sustainable approach with lower environmental footprints across various endpoints. These findings provide valuable guidance for stakeholders in the chitosan industry to enhance sustainability practices and minimize environmental impacts.

1. Introduction

Chitosan, a natural biopolymer, has garnered attention for its diverse applications in food, agriculture, medicine, pharmaceutics, and cosmetics, owing to its physicochemical and biological properties (Jiménez-Gómez et al. 2020). Chitosan is a water-soluble, non-toxic, biocompatible substance with antibacterial, antifungal, and antitumor properties, making it a highly intriguing product (Harugade et al. 2023). Chitosan can be obtained by deacetylation of chitin, a natural substance found in various organisms (Triunfo et al. 2022). Chitin is present in the exoskeletons of crustaceans such as shrimps, the endoskeletons of mollusks such as squids, and many invertebrates such as nematode eggshells (Muñoz et al. 2018). Additionally, chitin is found in the cell walls of fungi and some diatom algae (Durkin et al. 2009). Due to its widespread presence, chitin is considered one of the most abundant renewable resources (Piekarska et al. 2023).

Chitosan is in high demand, resulting in a reliable market. In 2019, the global market size for chitosan was valued at USD 6.8 billion. It is expected to grow at a compound annual growth rate (CAGR) of 24.7% between 2020 and 2027 (Aranaz et al. 2021). Chitosan is primarily obtained from crustacean shells such as those of crabs, prawns, and shrimp, which are available as waste from the food processing industry, making it a potential contributor to a circular economy. However, its production processes may have significant environmental impacts.

Conventional chemical extraction methods of chitin and chitosan are harsh, require strong acids and bases, and produce toxic waste products (Muñoz et al. 2018). Two distinct stages are involved in the conventional chemical treatment for chitin extraction from shrimp shells. Initially, chemical methods are employed for deproteinization, with various basic chemicals tested as reagents. Reaction conditions vary across studies, but sodium hydroxide (NaOH) is the preferred reagent, applied at concentrations ranging from 0.125 to 5.0 M, at temperatures up to 160 °C, and with treatment durations spanning from minutes to days. However, besides deproteinization, using NaOH inevitably leads to partial deacetylation of chitin and hydrolysis of the biopolymer, resulting in a reduced molecular weight. Demineralization, the second stage, involves the removal of minerals, primarily calcium carbonate. Typically, demineralization is carried out through acid treatment, mainly using hydrochloric acid (HCl) (Pellis et al. 2022). Due to the heterogeneity of the solid, complete mineral removal is challenging, prompting the use of larger volumes of or more concentrated acid solutions. However, it is essential to acknowledge that this process has certain drawbacks, notably its high cost and environmental unfriendliness due to the elevated application of acidic and basic chemicals (Casadidio et al. 2019). Therefore, exploring alternative processes becomes imperative to address these concerns and enhance the sustainability of chitin extraction.

One way to improve the sustainability of a process is to enhance production efficiency. It could be achieved by increasing the production yield. The efficiency of any extraction process is influenced by material pretreatments, leading to alterations in extraction yields, physicochemical properties, and biological activities of the compounds. Some researchers opt for various physical treatments instead of conventional heat methods to enhance extraction yields and the bioactive properties of a compound (Silva et al. 2024). Pretreatment methods include ultrasound, microwaves, and high pressure, with high-pressure processing (HPP) emerging as a promising technique in the food industry (Li et al. 2021). HPP offers a uniform treatment across the entire product, making it economically advantageous. From a green perspective, HPP is considered environmentally friendly due to its low energy cost and lack of chemicals. Once the desired pressure is attained, it can be sustained in the HPP chamber without additional energy input. Consequently, the key advantages of HPP over other thermal processing technologies lie in its lower energy cost and higher extraction efficiency (Mannozzi et al. 2023).

A combination of HPP and chemical extraction of chitin and chitosan has the potential to result in a higher production yield. It becomes crucial to evaluate the environmental performance of this method to comprehend its environmental impact. Life cycle assessment (LCA) is a prevalent tool for investigating sustainability in various industrial, agricultural, and aquaculture sectors (Ghamkhar et al. 2021; Ruiz-Salmón et al. 2021; Saerens et al. 2021). It is a robust tool for evaluating environmental impacts across the entire life cycle of a product. Utilizing LCA during the initial phases of product or service design and development provides insights to optimize resource consumption and mitigate emissions (Pacana et al. 2023).

Several studies have applied LCA to assess the environmental impacts of chitosan production using conventional methods on a large scale. Muñoz et al. (2018) identified that the production of chemicals NaOH and HCl is an important hotspot in the environmental impact of chitosan production and supply chain in India. Similarly, Meramo-Hurtado et al. (2020) reported comparable findings when evaluating large-scale chitosan production from shrimp exoskeletons in Colombia, employing exergy and environmental analyses. Their results suggested that the large-scale production process yielded environmental benefits, with a net reduction in potential environmental impact. Furthermore, Riofrio et al. (2021) conducted an environmental impact analysis of chitosan production in Ecuador using the ReCiPe Midpoint (H) method, version 2016. Among the 18 parameters considered, marine and freshwater ecotoxicity and human carcinogenic toxicity emerged as the most significant categories. In a study by Silva et al. (2024), microwave-assisted deacetylation was explored as a greener alternative to conventional methods. The study assessed morphological, thermal, and chemical properties, yield, and environmental impacts. The results showed that microwave-assisted deacetylation produced high-quality chitosan with a deacetylation degree close to 90% and a yield over 50%. The life cycle impact assessment (LCIA) highlighted significant environmental impacts, including fossil resource scarcity, climate change, and toxicity. Microwave-assisted deacetylation demonstrated a shorter reaction time (16 minutes vs. 240 minutes) and significantly reduced energy consumption (382.1 kJ to 8.9 kJ per 1 gram of chitosan). Despite the environmental challenge posed by NaOH usage, microwave technology showed promise in reducing overall environmental impact, offering a more sustainable approach to chitosan production. Huang and Tsai (2020) have studied the extraction of chitosan from squid pen waste by HPP. They have investigated the effects of HPP on the physicochemical properties and antioxidant activity of chitosan. However, the LCA of HPP-assisted chitosan production has not been investigated so far.

Thus, we aimed to assess the environmental impact of laboratory-scale chitosan production from shrimp shells (*Melicertus kerathurus*) by comparing the LCA of conventional and HPP-assisted processes. *Melicertus kerathurus* typically occurs in marine coastal and brackish waters. The species is classified as a tropical-warm temperate decapod, with a geographic distribution extending along the eastern Atlantic coasts from northern Angola to southern England, including the Mediterranean (Kevrekidis and Thessalou-Legaki 2011).

2. Materials and methods

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 (Summa et al. 2023). LCA scope was defined in this stage, which involved establishing system boundaries, determining the functional unit (FU), and considering the relevant assumptions. This research project had three main goals:

- The first goal was to gain an overview of the environmental impacts of producing chitosan through an HPP-assisted chemical process. This enables us to recognize the hotspots and the most important contributing flows to the environmental impacts.
- The second goal was to compare the environmental performance of producing chitosan through an HPP-assisted chemical process to a conventional process.
- The third goal was to investigate the sensitivity of the LCA results to the geographical location of production. The process was run at the University of Bologna, Italy. For sensitivity analysis, we chose Norway and Estonia as alternative production regions. Norway was selected because it has one of the greenest electricity mixes in Europe (Bashiri et al. 2024). Estonia was selected as about 60% of the electricity in Estonia is generated using oil shale, which is not a clean source of energy (Gavrilova et al. 2010). Therefore, in this analysis, we aim to investigate two extreme situations in Europe as potential alternatives. Detailed explanations are provided in Section 2.3. Due to the small scale of the production in this study, the chosen FU was one gram (1 g) of chitosan.

2.2. System boundary and process description

To be able to compare the potential environmental impact of HPP-assisted chitosan extraction to the conventional process, both processes were run on the lab scale. The conventional process began with raw shrimp shells. The shrimp species used in this study was *Melicertus kerathurus* (common name is striped prawn or caramote prawn). The shells were first dried in an oven at 60 °C for 24 hours to remove any moisture content. Once dried, the shells were milled for 10 minutes at room temperature using a Thermomix TM31. The milled shells then underwent demineralization. This involved treating the material with a 37% HCl solution. The demineralization process occurred on a magnetic stirrer with heating for 1 hour and 30 minutes at room temperature. It was found that the mineral content was already greatly reduced after the first hour, so the time was extended by 30 min, and it was enough to remove all the minerals. After demineralization, the material was deproteinized. This was achieved by treating the material with NaOH (pH 13.5) while stirring for three hours on a magnetic stirrer and heating it at 80 °C. Once deproteinization was complete, the material was dried again in an oven at 60 °C for 24 hours to remove excess moisture and obtain dried chitin. The dried chitin was then subjected to deacetylation to convert it into chitosan. Deacetylation involved treating the chitin with a high concentration of NaOH (pH 14, 50% w/v) while stirring for 18 hours on a magnetic stirrer with heating at 70 °C. Finally, the chitosan obtained from the deacetylation process was dried in an oven at 60 °C for 24 hours to remove any remaining moisture, resulting in dried chitosan.

The HPP-assisted process for producing chitin and chitosan closely resembles the conventional process with one notable difference: the incorporation of high-pressure processing treatment prior to the demineralization step. In this modified process, samples undergo HPP treatment at room temperature for five minutes using the HPP Hyperbaric 420 machine. The remaining steps of the process, including demineralization, deproteinization, drying, and deacetylation, follow the same procedures as in the conventional process. Figure 1 presents the process flow diagram and system boundary of the conventional process and HPP-assisted chitosan production.

Table 1 provides the amount of input and output flows at each stage of the production process per 1 gram of chitosan. 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:

$$\text{electricity demand (kWh)} = \text{power (kW)} \times \text{operational time (h)}. \quad (1)$$

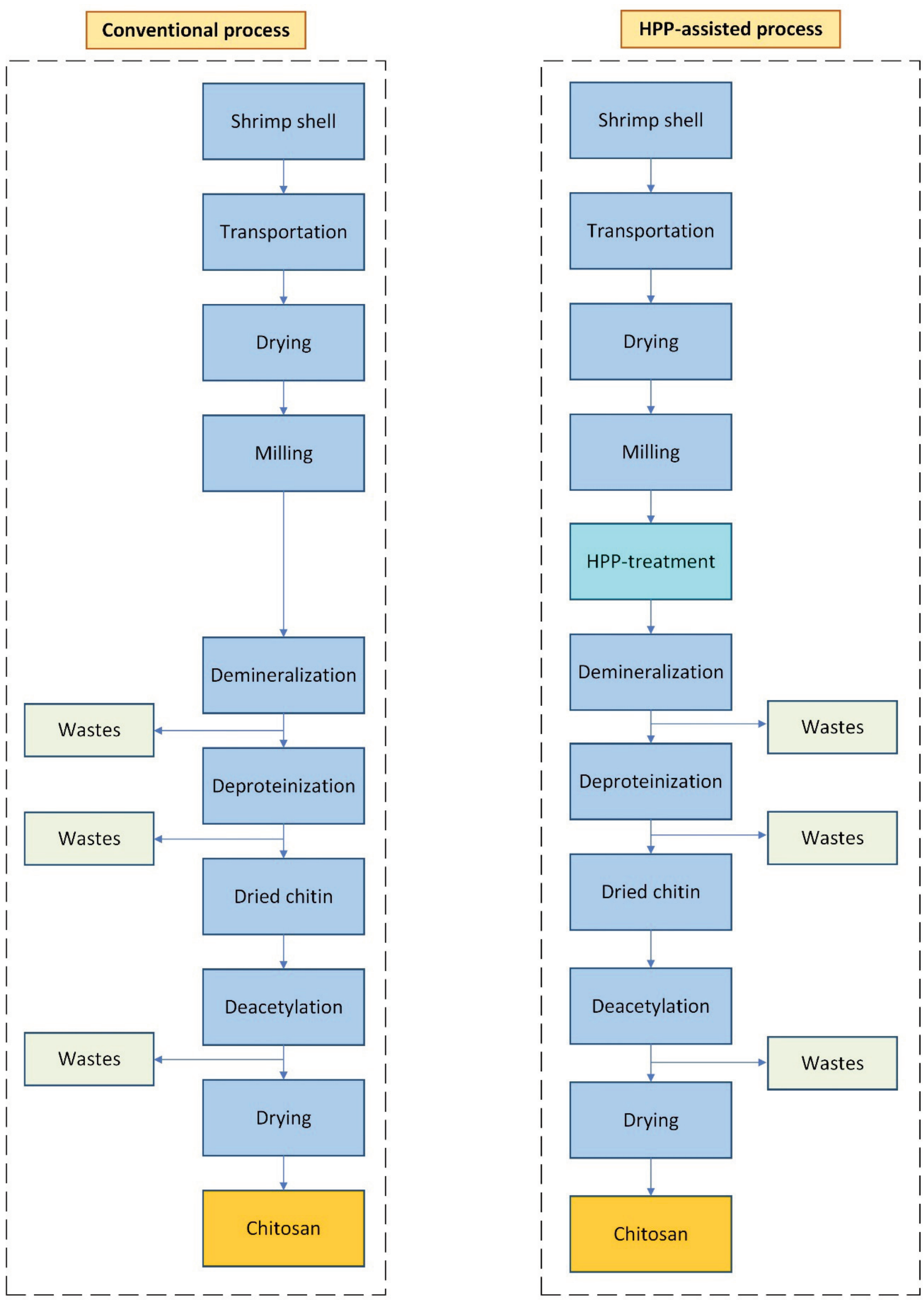

Fig. 1. Process flow and system boundary (dashed line) of conventional and HPP-assisted chitosan extraction.

Table 1. Life cycle inventory table of conventional and HPP-assisted chitosan production. The table includes production stages, input-output flows, and quantities

Production stages	Flows (unit)		Conventional	HPP-assisted
Preparation	Input	Frozen shell (g)	23.80	14.93
		Transportation (km)	404.76	253.88
	Output	Transported shell (g)	23.80	14.93
Drying	Input	Transported shell (g)	23.80	14.93
		Electricity (kWh)	0.017	0.010
	Output	Dried shell (g)	7.14	4.48
		Vapor (g)	16.66	10.45
Milling	Input	Dried shell (g)	7.14	4.48
		Electricity (kWh)	7.44×10^{-5}	4.66×10^{-5}
	Output	Milled dried shell (g)	7.14	4.48
HPP treatment	Input	Milled dried shell (g)	–	4.48
		Electricity (kWh)	–	6.8×10^{-4}
	Output	HPP-treated shell (g)	–	4.48
Demineralization	Input	Milled/HPP-treated shell (g)	7.14	4.48
		HCl (g)	17.21	10.12
		Water (g)	311.72	183.30
		Electricity (kWh)	0.081	0.048
	Output	Demineralized shell (g)	3.57	2.77
		Waste (g)	332.50	195.13
		Biogenic CO_2 (g)	3	1.88
Deproteinization	Input	Demineralized shell (g)	3.57	2.77
		NaOH (g)	1.82	1.075
		Water (g)	152.38	89.60
		Electricity (kWh)	0.108	0.064
	Output	Deproteinized shell (g)	3.571	1.805
		Waste (g)	154.209	90.681
Drying (chitin)	Input	Deproteinized shell (g)	3.571	1.805
		Electricity (kWh)	0.017	0.010
	Output	Dried chitin (g)	1.25	1.25
		Vapor (g)	0.94	0.55
Deacetylation	Input	Dried chitin (g)	1.25	1.25
		NaOH	14.69	12.80
		Water	32.65	25.60
		Electricity (kWh)	0.017	0.13
	Output	Deacetylated chitin (g)	1.25	1.25
		Waste (g)	47.34	38.40
Drying (chitosan)	Input	Deacetylated chitin (g)	1.25	1.25
		Electricity (kWh)	0.017	0.010
	Output	Vapor (g)	0.25	0.25
		Dried chitosan (g)	1	1

The emission of biogenic CO_2 from treating shells with acid was estimated at 0.14 kg CO_2 per 1 kg of shrimp, assuming an initial mineral content of approximately 90% $CaCO_3$. Consequently, the release of biogenic CO_2 per FU was calculated to be 0.003 kg CO_2. The chemical reaction of biogenic CO_2 emission is as follows (Kou et al. 2021):

$$CaCO_3 + HCl \longrightarrow CaCl + CO_2 + H_2O. \quad (2)$$

In the execution of this LCA, the OpenLCA software v1.11.0 (GreenDelta, Berlin, Germany) was employed, utilizing the Ecoinvent v3.8 database produced by the Ecoinvent organization, Zurich, Switzerland. Road transportation by lorry was used for transporting materials. Waste produced was sludge and liquid waste resulting from different acid and base treatments. Therefore, the waste was assumed to be treated as wastewater. A list of flows and the corresponding providers that were used for the life cycle modeling is provided in Table 2.

Table 2. List of the flows and the corresponding providers used in the life cycle modeling

Flows	Flow providers
Transportation	Market for transport, freight lorry 3.5–7.5 metric tons, EURO 4, APOS*, U, RoW**
Electricity	Market for electricity, low voltage | electricity, low voltage | APOS, U, IT***
Vapor	Water vapor, emission to air, high population density
HCl	Market for hydrochloric acid, without water, in 30% solution state, APOS, U, RoW
NaOH	Market for sodium hydroxide, without water, in 50% solution state, APOS, U, GLO****
Water	Market for tap water, tap water, APOS, U, Europe without Switzerland
Waste	Treatment of wastewater, average capacity, APOS, U, Europe without Switzerland

*allocation at the point of substitution, **rest of the world, ***Italy, ****global

Allocation at the Point of Substitution (APOS) is a system model that sets the methodological rules for calculating life cycle inventories used by Ecoinvent. It follows an attributional approach where the responsibility for waste (burdens) is shared between producers and subsequent users. APOS employs product system expansion to avoid allocation within treatment systems. This differs from the *cut-off by classification* method where the primary production of materials is always allocated to the primary user of the material. Using the APOS system results in a more accurate assessment of environmental impacts because the inputs that are by-products of primary products also carry a burden and are not considered impact-free (Wernet et al. 2016).

2.3. Sensitivity and scenario analysis

Sensitivity and scenario analyses were conducted to assess the impact of assumptions, calculations, and uncertainties on the reliability of findings and conclusions. Two key points that could potentially affect the overall outcomes:

(a) The sensitivity of LCA results to changing the electricity production region to Norway and Estonia. For this reason, the electricity provider is changed to the following:

market for electricity, low voltage | electricity, low voltage | APOS, U, NO;
market for electricity, low voltage | electricity, low voltage | APOS, U, EE.

(b) Utilization of an alternative LCIA method. While the primary study results are derived using ReCiPe 2016 v1.08 (Huijbregts et al. 2017), the results of the LCA analysis at the midpoint level are also regenerated using the IMPACT World+ (Bulle et al. 2019) impact assessment method during the sensitivity analysis to validate the primary study findings.

2.4. Uncertainty analysis

As a significant portion of the models in the life cycle inventory phase rely on secondary data, the results inherently carry a level of uncertainty. To assess the robustness of the findings, an uncertainty analysis was conducted in OpenLCA through 1000 simulation runs of the Monte Carlo analysis (Mahmood et al. 2022). This approach utilizes a pedigree matrix to scrutinize the uncertainty across all midpoint impact categories. Key results of this analysis include the mean, standard deviation (SD), and coefficient of variation (CV). CV is the ratio of SD to the mean. CV serves as a measure of the relative magnitude of uncertainty. A high CV suggests a lack of robust conclusions for the specific midpoint category (Saerens et al. 2021). This analysis integrates all uncertainty distributions defined in the flows, parameters, and characterization factors for the simulation. Statistically significant difference between the Monte Carlo distributions was assessed by using the Kolmogorov–Smirnov test in R 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Midpoint characterization factor

The comparison between the conventional process and the HPP-assisted process unveiled significant disparities in their midpoint categories (Table 3). The HPP-assisted process demonstrated better environmental performance in all categories. In the climate change category, HPP-assisted production of chitosan represented 64% lower impacts compared to the conventional production. The results showed saving or credits (negative impact values) in natural land transformation. It means that the land saving is higher than the land use. This is because the application of HPP would increase the yield of chitosan extraction. According to the inventory table (Table 1), the yield of

Table 3. Results of midpoint impact categories. The results are expressed per 1 g of chitosan. The impact assessment method is ReCiPe 2016 Midpoint (H) v1.08

Impact categories	Unit	Conventional process	HPP-assisted process
Climate change	kg CO_2-eq	0.177	0.064
Particulate matter formation	PM_{10}-eq	2.8×10^{-4}	1.2×10^{-4}
Ozone depletion	kg CFC-11-eq	3.82×10^{-8}	1.95×10^{-8}
Fossil depletion	kg oil-eq	0.066	0.022
Marine eutrophication	kg N-eq	4.95×10^{-5}	2.00×10^{-5}
Freshwater ecotoxicity	kg 1.4-DCB	10.9×10^{-3}	3.63×10^{-3}
Urban land occupation	m^2a	1.81×10^{-3}	0.67×10^{-3}
Agricultural land occupation	m^2a	15.2×10^{-3}	4.99×10^{-3}
Natural land transformation	m^2	-7.96×10^{-6}	-3.57×10^{-6}
Water depletion	m^3 water-eq	2.99×10^{-3}	1.23×10^{-3}
Marine ecotoxicity	kg 1.4-DCB	9.7×10^{-3}	3.24×10^{-3}
Ionizing radiation	kg U235-eq	24.9×10^{-3}	8.79×10^{-3}
Freshwater eutrophication	kg P-eq	6.27×10^{-5}	2.50×10^{-5}
Terrestrial ecotoxicity	kg 1.4-DCB	3.20×10^{-4}	2.8×10^{-4}
Human toxicity	kg 1.4-DCB	0.06318	0.0268
Terrestrial acidification	kg SO_2-eq	7.4×10^{-4}	2.8×10^{-4}
Metal depletion	kg Fe-eq	8.03×10^{-3}	3.33×10^{-3}
Photochemical oxidant formation	kg NMVOC-eq	4.6×10^{-4}	1.8×10^{-4}

Table 4. Comparison of the yield of chitosan extraction in conventional and HPP-assisted processes

Process	Yield of chitosan extraction (%)
Conventional	4.20
HPP-assisted	6.69

chitosan extraction in the HPP-assisted process has increased by 59% compared to the conventional process. Table 4 compares the yield of chitosan extraction in two extraction processes.

3.2. Contribution of different production steps

Figure 2 shows the contribution of different production steps in four representative midpoint impact categories, namely climate change, fossil depletion, freshwater eutrophication, and water depletion. All four impact categories had similar contribution profiles, suggesting that they share common characteristics during the categorization phase of the LCA modeling. Considering the conventional process, the roles of deacetylation, demineralization, and deproteinization were substantial in all represented impact categories. This considerable contribution could be related to the application of NaOH and HCl in these stages. NaOH and HCl are known for their large environmental impact, and it is in line with the conclusion made by Muñoz et al. (2018). Considering the HPP-assisted process, the contribution of the deacetylation and demineralization steps was still the greatest, and

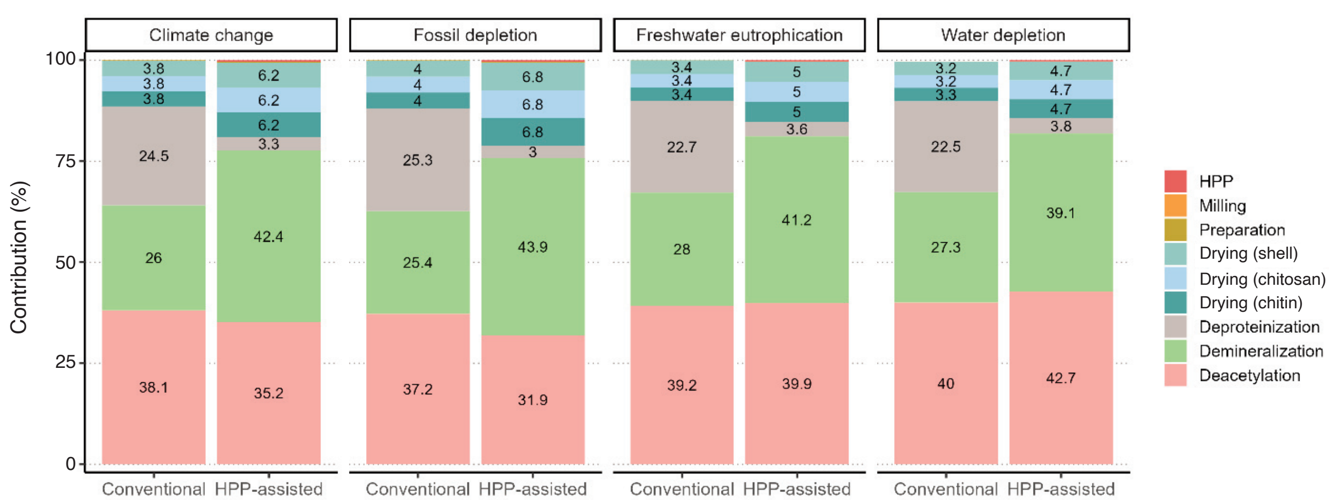

Fig. 2. Contribution of production stages in the represented midpoint impact categories. The results are generated using ReCiPe 2016 Midpoint (H) v1.08.

it could be explained by the application of HCl. Drying also had a notable contribution in all four impact categories, which is due to the use of electricity (Muñoz et al. 2018). After transitioning to the HPP-assisted process, the contribution of deproteinization diminished considerably. On the other hand, the role of demineralization increased. The contribution of deacetylation remained generally unchanged because even though the duration of deacetylation decreased from 18 hours to 4 hours, the consumption of NaOH in the HPP-assisted process was higher (see Table 1). Regardless, the contribution of the HPP stage was below 1% in all four midpoint impact categories represented in Fig. 2. Given that the global warming potential of CO_2 is considered as 1, the contribution of the biogenic CO_2 to the final impacts is deemed negligible. This conclusion is also supported by the conclusion drawn by Muñoz et al. (2018).

3.3. Endpoint characterization factor

The term "endpoint impacts" refers to the ultimate consequences or effects of a particular activity or process. The endpoint categories indicate the potential damage related to areas of protection, which in this study were human health, ecosystems, and resource scarcity, and were linked with midpoint categories through "damage pathways" (Saerens et al. 2021). It was observed that the HPP-assisted chitosan production had lower impact in all endpoint categories (Fig. 3). The conventional process showed about 60% to 90% higher impacts in all endpoint categories compared to the HPP-assisted process. Hence, the HPP-assisted process not only demonstrated a substantially lower CO_2-eq impact but also outperformed the conventional process in every endpoint category. Consequently, the HPP-assisted process emerges as a more environmentally sustainable option compared to conventional processes.

3.4. Single score product comparison

The analysis of the impacts of midpoint categories on endpoint single scores revealed significant insights into the environmental performance of both the conventional and HPP-assisted processes. As shown in Fig. 4, for the human health endpoint, factors such as climate change, particulate matter formation, and human toxicity were key contributors to the overall environmental impact. For the ecosystem category, climate change emerged as the primary driver of environmental impact, highlighting the need for measures to mitigate its effects. Additionally, agricultural and natural land occupation contributed significantly to the environmental burden. In terms of the resources category, fossil resource scarcity was a major concern, emphasizing the importance of transitioning to renewable energy sources and promoting resource efficiency.

3.5. Sensitivity and scenario analysis

We have conducted sensitivity and scenario analysis to assess the reliability of the study. Here we checked the sensitivity of the LCA model to the change in the production region and characterization method.

3.5.1. Change of production region to Norway and Estonia

The study systematically assessed the sensitivity of LCA to fluctuations in electricity production, as depicted in Fig. 5. This investigation scrutinized changes in the production region through a shift

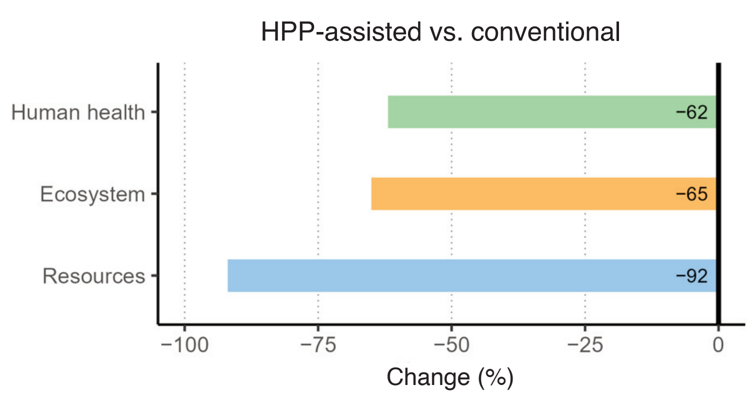

Fig. 3. Relative environmental impact of chitosan production processes. The highest impact (conventional process) is set at 100%, endpoint impact categories, functional unit is 1 g of chitosan, ReCiPe 2016 Endpoint (H) v1.08 method.

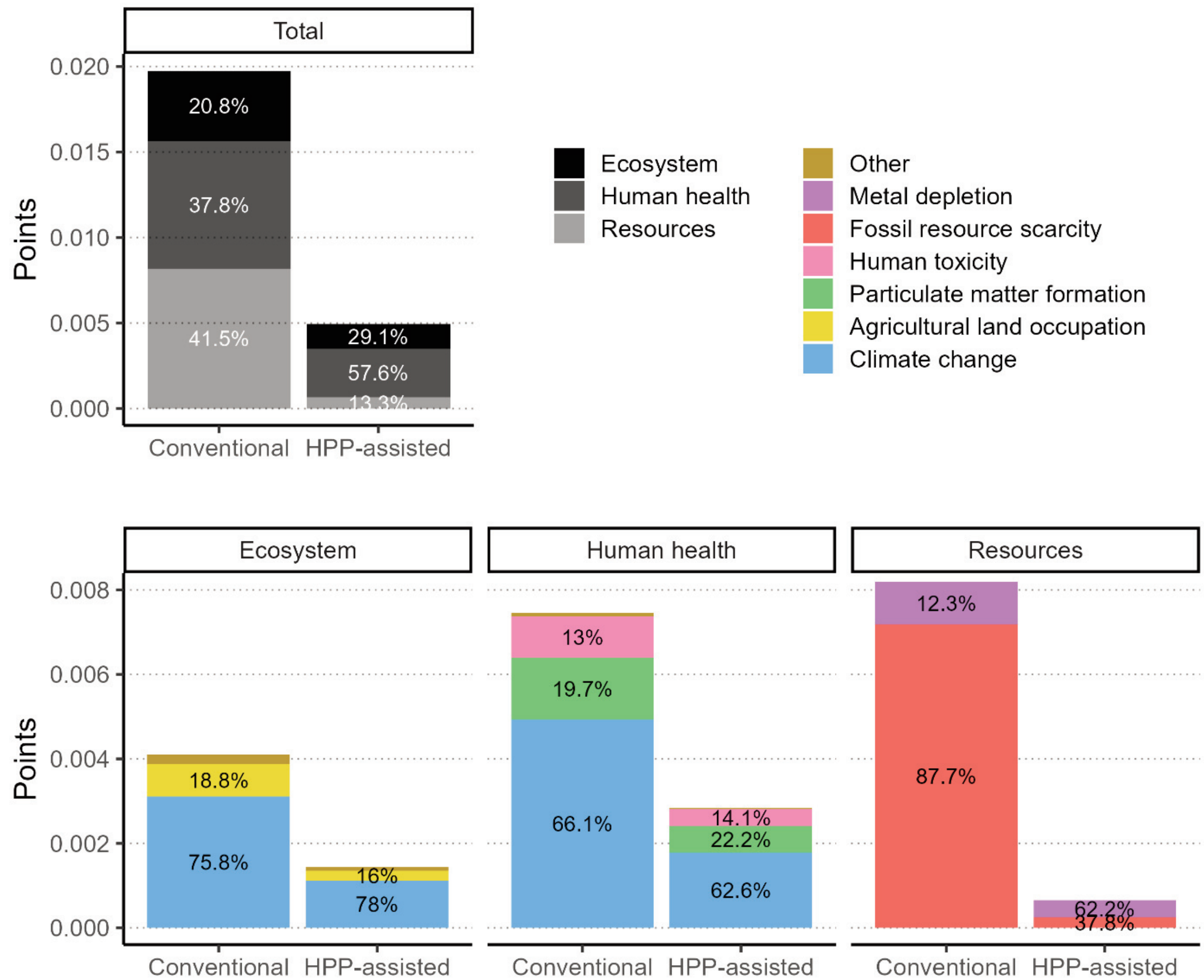

Fig. 4. Single score product comparison. The functional unit is 1 g of chitosan, ReCiPe 2016 Endpoint (H) v1.08, single score method.

in electricity providers. Utilizing the comprehensive Ecoinvent v3.8 database, detailed flows of various electricity generation methods across diverse geographical regions were available. Specifically, the study transitioned the electricity production region from Italy to Norway and Estonia.

The findings underscored a notable response to changes in electricity production. As shown in Fig. 5, changing the region of production from Italy to Estonia resulted in a significant increase in emissions and fossil depletion due to the large share of oil shale (about 60%) in the electricity mix

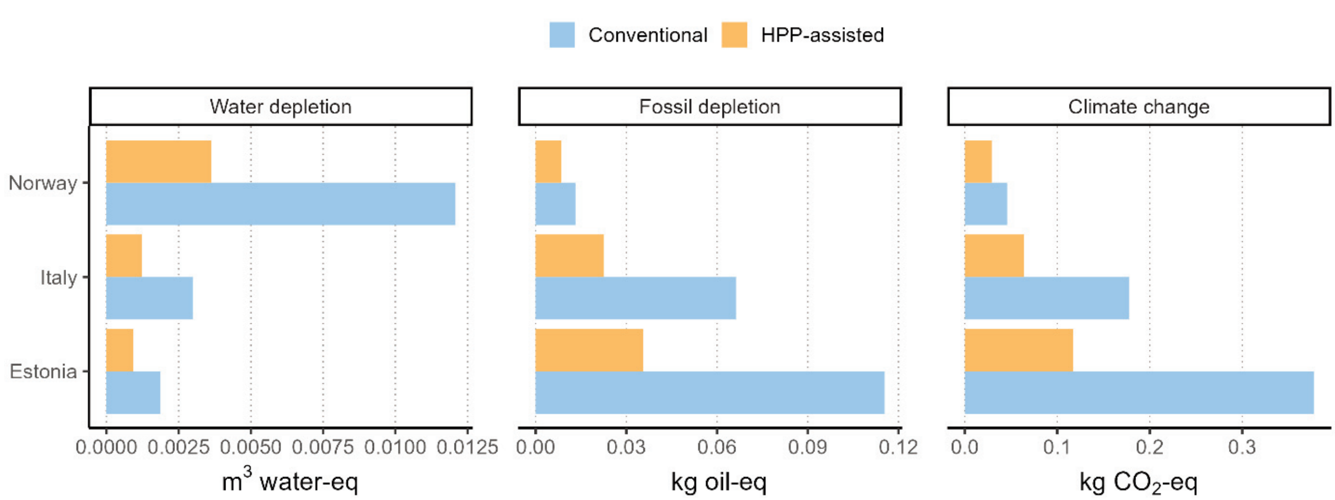

Fig. 5. Comparison of the midpoint impact categories of conventional and HPP-assisted processes when the region of production is switched to Norway or Estonia.

of Estonia (IEA; Gavrilova et al. 2010). On the other hand, transitioning from Italy to Norway would cause a reduction in the environmental impact of climate change and fossil depletion categories. However, changing the production region to Estonia would lead to a decrease in water depletion; in contrast, moving to Norway would increase the water depletion impacts. This could be explained by the higher share of hydropower in the Italian and Norwegian electricity mix compared to the Estonian energy mix. Mekonnen et al. (2015) showed that the water footprint of hydropower could be significantly higher than that of oil shale-based electricity production. Figure 5 also examines the regional transition from a production process perspective. If production was to occur in Estonia, transitioning to the HPP-assisted process would result in a stronger reduction of impact for the climate change and fossil depletion categories. Hence, it indicates that transitioning to an alternative production process would yield greater environmental benefits in regions that are less environmentally friendly (for example, in countries that have higher CO_2 emissions).

3.5.2. Alternative impact assessment method

The environmental impacts at the midpoint level are regenerated using the IMPACT World+ (Bulle et al. 2019) impact assessment method, as provided in Table 5. The results show that change in the impact assessment method does not affect the results at the midpoint level significantly.

3.6. Uncertainty analysis

The Monte Carlo sampling technique was employed to evaluate the reliability of the LCIA data for both processes. Table 6 displays the results. The impact categories that contributed less than 5% to the endpoint impact assessment were excluded. The uncertainty levels, as indicated by CV, varied across different environmental impact categories for both the conventional and HPP-assisted processes. Higher CV values indicate greater uncertainty. In most cases, the uncertainty levels were moderate. For climate change, fossil depletion, agricultural land occupation, particulate matter formation, and metal depletion, the CV values ranged from 2.72% to 24.5% for the conventional process and from 3.45% to 31% for the HPP-assisted process. For comparison, some studies have reported uncertainty levels of up to 47% (Saerens et al. 2021). The moderate levels of uncertainty suggest that the data obtained from the LCA analysis are reliable enough to draw specific conclusions regarding the environmental impacts of both processes in these categories. Visualization and statistical comparison of distributions obtained from the Monte Carlo simulation, as shown in Fig. 6, also confirmed that the difference between the conventional method and the HPP-assisted

Table 5. Comparing the midpoint environmental impacts generated by ReCiPe 2016 Midpoint (H) v1.08 and the IMPACT World+ impact assessment method

	Conventional chitosan production		HPP-assisted chitosan production	
	ReCiPe 2016 Midpoint (H) v1.08	IMPACT World+	ReCiPe 2016 Midpoint (H) v1.08	IMPACT World+
Climate change (kg CO_2-eq)	0.17	0.18	0.064	0.066
Ozone depletion (kg CFC-11-eq)	3.81×10^{-8}	4.45×10^{-8}	1.95×10^{-8}	2.14×10^{-8}
Photochemical oxidant formation (kg NMVOC-eq)	4.6×10^{-4}	4.6×10^{-4}	1.8×10^{-4}	1.8×10^{-4}

Table 6. Results of the uncertainty analysis for conventional and HPP-assisted chitosan production. The uncertainty is performed by 1000-run Monte Carlo analysis using the ReCiPe 2016 Midpoint (H) v1.08 impact assessment method

	Unit	Conventional process			HPP-assisted process		
		Mean	SD	CV	Mean	SD	CV
Climate change	kg CO_2-eq	0.177	5.11×10^{-3}	2.72%	0.064	2.35×10^{-3}	3.45%
Fossil depletion	kg oil-eq	0.066	5.54×10^{-3}	7.91%	0.022	1.57×10^{-3}	6.54%
Agricultural land occupation	m^2a	15.2×10^{-3}	2.96×10^{-3}	12.2%	4.99×10^{-3}	0.79×10^{-3}	14.4%
Particulate matter formation	PM_{10}-eq	2.8×10^{-4}	0.15×10^{-4}	5.19%	1.2×10^{-4}	0.08×10^{-4}	6.23%
Metal depletion	kg Fe-eq	8.03×10^{-3}	1.36×10^{-3}	24.5%	3.33×10^{-3}	0.94×10^{-3}	31%

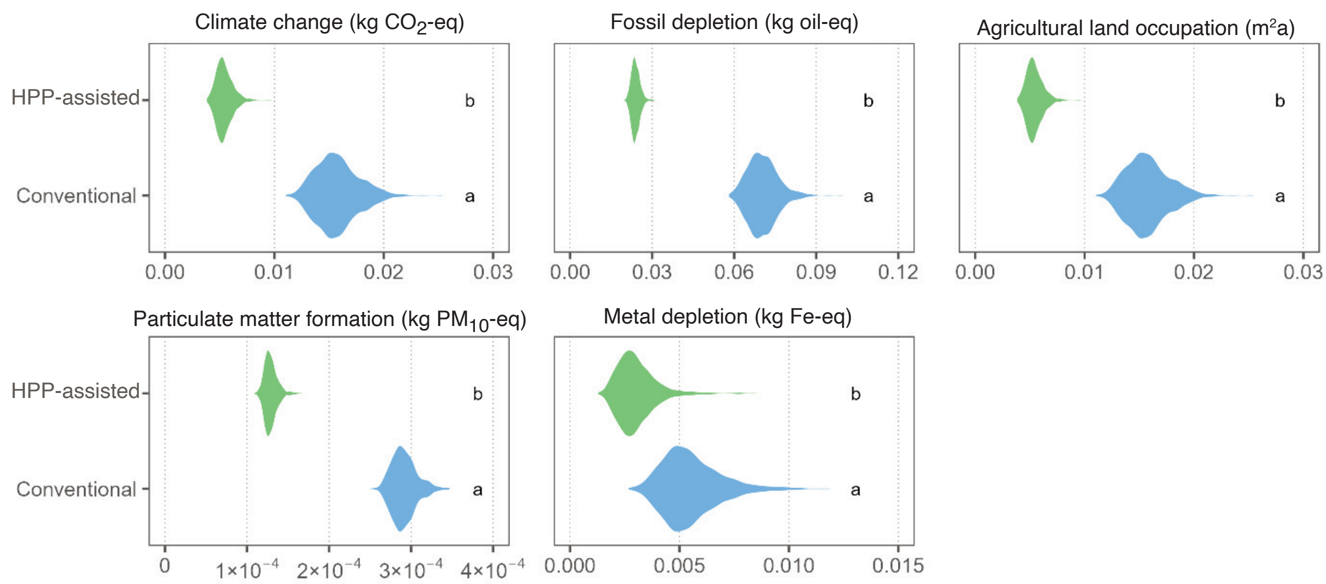

Fig. 6. Results of the uncertainty analysis are summarized as violins for comparison. Different letters within a panel indicate statistically significantly different distributions ($p < 0.05$) according to the Kolmogorov–Smirnov test. The data were obtained by 1000-run Monte Carlo analysis using the ReCiPe 2016 Midpoint (H) v1.08 impact assessment method.

method is significant, and, in all cases, the HPP-assisted process showed much lower environmental impacts. Only metal depletion showed a partial overlap of distributions.

3.7. Discussion

In evaluating the environmental performance of conventional chitosan production and the HPP-assisted process, distinct discrepancies were uncovered across midpoint categories. The HPP-assisted process consistently exhibited superior environmental outcomes across all categories, with a notable 64% reduction in climate change impacts compared to the conventional production method. This reduction can be attributed to the increased chitosan yield facilitated by HPP, leading to overall land savings exceeding the increased land use due to extraction intensification.

The environmental impacts calculated in the current study can be compared with the results of similar studies on chitosan production. Muñoz et al. (2018) investigated the environmental impacts of chitosan production in India and Europe. Riofrio et al. (2021) investigated chitosan production in Ecuador, and Fraterrigo Garofalo et al. (2023) investigated microwave-assisted chitosan production in Brazil. Although the analyzed product was chitosan, methodology, process, and scale of the production were different from the current study. The process of chitosan production in Ecuador includes shrimp farming, shrimp processing, ethanol production from sugarcane, and treatment of effluents generated by the process. While Muñoz et al. (2018) have not considered shrimp farming in their study, the treatment of effluents has been included. The comparison of some midpoint impact categories with these studies is provided in Table 7. Even though the results in some midpoint impact categories are similar, it is essential to recognize that our lab-scale processes likely exhibit lower production efficiency compared to industrial-scale operations. Additionally,

Table 7. Comparative analysis of the results of midpoint impact categories for chitosan against values reported in the literature. Impacts for conventional and HPP-assisted processes are calculated using ReCiPe 2016 Midpoint (H) v1.08. Values are per 1 g of chitosan

Impact categories	Conventional process	HPP-assisted process	Muñoz et al. (2018)	Riofrio et al. (2021)	Silva et al. (2024)
Climate change (kg CO_2-eq)	0.177	0.064	0.0468–0.0771	0.059	0.67–0.69
Fossil depletion (kg oil-eq)	0.066	0.022	–	0.011	–
Water depletion (m^3 water-eq)	2.99×10^{-3}	1.23×10^{-3}	2.2×10^{-3}–5.87×10^{-3}	34×10^{-3}	–
Freshwater eutrophication (kg P-eq)	6.27×10^{-5}	2.50×10^{-5}	2.02×10^{-5}–3.7×10^{-5}	1.39×10^{-5}	–

methodological differences and regional factors may contribute to observed variations. The LCA results of the current study are in good agreement with the results reported by Muñoz et al. (2018); however, Riofrio et al. (2021) and Silva et al. (2024) reported water depletion and climate change impacts about ten times higher. This difference could be attributed to the different production methods and regional differences in electricity production and water supply.

Analyzing the contributions of different production steps to environmental impacts elucidated key factors influencing the sustainability of each process. Both conventional and HPP-assisted processes showcased significant impacts from deacetylation, deproteinization, and demineralization steps, with notable roles played by chemicals such as NaOH and HCl. Silva et al. (2024) also recognized NaOH use during deacetylation as a hotspot and suggested minimizing its application. This is because the deacetylation process converts the chitin to chitosan, and it is the most NaOH-demanding process. Riofrio et al. (2021) analyzed the effect of different concentrations of NaOH (30, 40, 50 wt%) on the environmental impacts of chitosan production. They found that the environmental impacts increased as the concentration of NaOH increased. In contrast, Muñoz et al. (2018) indicated that HCl is among the highest contributors to chitosan production in India. However, in general, these findings support the results of the current study.

This study showed that transitioning to the HPP-assisted process reduced deproteinization contributions. Furthermore, the overall HPP contribution remained minimal across all impact categories, highlighting its limited environmental footprint within the production process. Moving beyond midpoint assessments, endpoint analyses revealed substantial advantages for the HPP-assisted process across all endpoint categories, including human health, ecosystem impacts, and resource scarcity. Not only did the HPP-assisted process exhibit significantly lower CO_2-eq impacts, but it also consistently outperformed the conventional method in all assessed endpoint categories. HPP-assisted food processing has been investigated from a sustainability point of view in comparison to other techniques. Studies demonstrate that HPP-assisted processing could be more environmentally friendly. Valsasina et al. (2017) showed that ultra-high pressure homogenization for sterilization of milk can lead to lesser environmental impacts compared to a conventional high-temperature process. Cacace et al. (2020) also showed that HPP pasteurization of food has lower environmental impacts in comparison to thermal pasteurization.

The sensitivity and scenario analyses included a regional shift and an alternative impact assessment method, and these analyses reaffirmed the reliability of our results. We showed that changing the region of electricity production made significant changes to the environmental impacts, supporting conclusions also made by Muñoz et al. (2018) and Silva et al. (2024). Despite moderate uncertainty levels in specific environmental impact categories, the Monte Carlo simulations reinforced the significant environmental advantages of the HPP-assisted process over the conventional method. These findings collectively support the conclusion that transitioning to HPP-assisted chitosan production offers substantial environmental benefits and underscores the importance of continued research and innovation in sustainable manufacturing practices.

4. Conclusion and prospects

In conclusion, the comparative analysis of chitosan production from a sustainability perspective using LCA methodology, focusing on high-pressure processing and conventional methods, revealed significant insights into the environmental impacts of each approach and demonstrated better environmental performance of the high-pressure-assisted process across multiple impact categories. The findings provide valuable insights for stakeholders in the chitosan production industry, guiding decision-making processes aimed at enhancing sustainability and mitigating environmental footprints. The findings show that integrating chitosan production with circular economy principles, such as utilizing waste streams from the food processing industry, could enhance sustainability and resource efficiency. Moving forward, further research could focus on optimizing the HPP-assisted process parameters to enhance its environmental sustainability. Additionally, exploring the scalability and feasibility of implementing HPP-assisted methods at an industrial scale would be valuable for practical applications.

Author contributions

Bashir Bashiri: conceptualization, methodology, software, validation, resources, data curation, writing – original draft preparation, visualization; **Ana Cristina De Aguiar Saldanha Pinheiro**: investigation, resources, data curation, writing – review and editing; **Silvia Tappi**: investigation, resources, data curation, writing – review and editing, supervision; **Pietro Rocculi**: resources, data curation, writing – review and editing, supervision; **Aleksei Kaleda**: writing – review and editing, visualization; **Raivo Vilu**: conceptualization, validation, writing – review and editing, supervision, funding acquisition, project administration.

Data availability statement

All data utilized for the assessment have been cited and are accessible online.

Acknowledgments

This study was conducted as a part of the ERA-NET BlueBio project "ImPrESsiVE – Improved Processing to Enhance Seafood Sidestream Valorization and Exploration". It was funded by the Estonian Research Council and ERA-NET BlueBio grant No. 341732 of the Research Council of Norway. The publication costs of this article were partially covered by the Estonian Academy of Sciences.

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Laboratoorsel skaalal kitosaani tootmise elutsükli hindamine: kõrgsurvetöötluse ja tavapäraste meetodite võrdlus

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Kitosaani järele on suur nõudlus selle laia kasutusala tõttu, mille tulemuseks on usaldusväärne turg. Kitosaani tavapärased keemilised ekstraheerimismeetodid ei ole keskkonnasõbralikud, nõuavad tugevaid happeid ja aluseid ning tekitavad mürgiseid jäätmeid. Kitosaani keemiline ekstraheerimine kõrgsurvetöötlusega (HPP) võib anda suurema tootmissaagise. Seetõttu on selle meetodi keskkonnamõju hindamine ülioluline. Selles artiklis esitatakse kitosaani tootmismeetodite põhjalik ja võrdlev analüüs keskkonna vaatenurgast, keskendudes HPP-toega ja tavapärastele tehnikatele. Uuringus hinnatakse tavapäraste ja HPPga kitosaani tootmisprotsesside keskkonnajalajälge, kasutades elutsükli hindamise (LCA) metoodikat. Tulemused näitavad, et võrreldes tavapäraste meetoditega on HPP abil tootmisel positiivsem keskkonnamõju, iseäranis väheneb kliimamuutuste mõju (64%). Tundlikkus- ja stsenaariumianalüüsid kinnitavad leidude õigsust, võttes arvesse elektri tootmise piirkondade muutmist ja alternatiivseid iseloomustusmeetodeid. Määramatuse analüüs näitab mõõdukat määramatuse taset, mis kinnitab andmete usaldusväärsust. Uuringust järeldub, et HPPga kitosaani tootmine pakub erinevates näitajates säästvamat ja väiksema keskkonnajalajäljega lähenemisviisi. Need leiud annavad kitosaanitööstuse asjaosalistele väärtuslikke juhiseid säästvama arengu edendamiseks ja keskkonnamõju minimeerimiseks.