DOCTORAL THESIS

Valorization of Blue Mussels in the Baltic Sea

Indrek Adler

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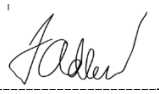
Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Indrek Adler



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List of publications

The list of author's publications, on the basis of which the thesis has been prepared:

I **Adler, I.,** Kotta, J., Tuvikene, R. and Kaldre, K. (2022). Optimizing the processing of shellfish (*Mytilus edulis* and *M. trossulus* hybrid) biomass cultivated in the low salinity region of the Baltic Sea for the extraction of meat and proteins. *Applied Sciences*, 12, 5163. https://doi.org/10.3390/app12105163

II **Adler, I**., Kotta, J., Tuvikene, R. and Orav-Kotta, H. (2024). Unlocking the potential of shellfish biomass: Refining protein extraction from Baltic blue mussels for sustainable food applications. *Cogent Food & Agriculture*, 10(1). https://doi.org/10.1080/23311932.2024.2405880

III **Adler, I.,** Kotta, J., Robal, M., Humayun, S., Vene, K. and Tuvikene, R. (2024). Valorization of Baltic Sea farmed blue mussels: Chemical profiling and prebiotic potential for nutraceutical and functional food development. *Food Chemistry: X*, 23, 101736. https://doi.org/10.1016/j.fochx.2024.101736

IV **Adler, I.,** Kotta, J. and Vene, K. (2025). Micronization of low-salinity Baltic Sea blue mussels: Enhancing whole-biomass utilization and nutritional viability. *Fishes*, 10, 199. https://doi.org/10.3390/fishes10050199

Author's contribution to the publications

I am the first and corresponding author of all four publications included in this dissertation.

In *Publication I*, I was responsible for the conceptualization of the study, development of the processing workflow, and the execution of laboratory trials for sedimentation-based biomass recovery. I also led the data analysis and the preparation of the manuscript, figures, and tables, with co-authors contributing to method refinement and critical review.

In *Publication II*, I designed and conducted the laboratory experiments on acid rinsing and protein extraction efficiency, analyzed the resulting data, and drafted the manuscript. The co-authors contributed to sensory evaluation methodology, chemical analysis, and manuscript editing.

In *Publication III*, I conducted the bioactive compound and prebiotic potential evaluations, including enzymatic hydrolysis protocols and *in vitro* probiotic assays. I led the data interpretation and manuscript preparation, with my co-authors contributing to compositional analysis, microbiological testing, and peer review of the draft.

In *Publication IV*, I developed the whole-biomass micronization methodology, coordinated industrial-scale processing trials, and analyzed the resulting powder characteristics. I prepared the figures, interpreted the results in the context of zero-waste aquaculture valorization, and led the drafting and revision of the manuscript in close collaboration with co-authors who assisted with statistical analysis and laboratory validation.

All publications reflect my original contributions as the principal investigator and doctoral candidate, with supervision and input from the co-authors.

1 INTRODUCTION

1.1 Broad framing: EU Green Deal and Blue Growth

The European Green Deal, adopted in 2019, sets the pathway for the European Union (EU) to become climate-neutral by 2050 (European Commission, 2019). One of its central pillars is the transformation of food systems towards sustainability, resilience, and biodiversity conservation. The Farm to Fork Strategy, launched as part of the Green Deal, targets a 50% reduction in the use of chemical pesticides, a 20% reduction in fertilizer use, and a 25% share of organic farming by 2030 (European Commission, 2020). Sustainable aquaculture is explicitly encouraged as a means to diversify food production with lower environmental impacts compared to traditional agriculture.

The strategy emphasizes the need for resource-efficient production models that minimize pollution, support ecosystem recovery, and reduce dependency on critical inputs such as freshwater resources and feed. In this context, the development of nature-positive aquaculture is seen as essential for achieving the Green Deal's broader goals of sustainable food security.

The Blue Growth strategy, initially launched in 2012 and updated to align with the European Green Deal, seeks to promote the sustainable use of ocean resources for economic growth, improved livelihoods, and ecosystem health (European Commission, 2021). Blue Growth identifies aquaculture as a key sector capable of delivering both economic value and environmental services when designed sustainably.

By focusing on innovation in aquaculture, blue biotechnology, and marine ecosystem restoration, Blue Growth supports the development of new, low-impact food systems. It encourages diversification beyond high trophic species to farming practices that have minimal ecological footprints, aligning economic development with marine conservation goals. In parallel, methodological innovation plays a key role in unlocking the potential of low-trophic biomass. Analytical approaches from food and marine sciences, such as advanced chromatography and spectroscopy, are increasingly applied to profile and valorize underutilized marine species (Otles, 2011; Bayona et al., 2022).

Low trophic aquaculture (LTA) refers to the cultivation of organisms low in the food web, such as mussels and seaweed, which require no external feed, fertilizers, or freshwater inputs. These systems not only provide biomass for food, feed, and other applications but also deliver measurable ecosystem services. Mussels, through filter-feeding, can remove significant quantities of nitrogen and phosphorus from coastal waters, contributing to nutrient load reduction (Petersen et al., 2016). Seaweed cultivation can sequester carbon, absorb excess nutrients, and increase local biodiversity (Duarte et al., 2017).

The integration of LTA within Blue Growth initiatives directly supports the objectives of the European Green Deal by offering production systems that improve water quality, enhance marine habitats, and strengthen coastal economies. Several European research and innovation programs, including Horizon Europe's Mission Ocean, recognize mussel and seaweed farming as essential tools for building sustainable, resilient marine ecosystems.

Against this policy and strategic backdrop, the present thesis focuses on advancing the utilization of low trophic aquaculture species, particularly Baltic Sea blue mussels. The research aims to explore their potential for sustainable biomass valorization, ecosystem service delivery, and contribution to food and feed innovation. Understanding and optimizing these systems can support both environmental restoration goals and the economic diversification targeted by EU strategies.

Blue mussels are part of a broader group of marine macroorganisms increasingly explored for aquafeed and food applications. Alongside species such as starfish, amphipods, jellyfish, and macroalgae, mussels have been studied for their protein content, fatty acid composition, and functional properties (Biandolino & Prato, 2006). Like many of these low-trophic, filter-feeding organisms, blue mussels offer both valuable biomass and ecosystem services that support environmental health. While species such as amphipods, starfish, and jellyfish are gaining attention due to unique protein or lipid profiles, many of these require artificial enrichment, specialized harvesting techniques, or intensive processing (Suhaimi et al., 2024). In contrast, mussels provide a naturally occurring biomass that can be valorized with relatively low inputs and operational complexity (Eroldoğan et al., 2022), making them especially well-suited for circular and sustainable aquaculture systems (Arantzamendi et al., 2023).

1.2 Mussel biology and ecology

Mussels are bivalve mollusks (phylum Mollusca, class Bivalvia) belonging to the family Mytilidae, which includes numerous marine species. For example, the blue mussel (*Mytilus edulis*), Mediterranean mussel (*M. galloprovincialis*), and Pacific blue mussel (*M. trossulus*) are all members of this group and are commonly found and farmed in temperate seas worldwide (National Research Council, 2010). Like all bivalves, a mussel's body is enclosed between two hinged shells (valves) composed largely of calcium carbonate. The shells can be clamped shut by strong adductor muscles, allowing mussels to conserve water and withstand short-term environmental stresses (e.g., exposure to air at low tide or to low oxygen in water). Mussels lack a head and centralized brain, but possess a muscular foot used for mobility and secretion of byssal threads, tough, collagen-like fibers that tether the animal to rocks or other substrates. These threads enable mussels to form persistent attachments, often clustering in dense aggregations on intertidal rocks, ship hulls, or aquaculture ropes. Indeed, many mussel species are gregarious, forming extensive beds or reefs with multiple layers of individuals bound together by byssus (Seed & Suchanek, 1992). Such mussel beds create complex three-dimensional habitats that provides niches for other organisms (e.g., small invertebrates and algae), earning mussels a reputation as ecosystem engineers that enhance local biodiversity (Hild & Günther, 1999). In natural settings, an established mussel bed can persist for years, although overcrowding and accumulation of silt and waste can cause inner individuals to die, occasionally leading to sections of the bed detaching and eroding away.

Natural predators of mussels (including starfish, crabs, wading birds, and ducks) are an important ecological factor controlling mussel populations, especially on exposed shores. Predation pressure tends to decrease as mussels grow larger, since many predators prefer smaller prey; this means a mussel that survives to a large size may enjoy a refuge from further predation (Seed, 1993).

As filter feeders, mussels obtain food by pumping water over their gills and filtering out suspended particles. They consume primarily phytoplankton (microscopic algae) and organic detritus, positioning them at a low trophic level as secondary producers (herbivores) in marine food webs (Duarte et al., 2009; National Research Council, 2010). Mussels can filter impressive volumes of water relative to their size (often several liters per hour per adult mussel). In doing so, mussels not only feed themselves but also significantly influence water quality and nutrient cycling. As they filter out plankton and

suspended solids, mussels clarify the water and their feces and pseudofeces (rejected particles bound in mucus) are deposited to the seafloor. This filtration and biodeposition activity effectively couples the pelagic and benthic environments, transferring organic material and nutrients from the water column to the sediments (Dame, 1996; National Research Council, 2010). Mussels excrete dissolved ammonium as metabolic waste, returning usable nitrogen to the ecosystem, which can fertilize local primary producers (Newell et al., 2005). In areas with substantial mussel populations or farms, these processes can enhance water clarity and promote the growth of seagrasses and other aquatic vegetation by reducing turbidity and recycling nutrients (Kaiser, 2001; Dame & Olenin, 2005). Mussel filtration is thus generally considered beneficial for water quality, and mussel beds provide ecosystem services akin to those of oysters in estuaries (National Research Council, 2010). Moreover, by harvesting and removing mussel biomass, humans can also remove some of these accumulated nutrients from the marine system. For instance, mussel farming has been proposed as a tool for bioremediation of nutrient-enriched waters – as mussels grow, they incorporate nitrogen and phosphorus into their flesh and shells, and harvesting them extracts these nutrients from the ecosystem (Lindahl et al., 2005). One Swedish study demonstrated that cultivating blue mussels in eutrophic waters could improve marine water quality by removing significant amounts of nitrogen when the mussels were harvested, while simultaneously yielding a usable biomass (Lindahl et al., 2005; Petersen et al., 2014). This highlights the elegant synergy between mussel ecology and human use: what mussels naturally do to feed and grow can also help manage nutrients and provide resources for people.

Mussels have a simple life cycle typical of many marine bivalves, involving a dispersive larval stage. Most mussel species have separate sexes (male and female), and fertilization occurs externally in the water column. Mature adults broadcast spawn by releasing eggs and sperm into the water, usually in synchrony triggered by environmental cues like temperature and phytoplankton abundance. A single female mussel can release on the order of millions of eggs in a spawning event (e.g., 5–10 million from a mid-sized mussel), reflecting an evolutionary strategy of high output to offset high mortality in early life stages. After fertilization, the developing embryos become free-swimming larvae (trochophore and then veliger stages) that drift with plankton for a period of a few weeks. During this time the larvae feed on microalgae and undergo development, eventually metamorphosing into juvenile mussels (spats) that settle out of the plankton. The larval phase allows mussels to disperse to new areas, but also means they are at the mercy of currents and predation until they find a suitable habitat to settle. Upon settlement, the juvenile mussel attaches to a firm surface using byssal threads and begins its benthic life. From there, growth to adulthood is relatively rapid under favorable conditions. Growth rates in mussels, however, show high variability, depending especially on environmental conditions but also on genetic factors (Seed & Suchanek, 1992). Under optimal conditions (e.g., full-strength seawater, moderate temperatures, and abundant food), young mussels can attain ~6–8 cm shell length within about two years (Seed & Suchanek, 1992). By contrast, in harsher environments growth can be stunted. For instance, mussels living high on the shore (exposed to air for long periods each day) or in low-salinity brackish waters grow much more slowly and may reach only a few centimeters across in several years (Kautsky, 1982; Westerbom et al., 2002). Environmental factors that strongly affect mussel growth and survival include temperature, salinity, food availability (plankton concentration), degree of tidal exposure, and biological interactions such as competition and parasitism. These factors

often act together. For example, food supply might mitigate some effects of suboptimal temperature or salinity, but only up to a point, beyond which physiological stress limits growth. In general, mussels tend to thrive in cool-temperate, saline waters with plentiful plankton. Most *Mytilus* species are eurythermal (tolerating a range of temperatures) and euryhaline to a degree, but each species has its limits. Blue mussels, for instance, can survive near-freezing winter temperatures and can also withstand summer peaks above 20–25 °C, though sustained water temperatures above roughly 30 °C can be lethal or cause reproductive failure (Almada-Villela et al., 1982). Similarly, while adults of *M. edulis* can acclimate to reduced salinities, growth and filtration rates drop significantly below about 8–10, and prolonged exposure to very low salinity (less than 6) may eventually exceed their osmotic tolerances (Riisgård et al., 2013). To cope with short-term stresses like a sudden drop in salinity or oxygen, mussels exhibit behavioral and physiological responses: they may close their shells to isolate themselves from unfavorable water, and their metabolism can shift to anaerobic pathways to sustain them during these closed periods. This allows intertidal mussels to survive several hours of exposure out of water (including temperature extremes and desiccation). However, staying closed also means a mussel cannot feed or respire normally, so there are trade-offs and time limits to this strategy. Generally, mussels must reopen and resume filtration once conditions improve, or else face starvation or asphyxiation.

The physiological tolerances of mussels are a key consideration for mariculture: successful mussel farming depends on selecting sites where salinity, temperature, oxygen levels, and plankton food are within the range that mussels can handle for healthy growth (National Research Council, 2010). Farmers avoid areas prone to severe low-salinity influxes or chronic pollution, because stress conditions suppress mussel feeding, growth, and can even cause mass mortality. Likewise, sites with excessive silt or frequent resuspension of sediments can clog mussel gills or lead to high pseudofeces production, reducing feeding efficiency. By understanding mussel physiology, for instance, knowing that *M. edulis* needs higher salinity for optimal growth, or that warm water speeds growth up to a point before heat stress occurs, aquaculturists can better match mussel species and stocks to suitable environments. This ensures not only good yields but also animal welfare, since mussels in appropriate conditions will have stronger immune function and a higher condition index, making them less susceptible to disease and predation.

Another important aspect of mussel biology is their biochemical composition, which underpins both their ecological role and their value to humans. Mussel tissues are rich in organic nutrients, which is one reason predators (including humans) find them so nutritious. The proximate composition of mussel meat (the soft tissue) is typically high in protein and low in fat. On a dry-weight basis, mussel flesh often contains 50–70% crude protein, with most of the remaining mass being glycogen (a carbohydrate reserve) and lipids in the range of ~5–10% (Smoothey, 2013; Saritha et al., 2015). Even though the total lipid content is relatively low, the type of fats present is noteworthy: mussels are a good source of omega-3 polyunsaturated fatty acids (especially eicosapentaenoic acid, EPA, and docosahexaenoic acid, DHA) as well as omega-6 fatty acids, making them a heart-healthy food choice (King et al., 1990; Ersoy & Sereflisan, 2010). In fact, mussels and other bivalves are often touted as a low-fat, high-protein seafood with significant levels of beneficial long-chain omega-3s that can support human health. They also provide essential minerals (like iron, zinc, selenium, and iodine) and vitamins (particularly B-complex vitamins such as B_{12}). The biochemistry of mussels further extends to various

bioactive compounds. For instance, mussels produce antioxidant enzymes to cope with reactive oxygen species generated during their high filtering activity, and their diet of phytoplankton can introduce algae-derived compounds into their system. Some mussel species have become sources of nutraceutical products: a prime example is the New Zealand green-lipped mussel (*Perna canaliculus*). The green-lipped mussel is processed into extracts (both powdered and oil forms) that are used as dietary supplements for joint health, due to their anti-inflammatory properties. Research has shown that lipid extracts from *P. canaliculus* are rich in anti-inflammatory molecules (including omega-3 fatty acids and unique furan fatty acids) that can inhibit inflammatory pathways in the body, offering relief in conditions like arthritis (McPhee et al., 2007; Wakimoto et al., 2011). This is an excellent illustration of how the chemistry of mussel tissues (in this case, their fatty acid profile) supports valorization: a natural product from mussel biology becomes a high-value health supplement in the marketplace. The mussel's shell is another component of interest. Mussel shells are composed of calcium carbonate ($CaCO_3$) crystals (a combination of calcite and aragonite forms) embedded in a matrix of proteins and polysaccharides. In fact, about 95% of the shell weight is $CaCO_3$, with only ~5% organic material (Cinelli et al., 2020). This makes mussel shells a rich source of biogenic calcium carbonate. Traditionally, discarded shells from mussel farms or processing have been treated as waste, but increasingly they are viewed as a valuable by-product that can be repurposed. Crushed mussel shells can be used as a soil amendment or fertilizer (to neutralize acidic soil and add calcium) and as a calcium supplement in animal feed (for example, poultry farmers have used ground shell to provide laying hens with calcium for eggshell formation, analogous to oyster shell supplements). Ground shells have also been explored as a raw material in construction (cement and concrete additives) and in manufacturing biocomposites. For instance, recent studies have added powdered mussel shell as a filler in biodegradable plastics, effectively recycling aquaculture waste into new materials (Cinelli et al., 2020). The high purity of calcium carbonate in mussel shells means they can substitute for mined limestone in certain applications, which has economic and environmental appeal.

1.3 Farming and valorization of mussels and algae for ecosystem restoration and sustainable food and feed

Mussel farming offers important ecosystem services that align with marine restoration objectives. Through their filter-feeding activity, mussels remove suspended particles and phytoplankton from the water column, thereby also reducing excess nutrients such as nitrogen and phosphorus from the ecosystem. This process can improve water clarity, reduce eutrophication, and promote the recovery of seagrass beds and other benthic habitats (Newell, 2004). Mussel structures also provide habitat complexity, supporting increased biodiversity of associated fish and invertebrate communities (Petersen et al., 2016).

Seaweed farming similarly contributes to nutrient removal by directly absorbing dissolved nutrients from seawater. Additionally, seaweed cultivation offers carbon sequestration potential and can moderate local ocean acidification levels (Duarte et al., 2017). Together, mussel and seaweed farming can be integrated into marine management strategies aimed at achieving the goals of the EU Marine Strategy Framework Directive and other regional restoration initiatives.

Globally, mussel farming has demonstrated its potential as a sustainable industry with both environmental and economic benefits. In Denmark, large-scale mussel farms have been established for nutrient bioextraction, particularly in areas affected by agricultural runoff, with demonstrated reductions in coastal nitrogen loads (Petersen et al., 2016). In New Zealand, mussel aquaculture is integrated into coastal resource management, contributing both to marine biodiversity enhancement and significant economic output through export markets (Forrest et al., 2009).

In the broader search for sustainable aquafeed and food inputs, a wide range of marine species have been explored, including microalgae, thraustochytrids, fungi, amphipods, and jellyfish, each offering distinct advantages in protein and lipid content (Odabaşı et al., 2016; Khong et al., 2016; Bonfanti et al., 2018; Jaseera & Kaladharan, 2019; Suhaimi et al., 2024). Blue mussels represent a relatively accessible, autochthonous biomass source that can be harvested at comparatively low cost. Their overall simplicity and ecological compatibility make mussel farming a strategically valuable option, particularly in regions where nutrient reduction and environmental remediation are of high priority (Gren, 2019).

A broad range of methodological approaches, such as amino acid profiling, digestibility assays, and functional bioactivity screening, are now applied across marine species to assess their suitability for feed and nutraceutical applications (Shahidi & Saeid, 2025). Positioning mussels within this comparative framework could enhance their visibility and industry relevance in both farming and valorization efforts (Eroldoğan et al., 2022).

In Europe, integrated multi-trophic aquaculture (IMTA) systems are increasingly promoted, combining mussels, seaweed, and finfish to optimize resource use and minimize environmental impacts. Successful models from Norway and France illustrate how LTA species can contribute to diversified production systems with improved ecological outcomes. Recent research on aquafeed and food systems consistently highlights the value of low-trophic marine species, not only for their sustainability, but also for their role in supporting diversified and climate-resilient food systems. Mussels fulfill these criteria while also delivering ecosystem services, a combination not commonly found in other candidate species (Eroldoğan et al., 2022).

Mussel biomass offers high-quality protein, essential fatty acids, and micronutrients, positioning it as a valuable contributor to future food and feed systems. Mussels have a low feed conversion ratio, requiring no added feed inputs, and can be harvested sustainably with minimal environmental footprint compared to terrestrial livestock (SAPEA, 2017). The nutritient density of mussel biomass has led to increased interest in its biochemical profiling for functional food and nutraceutical development. Recent work suggests that advanced molecular and chromatographic techniques are required to accurately quantify bioactive peptides, fatty acids, and glycogen, given their potential role in health-promoting formulations (Suleria et al., 2015; Eroldoğan et al., 2022).

Beyond direct human consumption, mussel meal is increasingly explored as a sustainable ingredient for aquafeeds and animal feeds. Its amino acid profile and nutrient density make it a promising alternative to traditional fishmeal, supporting the development of more sustainable aquaculture and livestock industries.

Seaweed biomass also offers potential as a feed supplement due to its mineral content, bioactive compounds, and capacity to improve animal gut health. Together, mussel and seaweed cultivation can contribute to diversified and resilient food systems, addressing challenges of food security and environmental sustainability.

1.4 Specificities of the Baltic Sea context

The Baltic Sea is characterized by low salinity, ranging from approximately 2 to 20, from the inner parts to the Danish Straits (Schubert et al., 2017). This brackish environment limits the growth potential of marine species, including mussels. The dominant mussel populations consist of hybrids between *Mytilus edulis* and *Mytilus trossulus*, which show smaller size and slower growth compared to their counterparts in fully marine environments (Kautsky et al., 1990). Typical adult mussels in the Baltic Sea reach shell lengths of only 20–30 mm, significantly smaller than those farmed in Atlantic waters.

The Baltic Sea has experienced decades of nutrient enrichment from agricultural runoff, wastewater discharge, and industrial pollution, resulting in widespread eutrophication (HELCOM, 2018). Mussel and seaweed farming offer promising approaches to mitigate these impacts through nutrient bioextraction. Mussels can effectively remove nitrogen and phosphorus through filtration and subsequent biomass harvest, providing a direct ecosystem service that aligns with regional action plans such as the Baltic Sea Action Plan.

Similar nutrient-removal functions have been investigated in other marine organisms such as macroalgae and various filter-feeding invertebrates. However, mussels remain one of the few options that simultaneously provide marketable biomass and high nitrogen and phosphorus uptake per unit area (Jansen et al., 2019). In contrast to species like jellyfish or sea cucumbers, which require active harvesting and post-capture stabilization, mussels offer more consistent deployment, maintenance, and recovery (Petersen et al., 2019).

Environmental stressors, including low salinity, seasonal temperature fluctuations, and varying nutrient availability, affect the physiological condition of mussels in the Baltic Sea. Thin shells and relatively low meat yields are common features in farmed populations (Lindahl, 2012). These characteristics present challenges for the economic viability of mussel farming, particularly when targeting traditional markets where larger, meatier mussels are preferred. These region-specific constraints necessitate tailored methodologies. Standard aquaculture processing workflows fail to recover sufficient biomass from small, fragile mussels, prompting the development of redesigned techniques grounded in marine biochemical analysis and fractionation processes (Bayona et al., 2022; Maar et al., 2023).

Comparable processing challenges have been documented for other underutilized marine inputs such as starfish, microalgae, and crustacean shells, where fragile structures or low raw yields complicate mechanical and chemical conversion. Research in these areas increasingly turns to targeted fractionation, gentle disruption, and enzymatic release (Venugopal, 2021), mirroring the trajectory followed in the present thesis.

Despite these constraints, Baltic Sea blue mussels remain valuable for alternative valorization pathways, such as nutrient recycling, feed production, and functional ingredient extraction. Even if mussel growth and final sizes are not optimal, farming in the Baltic Sea remains essential, as it is one of the few viable methods to extract legacy nutrients from the marine environment. Understanding and adapting farming and processing methods to these environmental specificities is essential for building a sustainable mussel industry in the region.

1.5 Current challenges and scientific framework: State-of-the-art in Baltic Sea blue mussel farming

Low-trophic aquaculture (LTA) focuses on cultivating species at the base of the food web, such as bivalves and macroalgae, which require no external feed inputs and can improve water quality through nutrient assimilation (Petersen et al., 2014; FAO, 2020). Within this context, blue mussels (*Mytilus edulis/trossulus* hybrids) in the low-salinity Baltic Sea provide both key ecosystem services and a supply of biomass for processing (Kotta et al., 2020). Their cultivation aligns with ecosystem-based management objectives by coupling seafood production with nutrient removal from eutrophic waters (Lindahl et al., 2005; Petersen et al., 2014), mitigating eutrophication while generating biomass.

Despite this ecological potential of Baltic mussel farming (Kotta et al., 2020), valorization pathways for the small-sized mussels remain underdeveloped. Most existing efforts have emphasized nutrient removal and ecosystem service provision, with limited initiatives directed toward developing high-value products for human consumption or feed markets (Lindahl, 2012; Petersen et al., 2016). While prior studies have highlighted the role of Baltic Sea blue mussels in nutrient bioextraction, they fall short of addressing how this biomass can be systematically converted into viable food, feed, or functional products.

This thesis focuses on hybrid *M. edulis/trossulus* mussels because of their ecological relevance and dominance in Baltic waters, with method development informed by marine metabolomics and food chemistry studies (Otles & Pire, 2001; Otles, 2011). The mussel valorization strategy was based on a biorefinery approach, which fractionates raw material into multiple product streams to maximize value and reduce waste (Cherubini, 2010). For Baltic blue mussels, such a strategy could yield high-protein meat fractions, lipid extracts, bioactive peptides, and mineral-rich shell powder, each targeting different market segments (Naik et al., 2019). This multi-stream use reflects a broader shift in marine bioproduct research from raw extraction to targeted conversion, as seen in work on krill, fish by-products, and microbial biomass, where innovative processing helps overcome biological limits and realise value (Bleakley & Hayes, 2017).

One of the main challenges in Baltic Sea mussel farming stems from the biological characteristics of the mussels themselves. In the low-salinity Baltic, *M. edulis/trossulus* grows slowly, remains small (typically less than 2 cm shell length), and develops fragile shells. These features severely limit the applicability of conventional processing methods, such as depuration, mechanical shucking, and meat extraction, because those methods were designed for larger, oceanic mussel species (Naylor et al., 2008; Kotta et al., 2020). Globally, manual shelling or meat extraction machinery can achieve high yields from large mussels, but only about 15–20% from the small Baltic mussels, with the remaining biomass (mainly shell) often discarded as waste (Lindahl et al., 2005; Nielsen et al., 2016). As a result, existing methods fail to achieve acceptable recovery yields or consistent product quality when applied to the Baltic Sea populations – a technological mismatch that restricts the sector's capacity to scale up, access markets, and generate value-added products (Maar et al., 2023).

Furthermore, the literature reveals a distinct gap in developing zero-waste processing models and in the detailed biochemical profiling of Baltic Sea blue mussels for higher-value applications (Gren, 2019; Paper III). Research remains scarce on whole-biomass utilization of these mussels, for instance, on scalable techniques for protein isolation and hydrolysate production, on understanding seasonal variations in their biochemical composition, and

on incorporating micronized shell fractions into value-added products without quality loss (Petersen et al., 2014; Eroldoğan et al., 2022). This absence of integrated valorization strategies hinders the economic viability of mussel farming in the region and contributes to the perception of Baltic blue mussels as a low-value biomass rather than as a resource for premium product development. Such perceptions parallel challenges observed with other "orphan" marine biomasses (e.g., tunicate waste or invasive green crabs), where the lack of downstream valorization frameworks has similarly limited investment and innovation despite clear ecological and nutritional potential (Eroldoğan et al., 2022).

Nevertheless, growing interest in low-input aquaculture and sustainable marine biorefinery concepts presents a timely opportunity to reposition Baltic Sea blue mussel farming. By targeting novel applications, including the production of protein-rich ingredients, functional foods, and nutraceuticals, this sector can overcome many of its current limitations. Valorizing mussels for direct human consumption, rather than solely as a means of ecosystem service delivery or low-grade feed, would significantly enhance the industry's profitability and resilience. To unlock this potential, it is essential to develop innovative, scalable processing methods (Otles, 2011; Chen et al., 2022) tailored to the biological constraints of Baltic Sea mussels. The latter can support both industry and policy, advancing the mussel farming value chain as part of sustainable, zero-waste LTA systems. These efforts also directly support the ambitions of the European Green Deal and the Blue Growth strategy by coupling marine restoration with food system transformation and regional economic development. Importantly, repositioning mussel biomass within a marine biorefinery framework aligns with the growing recognition that sustainability alone does not ensure industry adoption. As shown in other marine inputs, scalability and commercial feasibility hinge on the ability to generate differentiated, higher-value end products, particularly in nutraceutical and functional food markets (Rustad et al., 2011).

1.6 Research aim, hypotheses, and questions

The overarching aim of this dissertation is to develop and optimize scalable, sustainable valorization pathways for small-sized *Mytilus edulis/trossulus* mussels cultivated in the low-salinity conditions of the Baltic Sea. This objective addresses two interlinked challenges identified in the scientific literature:

1. The limited integration of low-trophic aquaculture biomass into circular bioeconomy frameworks, and
2. The lack of processing techniques tailored to the biological constraints of Baltic Sea blue mussels, including their small size, thin shells, and variable biochemical composition.

This research was driven by the need to integrate ecological, biochemical, and processing perspectives to advance the blue mussel value chain in the Baltic Sea region. To achieve this, the thesis combines classical aquaculture approaches with modern processing techniques and analytical tools, such as FTIR spectroscopy, gas chromatography, and colorimetric assays, to explore the scope and potential of Baltic Sea blue mussel valorization.

The sub-objectives are:

1. To explore the chemical and nutritional potential of Baltic Sea blue mussel biomass, by conducting detailed compositional analyses.
2. To develop and optimize mussel biomass valorization pathways for human consumption.
3. To improve processing methods for small-sized mussels.
4. To provide future perspectives for sustainable low trophic aquaculture development in the Baltic Sea.

To guide the empirical investigations, the following research questions were formulated:

Research Questions

1. What are the technical and nutritional limitations of current methods for processing Baltic Sea blue mussels, and how can novel workflows improve biomass recovery and product quality?
2. Can a scalable processing approach based on mechanical disruption, sedimentation, and centrifugation efficiently separate high-value organic biomass from shell material in small mussels?
3. To what extent does whole-organism micronization facilitate zero-waste valorization, and what are the functional and sensory characteristics of the resulting powders?
4. Do enzymatically hydrolyzed mussel fractions support the growth of probiotic bacteria, and how does their bioactivity vary with seasonal changes in biomass composition?
5. How do environmental and seasonal factors affect the nutritional and functional suitability of Baltic Sea blue mussel biomass for food and feed applications?

Based on these questions, four central hypotheses were proposed:

Research Hypotheses

- **H1**: Mechanical slurry-based processing workflows significantly increase biomass recovery and protein yield from small-sized Baltic Sea blue mussels compared to traditional manual shelling methods.
- **H2**: Whole-biomass micronization produces fine powders (less than 63 µm) with acceptable sensory characteristics and high nutrient density, enabling zero-waste product development.
- **H3**: Enzymatic hydrolysates derived from Baltic Sea blue mussel biomass contain low molecular weight peptides with measurable prebiotic effects on probiotic strains such as *Lactobacillus rhamnosus* and *Bifidobacterium animalis*, *in vitro*.
- **H4**: Seasonal physiological differences in mussels, such as post-spawning recovery in autumn, significantly influence the biochemical composition and valorization potential of the harvested biomass.

Structure of the Empirical Work

The empirical component of this dissertation is based on four peer-reviewed scientific publications, each targeting a specific constraint in the valorization of Baltic Sea blue mussel biomass. The publications follow a sequential structure that reflects the stepwise development of an integrated processing framework suitable for small-sized *Mytilus edulis/trossulus* mussels under low-salinity conditions.

Each publication corresponds to one or more of the above hypotheses and addresses a defined stage in the valorization pathway:

1. **Publication I** investigates scalable mechanical processing techniques, focusing on sedimentation and centrifugation to recover organic biomass without the need for manual shelling.
2. **Publication II** examines protein extraction efficiency and flavor improvement, introducing citric acid treatment to reduce undesirable off-flavors and enhance product quality.
3. **Publication III** evaluates the seasonal biochemical composition of mussel biomass and explores the prebiotic potential of enzymatically hydrolyzed fractions *in vitro*.
4. **Publication IV** assesses the feasibility of whole-biomass micronization, converting entire mussels, including shell material, into fine powders suitable for use in food and feed applications.

These publications are aligned with distinct case study foci and contribution areas, as presented in **Table 1**. Collectively, they establish a scalable, resource-efficient approach to the valorization of Baltic Sea blue mussel biomass, addressing both technological and functional dimensions.

Table 1. *Alignment of publications with the dissertation.*

Case Study Focus	Contribution Area of the Publication	Publication
Biomass Processing	Developing mechanical processing workflows tailored to Baltic Sea blue mussels, focusing on sedimentation and centrifugation techniques to improve yield and usability.	I
Protein Extraction	Refining extraction protocols to increase protein recovery and sensory quality, including acid rinsing and dry matter optimization.	II
Bioactivity & Composition	Evaluating seasonal impacts on biochemical composition and assessing prebiotic effects of enzymatic hydrolysates.	III
Whole-Biomass Micronization	Developing a zero-waste processing model by micronizing whole mussels, including the shell, into functional powders suitable for food and feed applications.	IV

TERMS

Term	Definition
Sedimentation	Separation technique using gravity to settle particles in a suspension
Decantation	Process of separating liquid from settled solids
Micronization	Mechanical process of reducing particles to micron scale
Mechanical Shucking	Traditional method of removing mussel meat from the shell
Enzymatic Hydrolysis	Use of enzymes to break down protein structures
Sensory Evaluation	Human panel testing for taste, smell, and mouthfeel
Meat Yield	Proportion of mussel mass that can be recovered as meat
Glycogen Analysis	Measurement of carbohydrate storage form in mussels
Kjeldahl Method	Standard method for measuring total nitrogen/protein
Functional Food	Food with health benefits beyond basic nutrition
Prebiotic Activity	Effect of ingredients that support the activity of beneficial gut bacteria
Whole-Biomass Processing	Valorization without separating meat from the shell
Eutrophication	Excess nutrient loading in water bodies leading to algal blooms
Low Salinity Stress	Growth-limiting factor in Baltic Sea blue mussels due to brackish conditions
Mussel Slurry	Crushed mussel biomass mixture used in processing
Thermal Drying	Controlled heating used to reduce moisture content
Biovalorization	Process of converting biomass into higher-value products

Explanations of terms used in the thesis.

2 METHODS

2.1 Raw materials used in tests

All experimental trials in this study were based on *Mytilus edulis/trossulus* hybrids cultivated under low-salinity conditions of the Baltic Sea. Mussels were primarily sourced from two farming sites:

1. Tagalaht Bay, Saaremaa Island, Estonia

Mussel biomass for protein and bioactive compound extraction experiments (see Papers I–III) was sourced from Tagalaht Bay (58.456° N, 22.054° E). Mussels were grown on longline structures suspended at depths of 0–3 m and harvested seasonally from 2020 and 2022. The biomass was immediately cleaned, packed wet, and frozen at −18 °C until analysis. Four harvesting time points were used to capture seasonal variability.

2. Sankt Anna Archipelago, Sweden

Biomass used for whole-mussel micronization experiments (Paper IV) was harvested from a low-salinity mussel farm in the central Baltic region. Mussels were frozen on-site and stored at −18 °C for up to 11 months prior to processing.

In addition to mussels, barnacles growing on the shell surfaces were separately analyzed during flesh separation procedures to assess their impact on final biomass composition (Paper II).

All laboratory reagents and solvents used in the trials were of analytical grade (Sigma-Aldrich, VWR, or Merck), and all analytical instruments were calibrated and maintained according to manufacturers' standards and ISO requirements.

2.2 Laboratory trials

This study included multiple laboratory experiments aimed at optimizing the processing of small-sized Baltic Sea blue mussels and characterizing their biochemical, nutritional, and functional properties. All trials followed a systematic sequence of biomass pretreatment, fractionation, drying, and analysis (Figure 1).

2.2.1 Pretreatment and mechanical processing

Frozen mussels were thawed overnight at 4 °C, rinsed with tap water, and mechanically crushed using a high-speed blender. Three water-to-biomass ratios (1:2, 1:3, 1:4 w/v) were tested to prepare slurries with varying consistency.

Following blending, slurries were poured into graduated cylinders and subjected to sedimentation trials (1, 5, and 15 minutes) to separate lighter organic matter from heavier shell fragments. Decanted upper fractions were retained for further centrifugation and drying (Figure 1). Full details of this pretreatment optimization process, including dry matter yields and protein recoveries, are provided in II.

2.2.2 Centrifugation, separation, and drying

Slurry fractions were subjected to low-speed and high-speed centrifugation. Resulting pellets were either:

- Dried in a convection oven at 70 °C for 10 h (moisture less than 5%)
- Freeze-dried under vacuum at shelf temperatures ranging from −20 °C to +20 °C for sensitive bioactive analyses.

Moisture levels were monitored using a moisture analyzer. This step yielded dry, stable biomass suitable for micronization, extraction, and biochemical analysis.

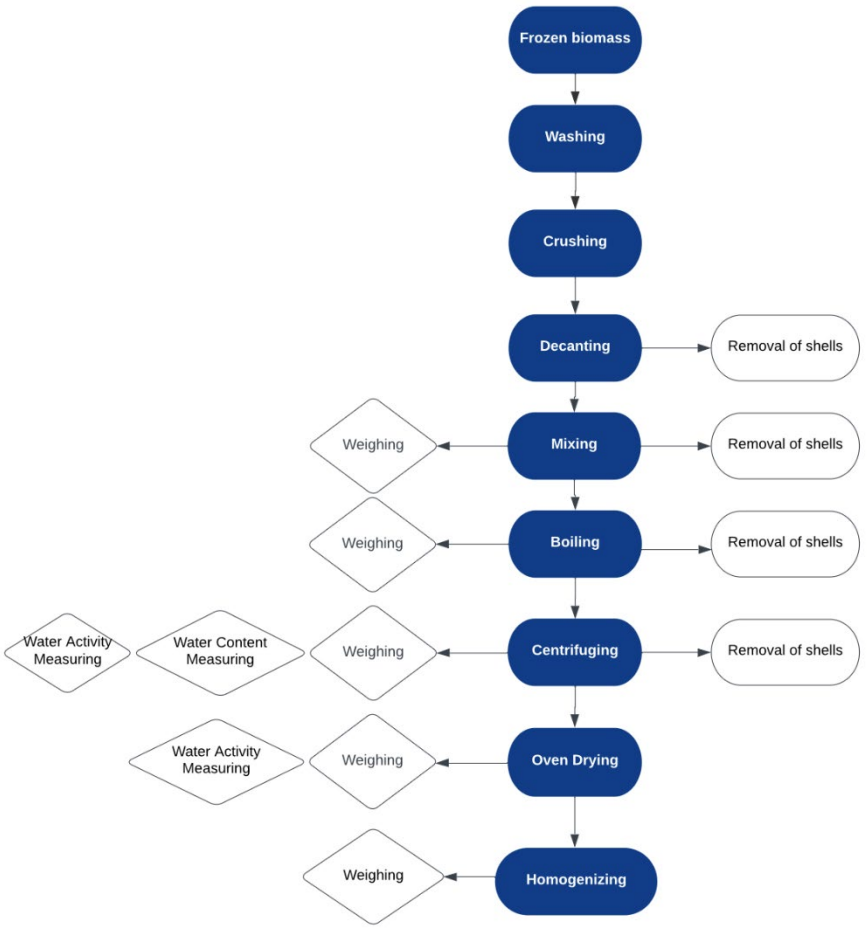

Figure 1. *Schematic diagram of the meat extraction and refinement process [Paper II].*

2.2.3 Reduction of off-flavors

A standard solution of 0.1% citric acid (w/v) in chilled tap water was prepared fresh for each trial. After sedimentation and centrifugation, biomass pellets were immersed in the acid solution for 5 minutes with gentle stirring to ensure full contact. This was followed by two sequential rinses with cold tap water to remove residual acid.

Control samples (no acid treatment) underwent identical rinsing with water only. Both treated and untreated samples were then oven-dried, ground to a powder, and rehydrated in hot water to 4% w/v concentration for sensory evaluation.

A trained sensory panel was assembled under blind testing conditions to evaluate aroma and flavor characteristics of rehydrated mussel powder. Each sample was rated on a 5-point scale for the presence of specific off-notes:

- 0 = not present
- 1 = very weak

- 2 = weak
- 3 = moderate
- 4 = strong

Descriptors included:

- Muddy/earthy
- Metallic
- Bitter
- Fishy

Panelists were also asked to indicate overall acceptability.

2.2.4 Evaluation of prebiotic properties

To evaluate the prebiotic potential of processed Baltic Sea blue mussel biomass, representative dried samples (micronized powder and centrifuged pellet) were subjected to controlled proteolysis using subtilisin (serine protease, 1% w/w, pH 8.0) under mild conditions (60 °C, 2 h). This protocol was chosen based on previous studies on molluscan hydrolysates (Je et al., 2007) and optimized for food-grade applications.

Post-hydrolysis, the samples were centrifuged (10,000 × g, 10 min) to remove insoluble fragments, and the resulting supernatant was freeze-dried. Degree of hydrolysis (DH) was assessed by measuring free amino nitrogen using the OPA method (Nielsen et al., 2006). The average DH reached 27.4 ± 2.2%, indicating effective cleavage into low molecular weight peptides.

These hydrolysates were then used for functional testing with target gut-associated bacteria to explore their prebiotic activity.

To assess whether mussel hydrolysates could stimulate beneficial microbial populations, two well-characterized probiotic strains were selected:

- *Lactobacillus rhamnosus* GG (ATCC 53103)
- *Bifidobacterium animalis* subsp. *lactis* BB-12.

These strains are widely used in clinical and food settings and serve as benchmark organisms for evaluating prebiotic and synbiotic potentials. *In vitro* growth assays were performed by supplementing modified MRS medium with 2% w/v of mussel hydrolysate as the sole nitrogen and carbon source. Bacterial growth was monitored at 600 nm over a 24-hour anaerobic incubation at 37 °C.

2.2.5 Micronization of whole biomass

Figure 2 illustrates the analytical steps and procedures involved in mussel micronization. Whole mussels (harvested from the Sankt Anna Archipelago and Tagalaht Bay), previously thawed, rinsed, dried (70 °C for 10 h), and stored under vacuum, were micronized using a Librixer industrial micronizer (Librixer AB, Sweden). Here, dried mussels were processed without prior shell separation. This equipment employs a dry, high-speed mechanical grinding principle based on air vortex impact, which allows gentle yet effective comminution of composite biological materials.

The following rotational speeds were tested:

- 1500 rpm (low)
- 2500 rpm (moderate)
- 4000 rpm (high).

For each batch, approximately 200 g of dried whole mussel biomass was loaded into the micronization chamber. The process duration was standardized at 2 minutes, with temperatures kept below 40 °C to prevent heat-induced degradation. Final particle size was assessed using mechanical sieving (Retsch AS200) with stainless steel sieve stacks ranging from 500 µm to 20 µm.

Post-micronization powders were visually inspected, sieved, and analyzed for texture, grittiness, and palatability. The finest particle fraction (less than 63 µm) was targeted for food product development and sensory evaluation. Further methodological details, including microbiological safety procedures and sensory testing, are available in Paper IV.

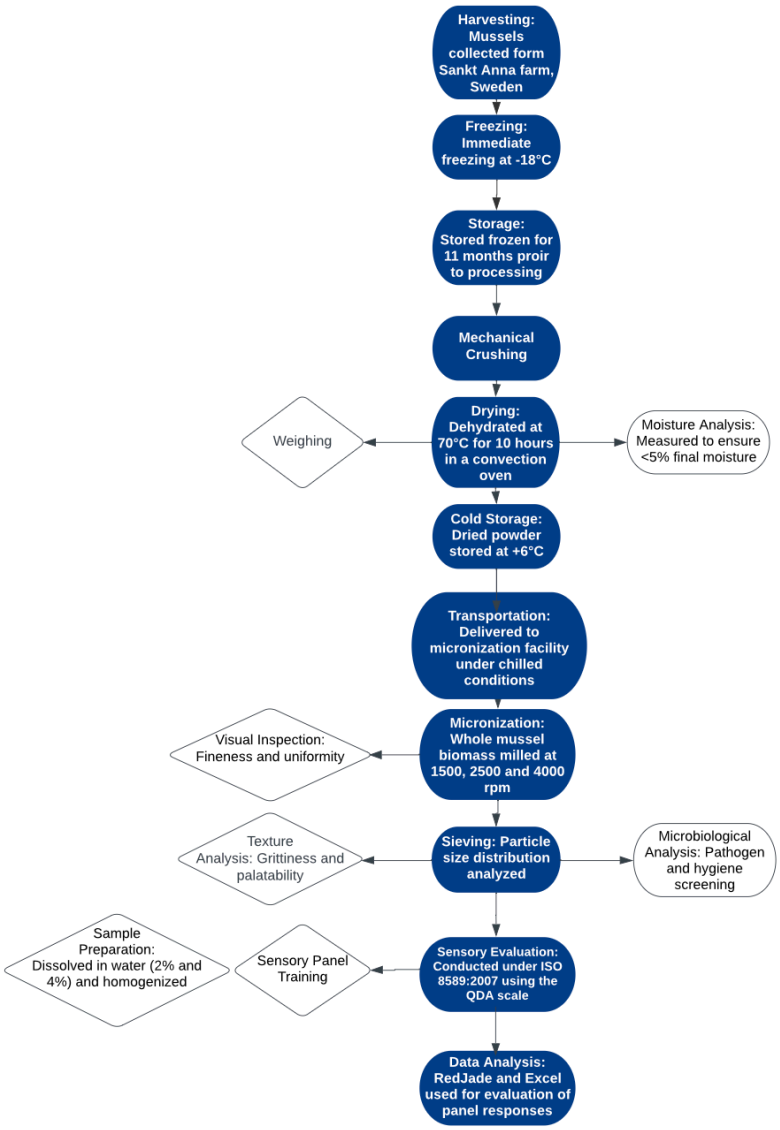

Figure 2. *Schematic diagram of the micronization process [Paper IV].*

2.2.6 Analytical methods

Total protein was measured using the Bradford assay (Sigma-Aldrich B6916) and the Kjeldahl nitrogen method (ISO 937:1978). Amino acid profiles were determined via HPLC after acid hydrolysis (6N HCl, 110 °C, 24 h), as described in III.

Total lipids were extracted using the Bligh and Dyer method. Fatty acids were methylated and analyzed by gas chromatography (Agilent 7890A, FID), focusing on EPA and DHA quantification (III).

Glycogen was quantified enzymatically using commercial assay kits (Sigma MAK016) via glucose detection at 505 nm.

Mussel powders were evaluated for food-grade safety by testing for:

- Aerobic mesophilic bacteria (ISO 4833-1:2013),
- Enterobacteriaceae (ISO 21528-2:2017),
- *Salmonella* spp. (ISO 6579-1:2017),
- *Listeria monocytogenes* (ISO 11290-1:2017).

All analyses were conducted by the Estonian National Centre for Laboratory Research and Risk Assessment (Paper IV).

Selected biomass fractions were enzymatically hydrolyzed using subtilisin (1% w/w) at 60 °C for 2 hours. Their ability to stimulate the growth of *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* BB-12 was tested in modified MRS medium. Optical density was measured at 600 nm after 24 h anaerobic incubation to determine growth stimulation effects. Prebiotic methodology and results are detailed in Paper III.

2.3 Statistical analysis

All laboratory trials were conducted in biological triplicates unless otherwise stated. Results are expressed as means ± standard deviation. To assess the effectiveness of different processing optimizations (e.g., water ratios, sedimentation times, centrifugation conditions), one-way ANOVA was used to evaluate differences between treatment groups.

Post-hoc comparisons were carried out using Tukey's HSD test, with significance set at $p < 0.05$.

Where appropriate, regression analysis was used to assess correlations between processing parameters (e.g., drying time vs. moisture content, protein yield vs. centrifugation speed). Details of statistical treatments for biochemical composition and microbiological data are included in the corresponding publications (Papers I–IV).

3 RESULTS

3.1 Development of processing workflows

The practical limitations of processing small, thin-shelled mussels such as *Mytilus edulis/trossulus* hybrids have posed a major barrier to their utilization in food and feed applications (Kotta et al., 2020). Unlike their Atlantic counterparts, Baltic Sea blue mussels rarely exceed 30 mm in shell length and have a low tissue-to-shell ratio, making conventional meat extraction inefficient and labor-intensive (Hedberg et al., 2018). This section presents the development of a low-input, scalable biomass separation method tailored for Baltic Sea blue mussel farming systems.

In contrast to large-scale mussel farming operations that rely on automatic shucking equipment, the manual or mechanical separation of soft tissue from the shell is not feasible for Baltic Sea blue mussels due to their size and strong shell-tissue adhesion (III). Earlier research efforts (Lindahl, 2012; Petersen et al., 2016) emphasized the potential of Baltic Sea blue mussels for ecosystem services, but few attempts have been made to address the technical barriers to their processing. Hence, this thesis developed an alternative processing workflow based on physical fractionation principles, targeting the recovery of usable biomass without requiring direct shell removal.

To address this, a two-step gravity-based separation system was developed and optimized. Whole mussel biomass was first mechanically disrupted using a high-speed blender to produce a uniform slurry. Blending was performed on thawed mussels. The blending time was standardized, with cooling intervals between batches to prevent thermal denaturation of proteins. Three water-to-biomass dilution ratios were tested. All slurries were homogenized and poured into glass cylinders where sedimentation trials were conducted.

The bottom layer consisted of large shell fragments and heavier mineralized particles. The middle layer comprised suspended tissue-rich slurry, while the top layer was typically a light aqueous phase with minimal visible solids. Subsequently, the top two-thirds of the cylinder volume were gently decanted using wide-bore pipettes, taking care not to disturb the settled bottom fraction. The decanted material was transferred to clean beakers and visually assessed for homogeneity and shell particle contamination.

The experiment showed that the choice of sedimentation time influenced both the efficiency and purity of the recovered fraction:

- 1-minute settling yielded the highest slurry volume but included more shell fines.
- 5-minute settling gave the best trade-off between volume and purity.
- 15-minute settling led to denser fractions, but some organic particles were also lost to sedimentation.

A typical decanted slurry appeared as a pale beige suspension, with minor residual grit but no large shell fragments. Upon standing further, this slurry could be used directly in feed formulations or subjected to centrifugation for concentration. Visual clarity and organoleptic properties of the decanted layer improved significantly at 1:3 dilution with 5-minute settling.

This gravity-driven fractionation method was reproducible across biomass samples from four different harvests and two geographic sources (Estonia, Sweden). Environmental variability (e.g., temperature, salinity) did not significantly affect sedimentation efficiency, although slightly more shell breakage was observed in later-season samples with thinner shells.

Notably, this approach requires only simple, non-specialized equipment and is scalable for use at coastal farming sites or in small processing units. Compared to filter-based separation (which showed clogging and protein losses of more than 40% in preliminary tests), gravity separation offered a cost-effective, gentle, and efficient alternative.

This method demonstrates that simple physical principles, mechanical disruption followed by sedimentation, can be used to separate usable biomass from shell material in small mussels. It also shows that the assumption that small mussels must always be discarded or downcycled is unfounded and opens the door for scalable valorization workflows suitable for low-trophic aquaculture systems.

3.2 Optimization of meat extraction yield

Traditional mussel processing focuses on removing the shell the organism and extracting the edible tissue. However, this approach becomes infeasible with *Mytilus edulis/trossulus* hybrids in the Baltic Sea, where mussels are typically small (shell lengths of 20–30 mm) and exhibit a high shell-to-meat ratio, with flesh often strongly adherent to the inner shell surface. Manual or mechanical separation is labor-intensive, inconsistent, and results in poor yields.

Here, the goal was to maximize the extraction of edible organic material through an alternative processing route, combining sedimentation and centrifugation. Yield was quantified in terms of dry matter recovery, organic purity, and protein concentration, with the intention of producing a concentrated biomass fraction suitable for either further processing or direct valorization.

Following the sedimentation trials described in the previous section, the decanted biomass slurries were subjected to a centrifugation protocol. Three experimental variables were investigated:

- Water-to-biomass ratio,
- Sedimentation time,
- Harvest season (spring vs. autumn) across two farming locations: Tagalaht Bay and Sankt Anna.

The results showed that the recovery of dry matter from whole mussel biomass varied considerably based on sedimentation time and dilution ratio (Table 2).

Table 2. *Dry matter recovery (% ± SD) by sedimentation time and water dilution ratio [Paper II].*

Sedimentation Time	1:2 Ratio	1:3 Ratio	1:4 Ratio
1 min	13.5 ± 0.8	14.9 ± 0.6	13.2 ± 0.7
5 min	15.8 ± 1.0	17.2 ± 1.1	14.6 ± 0.8
15 min	13.0 ± 0.9	14.3 ± 0.5	14.8 ± 0.6

The 1:3 dilution with 5-minute sedimentation provided the highest yield of recoverable dry matter. Lower ratios (1:2) resulted in less efficient separation due to higher viscosity, while 1:4 ratios produced more dilute slurries with lower pellet mass despite cleaner decantate.

Prolonged sedimentation (15 min) caused loss of fine organic particles into the bottom shell-rich layer, reducing usable biomass. Therefore, 5 minutes emerged as the optimal time for balancing separation and retention.

Protein content per gram of dried pellet was also assessed. Mean concentrations ranged from 48.1 ± 2.3% to 52.7 ± 1.9% (dry weight basis), depending on season and site. Autumn-harvested mussels (October–November) consistently yielded higher protein content and total protein per mussel, likely reflecting post-spawning nutritional recovery.

Notably, centrifuged pellets from spring-harvested mussels had more shell fragments and slightly lower protein concentration (~2–3%), suggesting thinner shells during that season contribute to less clean sedimentation.

For benchmarking, 20 randomly selected mussels from each harvest batch were manually shucked after boiling and freezing. The meat yield was weighed and dried to calculate dry matter content. Across all replicates, the average yield from manual separation was 7.4 ± 0.9% dry matter, significantly lower than the best-performing sedimentation + centrifugation treatment (17.2 ± 1.1%).

In addition, the manual process took approximately 4–5 minutes per 20 mussels, whereas the slurry method processed 300 g biomass in under 10 minutes, demonstrating a clear advantage in labor and scalability.

Centrifuge pellets appeared as dense, slightly sticky pastes with uniform consistency and a light brown color. In contrast to manually extracted flesh, the processed slurry showed higher levels of residual calcium particles, but no large shell fragments, under stereomicroscopy. To address these texture-related concerns, micronization and sieving were employed as subsequent processing steps, enabling refinement of the final product's mouthfeel and physical uniformity.

Thus, the processing sequence, from blending through sedimentation to centrifugation, significantly improved biomass yield from Baltic Sea blue mussels compared to manual methods. The optimized parameters (1:3 w/v dilution, 5-minute sedimentation, 4500 × g centrifugation) delivered a reproducible and scalable approach for the efficient extraction of high-protein biomass, overcoming one of the most pressing limitations in low-trophic aquaculture in the Baltic Sea.

3.3 Reduction of off-flavors using citric acid

One of the practical challenges in developing food or feed products from Baltic Sea blue mussel biomass is the presence of off-flavors, particularly in biomass harvested during autumn months when organic debris and detrital matter tend to accumulate within the shell cavity. These off-notes, often described as "muddy," "metallic," or "earthy", are attributed to the presence of absorbed sediments, microbial metabolites, and shell surface fouling organisms such as barnacles and filamentous algae. While not harmful, these flavors can significantly reduce consumer acceptability and limit the applications of mussel-based ingredients.

Previous work on other low-trophic seafood products (e.g., oysters, macroalgae) has demonstrated that mild acid rinses can reduce undesirable taste profiles by solubilizing surface-bound compounds and neutralizing basic odorants (Forrester et al., 2002). Inspired by this, a short citric acid rinse protocol was tested to evaluate its efficacy in improving the sensory profile of fractionated mussel biomass.

The citric acid treatment significantly reduced muddy and metallic notes ($p < 0.05$, paired t-test) while having limited effect on natural fishy aroma (Table 3). No acidic or sour taste was reported by the panel, indicating complete removal of citric acid and good taste neutrality.

Table 3. *Mean intensity scores (± SD) of off-flavors before and after citric acid treatment (n = 6) [Paper II].*

Descriptor	Untreated	Citric Acid Treated	% Reduction
Muddy/Earthy	3.2 ± 0.4	1.5 ± 0.3	53.1%
Metallic	2.8 ± 0.5	1.2 ± 0.3	57.1%
Bitter	2.0 ± 0.6	1.0 ± 0.2	50.0%
Fishy	2.3 ± 0.5	2.0 ± 0.4	13.0%
Overall Acceptability	2.0 ± 0.4	3.5 ± 0.3	↑ 75.0%

Citric acid likely acts by chelating metal ions (e.g., Fe^{2+}, Mn^{2+}) associated with shell or sediment particles, which contribute to metallic taste. It may also lower surface pH and neutralize basic volatile compounds (e.g., trimethylamine), thereby improving olfactory perception. Similar acid-rinse strategies are employed in algae and seafood processing to remove iodine and mineral residues (Klinmalai et al., 2021).

Given its status as a Generally Recognized As Safe (GRAS) compound and widespread use in food processing, citric acid offers a cost-effective, scalable pre-treatment option for mussel-based ingredients, especially in whole-biomass formats where shell surface contaminants may influence flavor.

The treatment requires only a basic immersion and rinsing step, with no need for heated water or mechanical agitation. It adds approximately 15 minutes to the overall processing workflow but substantially enhances sensory quality and product versatility. For industrial applications, treatment could be integrated into post-centrifugation rinsing systems without major equipment modification. Thus, citric acid pre-treatment offers a practical, food-safe method for reducing off-flavors in Baltic Sea blue mussel biomass, particularly in autumn-harvested samples. Its integration into the processing sequence enhances the palatability of dried or micronized mussel products (see the section below) and increases their suitability for use in functional food applications.

3.4 Impact of seasonal variability on biomass quality and processing

Seasonal variation plays an important role in shaping the biochemical composition and processing behavior of *Mytilus edulis/trossulus* mussels cultivated in the Baltic Sea (Riisgård et al., 2015). Mussels experience predictable physiological changes across the annual cycle, particularly in relation to reproductive status, feeding intensity, and environmental conditions. These changes directly influence both biomass yield and the quality of material recovered during processing.

To evaluate these effects, this study compared mussels harvested in spring (April–May) and autumn (October–November) using the same optimized sedimentation–centrifugation workflow. Notable differences were observed in both the physical characteristics and biochemical parameters of the biomass. Mussels harvested in spring exhibited smaller mean shell length (21.4 ± 2.6 mm) and lower tissue mass, consistent with post-spawning depletion. Autumn mussels were significantly larger (25.7 ± 3.1 mm), and exhibited higher soft tissue fullness and more robust shell structures.

These physical differences translated into measurable differences in processing performance. Spring samples showed reduced dry matter recovery (14.8 ± 0.7% of wet biomass), compared to 17.2 ± 1.1% for autumn samples and a correspondingly lower protein concentration in the recovered pellet (46.7 ± 2.1% DW vs. 52.3 ± 1.9% DW). The increased yield and protein density in autumn reflect a seasonal accumulation of energy reserves and improved physiological condition as mussels prepare for winter.

Shell fragility was notably higher in spring. Microscopic analysis of spring-derived slurries revealed greater contamination with fine calcium carbonate particles, a consequence of shell brittleness during the reproductive recovery phase. These fragments were more difficult to remove via sedimentation and occasionally migrated into the organic fraction, reducing visual and textural quality. By contrast, autumn-harvested mussels yielded cleaner organic fractions with minimal shell debris, contributing to improved product quality and reduced processing loss.

These seasonal patterns have direct implications for biomass valorization strategies. Autumn-harvested mussels are more suitable for high-value applications such as food and nutraceutical powders, due to their superior composition and cleaner processing behavior. Spring mussels, while still usable, may require additional clarification steps or may be more appropriately directed toward lower-value product streams such as animal feed, fertilizer, or calcium-rich supplements. Table 4 summarizes the observed differences in shell size, dry matter recovery, protein content, and shell integrity between the two harvest periods:

Table 4. *Seasonal differences in biomass recovery and biochemical profile of Baltic Sea blue mussels (mean ± SD) [Paper III].*

Parameter	Spring	Autumn
Protein (% DW)	46.7 ± 2.1	52.3 ± 1.9
Lipid (% DW)	6.8 ± 0.9	8.1 ± 1.0
EPA (% total FA)	15.4 ± 1.3	18.7 ± 1.2
DHA (% total FA)	3.1 ± 0.7	4.6 ± 0.9
Glycogen (% DW)	2.1 ± 0.5	3.4 ± 0.6

The findings support the recommendation that mussel farming and harvesting operations should be aligned with periods of optimal biomass condition. In the Baltic context, this means planning for late-summer or autumn harvests when biomass quality is highest and adjusting preprocessing parameters for spring harvests when shell fragility and protein depletion are more pronounced. Establishing seasonal intake specifications for processing facilities would further support resource efficiency and product standardization.

3.5 Micronization: Rethinking mussel processing

The conventional model of mussel processing relies heavily on the physical separation of meat from the shell, a practice rooted in large-scale farming of Atlantic *Mytilus edulis*, where mussels reach shell lengths of 50–70 mm. These larger mussels can be efficiently shucked either manually or via industrial shelling machines, making meat extraction both viable and economically justified.

In contrast, Baltic Sea *Mytilus edulis/trossulus* hybrids are very small and unsuitable for traditional shelling workflows. As shown in the previous sections, alternative fractionation methods offer a more efficient route to recover organic matter from small mussels. However, even these methods depend on partial shell removal or sedimentation-based discard of the inorganic fraction.

Micronization offers a fundamentally different approach. Instead of attempting to separate shell and flesh, it aims to utilize the whole mussel biomass, converting the entire organism, including its calcium-rich shell, into a fine, uniform powder. This zero-waste valorization strategy aligns with circular bioeconomy principles, reduces processing time and energy requirements, and opens up new possibilities for developing sustainable, nutrient-dense food and feed ingredients.

The degree of micronization was highly dependent on the rotational speed. Table 5 presents the distribution of particles by size class after sieving, showing a clear shift toward finer fractions with increasing speed. At 4000 rpm, over 40% of the biomass was reduced to a particle size below 63 µm which was the target threshold for smooth texture and fine suspension. The 1500 rpm setting resulted in a coarser, chalky product with visible shell fragments, while 2500 rpm offered an acceptable intermediate grade but retained some grittiness.

Table 5. *Particle size distribution of micronized mussel biomass (%, w/w) [Paper IV].*

Size Range (µm)	1500 rpm	2500 rpm	4000 rpm
>250	31.2%	18.7%	6.4%
125–250	39.5%	32.1%	17.9%
63–125	22.0%	31.6%	34.1%
<63	7.3%	17.6%	41.6%

Micronized powders produced at high speed exhibited a pale beige color and uniform consistency. They were free-flowing and did not exhibit clumping under standard storage conditions. When suspended in hot water (4% w/v), the 4000 rpm powder formed a stable dispersion with minimal sedimentation after 30 minutes, unlike coarser fractions, which rapidly settled.

Sensory evaluation by a trained panel (n = 6) showed a marked improvement in mouthfeel at higher micronization levels. The less than 63 µm powder was described as “smooth, creamy, with slight mineral tones” compared to “chalky” and “gritty” descriptors applied to 125–250 µm powders. No bitterness or shell-derived harshness was noted in the fine powder.

Considering nutritional value, whole-biomass micronization preserves the complete profile of mussels, including:

- High-quality protein and amino acids,
- Essential long-chain omega-3 fatty acids (EPA, DHA),
- Glycogen and micronutrients,
- Natural minerals from the shell, especially calcium and trace elements.

Calcium content increased proportionally with shell inclusion, reaching up to 18.4% (on a dry weight basis) in the powder with particles less than 63 µm, suggesting potential applications as a functional food additive for calcium enrichment.

The application of whole-organism micronization in mollusk processing remains rare. Studies by Hermund et al. (2019) explored similar methods in crustaceans, while seaweed-based powders have used analogous air-jet milling approaches. This study represents one of the first systematic attempts to micronize farmed Baltic Sea blue mussels for human consumption, with a focus on mouthfeel, nutritional completeness, and operational simplicity.

From a sustainability standpoint, this technique removes the need for shell disposal, typically an environmental burden, and converts it into nutritional or textural value. Furthermore, it reduces water use (no depuration, no waste streams) and energy consumption compared to meat–shell separation and traditional boiling.

The micronization system proved capable of processing up to 1000 kg/h of dried biomass at 4000 rpm under laboratory conditions. Energy consumption was recorded at approximately 0.4 kWh per kg biomass, significantly lower than wet-thermal shelling combined with drying. In industrial contexts, similar micronization systems could be powered by solar or biogas energy, enhancing circularity (Brandt et al., 2018).

However, equipment costs and maintenance (abrasion of internal surfaces by shell particles) remain considerations for commercial adoption. Pilot-scale trials are required to evaluate continuous feed systems, temperature stability under load, and product homogeneity.

Nevertheless, micronization offers a transformative alternative to traditional mussel processing, particularly well-suited to the Baltic context. By fully utilizing the whole organism, including the shell, it redefines the concept of waste in bivalve farming. The resulting powder has favorable functional, nutritional, and sensory properties, and the process is both scalable and resource-efficient. As interest grows in whole-biomass ingredients and low-impact aquaculture, micronization may provide the technological foundation for the next generation of mussel-based foods.

3.6 Chemical composition of mussels

Baltic Sea *Mytilus edulis/trossulus* mussels represent a highly underexplored nutritional resource. While environmental constraints, particularly low salinity and eutrophication, affect their size and shell morphology, their tissue biochemistry remains rich in essential nutrients, including amino acids, omega-3 fatty acids, and glycogen. This section summarizes the results of biochemical profiling of mussel biomass obtained through the optimized processing workflows described in the previous sections.

Biomass fractions analyzed included:

- Centrifuged pellets from sedimentation-processed slurries,
- Micronized whole mussel powders,
- Hydrolysates generated by enzymatic treatment.

Protein content, as previously established, ranged from 46% to 53% of dry matter, depending on season and processing method. To further assess nutritional value, amino acid profiles were determined for representative dried biomass samples.

Total amino acid content reached 42.5 ± 1.6 g/100 g DW, of which approximately 40–45% were essential amino acids (EAAs). Table 6 summarizes average concentrations of major amino acids from autumn-harvested mussels (dry weight basis).

The EAA profile closely matched FAO/WHO dietary requirements for high-quality animal protein. Particularly high levels of glutamic acid and aspartic acid contribute to umami flavor, while leucine and lysine are of interest for muscle synthesis and infant nutrition. No major amino acid degradation was observed during drying or micronization, indicating that the processing method preserves protein quality effectively.

Table 6. *Amino acid composition of processed Baltic Sea blue mussel biomass (g/100 g DW, mean ± SD) [Paper III].*

Amino Acid	**Content (g/100 g DW)**
Glutamic acid	6.8 ± 0.4
Aspartic acid	4.9 ± 0.3
Leucine (EAA)	3.8 ± 0.2
Lysine (EAA)	3.5 ± 0.3
Arginine	3.3 ± 0.2
Valine (EAA)	2.9 ± 0.2
Methionine (EAA)	1.5 ± 0.1
Histidine (EAA)	1.2 ± 0.1
Tryptophan (EAA)	0.9 ± 0.1
Others (sum)	13.0 ± 0.6

Lipid content of processed mussel biomass ranged from 6.8% to 8.1% of dry weight, depending on the harvest season and sample fraction. Total fatty acid profiling revealed a consistent presence of marine long-chain polyunsaturated fatty acids (LC-PUFAs), particularly eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). The proportion of EPA averaged 18.7 ± 1.2% of total fatty acids, while DHA was present at 4.6 ± 0.9%. Saturated fatty acids (SFA), such as palmitic acid, accounted for approximately 28%, monounsaturated fatty acids (MUFAs), including oleic acid, contributed around 25%, and omega-6 fatty acids, such as linoleic acid, comprised roughly 8–10% of the total fatty acid profile.

These figures are comparable to, or exceed, those of some commonly consumed oily fish, especially for EPA (Miller et al., 2014). This finding positions Baltic Sea blue mussels as a valuable local source of marine omega-3s, with relevance for cardiovascular and anti-inflammatory health claims in both human food and pet nutrition sectors.

No significant lipid oxidation was detected in the final dried or micronized products, as verified by peroxide value and anisidine index assays (data not shown), suggesting good stability under controlled storage.

Glycogen content, important as an energy source and functional polysaccharide, was measured using enzymatic assays. Mean concentrations ranged from 2.6% to 3.4% of DW, with higher levels in autumn samples. This is consistent with the seasonal feeding and metabolic cycles of mussels, with glycogen accumulating after summer phytoplankton blooms and depleting during reproduction.

While lower than in high-energy mollusks such as scallops (Liu et al., 2024), this value still contributes to the mild sweetness and nutritional energy density of mussel biomass. Moreover, glycogen has potential prebiotic properties (see the section below) and may play a role in improving gut microbial balance when consumed regularly.

The biochemical composition of mussels was strongly influenced by season, as shown in Table 7. Autumn samples consistently outperformed spring-harvested mussels in all nutritional categories. This reflects the post-spawning recovery and higher phytoplankton availability in late summer, supporting energy storage and tissue protein synthesis (Guillou et al., 2020). This seasonal effect must be considered when planning harvests for high-value applications, such as nutritional supplements, functional foods, or performance feeds. Spring harvests may still be suitable for bioremediation or low-grade feed inputs but offer reduced biochemical value.

Table 7. *Seasonal variability in mussel biochemical composition (mean ± SD, n = 3 per season) [Paper III].*

Parameter	Spring	Autumn
Protein (% DW)	46.7 ± 2.1	52.3 ± 1.9
Lipid (% DW)	6.8 ± 0.9	8.1 ± 1.0
EPA (% total FA)	15.4 ± 1.3	18.7 ± 1.2
DHA (% total FA)	3.1 ± 0.7	4.6 ± 0.9
Glycogen (% DW)	2.1 ± 0.5	3.4 ± 0.6

3.7 Prebiotic potential and bioactive compounds

While whole mussel biomass is rich in macronutrients and minerals, the biological availability of bioactive peptides and functional oligosaccharides is often limited by the structural matrix of proteins and glycogen storage forms. Enzymatic hydrolysis is a widely used method to enhance digestibility, release low-molecular-weight peptides, and potentially activate biofunctional properties such as antioxidative, immunomodulatory, or microbiome-modulating effects.

Enzymatically treated blue mussel fractions, particularly those hydrolyzed with subtilisin (ENZ-S and ENZ-S-UF-P), had strong prebiotic activity, especially in promoting the growth of beneficial gut bacteria such as *Bifidobacterium animalis* subsp. *lactis*. The enzymatic hydrolysis significantly increased the availability of free amino acids and bioactive peptides, which are known to selectively stimulate probiotic bacteria.

The ENZ-S fraction contained 42.5% total amino acids, indicating high solubility and protein hydrolysis efficiency. This translated into enhanced bioavailability, which is critical for nutritional and prebiotic applications. ENZ-S and ENZ-S-UF-P increased *B. animalis* survival by 37–40%, clearly indicating their capacity to support probiotic growth. Simultaneously, they reduced the growth of *Cutibacterium acnes* (a potentially pathogenic strain) by 12–18%, suggesting selective bioactivity of the peptides. In contrast, the larger molecular weight retentate (ENZ-S-UF-R) showed no prebiotic activity, highlighting that low molecular weight peptides (less than 1 kDa) are most effective in stimulating beneficial microbes. In addition, glycogen extracted from mussel biomass showed promise due to its structural integrity and fermentability by gut microbiota, aiding in short-chain fatty acid (SCFA) production, which is a hallmark of

prebiotic action. The study also confirmed that molecular size is a determining factor for fermentation efficiency by probiotic species. Preparations with molecular weights below 0.7 kDa exhibited the strongest prebiotic effects.

To assess whether mussel hydrolysates could stimulate beneficial microbial populations, two well-characterized probiotic strains were selected: *Lactobacillus rhamnosus* GG (ATCC 53103) and *Bifidobacterium animalis* subsp. *lactis* BB-12. Both strains exhibited significantly enhanced growth in the presence of mussel hydrolysates when compared to control medium lacking a nitrogen source. After 24 hours of incubation, *L. rhamnosus* GG achieved an optical density at 600 nm (OD_{600}) of 1.27 ± 0.05, while *B. lactis* BB-12 reached an OD_{600} of 1.09 ± 0.04, indicating robust bacterial proliferation. This growth corresponded to approximately 80–85% of the levels observed in standard glucose-supplemented MRS medium, suggesting a strong substrate utilization potential of the hydrolysates. No inhibitory effects or cytotoxic responses were observed during the experiments, further supporting the suitability of mussel-derived hydrolysates for prebiotic applications.

These results suggest that mussel-derived peptides and residual glycogen can serve as fermentable substrates or growth cofactors for probiotic bacteria, contributing to gut microbial balance. The mechanism is likely multifactorial, involving direct utilization of oligosaccharides and free amino acids, release of growth-enhancing peptides with quorum sensing or metabolic regulation functions, and possible mineral contribution (e.g., Zn^{2+}, Mg^{2+}) supporting bacterial enzyme function.

In this context, Baltic Sea blue mussel hydrolysates represent a regionally sourced, multifunctional ingredient, combining protein quality, mineral enrichment, and mild prebiotic function in a single matrix. Compared to plant-based prebiotics (e.g., inulin, GOS), mussel hydrolysates offer a protein-rich, allergen-light, and sustainably harvested alternative, particularly relevant for pet foods and functional snacks aimed at active or aging populations.

Thus, enzymatic hydrolysis of mussel biomass enhances bioavailability and peptide release. Mussel hydrolysates stimulate the growth of common probiotic strains in vitro. The material offers potential as a marine-derived functional food ingredient, with mild prebiotic activity and a good safety profile. Consequently, Baltic Sea blue mussels serve a dual role in ecosystem restoration and bioactive ingredient development, bridging marine sustainability and functional nutrition.

3.8 Summary of processing outcomes

The results presented in this chapter demonstrate the feasibility and performance of whole-biomass processing methods for Baltic Sea blue mussels, with particular emphasis on mechanical efficiency, recovery optimization, and final product quality. Sedimentation coupled with centrifugation enabled consistent recovery of high-protein fractions while reducing manual labor and operator-dependent variability. Compared to manual shucking, which processed approximately 250 g of biomass per hour, the mechanical slurry method achieved a 5–6-fold increase in throughput with minimal labor input, processing 300 g in under 10 minutes (Lee et al., 2008).

Visual and microscopic inspection of the recovered centrifuged pellets confirmed effective separation, with only minimal shell residues remaining, particularly when optimal dilution ratios and sedimentation times were applied. Though fine calcium particles were observed in some fractions, especially in spring-harvested material,

the final pellets were uniform in consistency, neutral in odor, and suitable for further processing.

Micronization emerged as a particularly significant innovation. The high-speed milling process produced free-flowing powders with more than 40% of particles below 63 µm, offering favorable rehydration and suspension properties. The resulting powders were rated positively in sensory testing for texture and flavor attributes, and biochemical analyses confirmed retention of valuable nutrients, including protein, omega-3 fatty acids, and calcium. Importantly, the whole-biomass approach also eliminated the need for shell separation, transforming what is typically considered processing waste into a functional component of the final product.

Thus, these outcomes reinforce the technical viability of integrated, waste-minimizing processing workflows for small-sized mussels in low-salinity regions. The combined use of mechanical disruption, sedimentation, centrifugation, and micronization provides a scalable framework for the development of mussel-based functional ingredients aligned with circular bioeconomy goals.

4 DISCUSSION

The overarching research aim was addressed by answering five interrelated research questions through a series of experimental investigations, each linked to specific hypotheses. The work contributes to both theoretical and methodological advancements supporting the development of a scalable and sustainable valorization model for small-sized Mytilus edulis/trossulus mussels in the Baltic Sea.

RQ1 – What are the technical and nutritional limitations of current methods for processing Baltic Sea blue mussels, and how can novel workflows improve biomass recovery and product quality?

This question was addressed by the development of a processing workflow integrating mechanical disruption, sedimentation, and low-speed centrifugation. The optimized protocol (1:3 dilution, 5-minute sedimentation, 4500 × *g* centrifugation) yielded up to 17.2 ± 1.1% dry matter recovery, with protein concentrations exceeding 50% of DW, compared to 7.4 ± 0.9% from manual shucking. These findings confirmed **H1**, demonstrating that slurry-based processing significantly improves recovery and throughput, and overcomes the limitations of size and shell fragility in Baltic Sea blue mussels.

RQ2 – Can a scalable processing approach effectively separate high-value biomass from shell waste in small mussels?

The developed method proved reproducible and effective across seasonal and geographic samples. It enabled the isolation of soft tissue fractions with minimal shell contamination, without requiring depuration or shell–meat separation. This supports both **H1** and the practical scalability of a low-input, site-adapted processing solution suitable for decentralized deployment.

RQ3 – To what extent does whole-organism micronization facilitate zero-waste valorization, and what are the functional and sensory characteristics of the resulting powders?

Micronization of dried mussel biomass at 4000 rpm produced powders in which more than 40% of particles were smaller than 63 µm, resulting in shelf-stable, free-flowing products. Sensory evaluation confirmed acceptable texture and mild flavor, with no significant off-notes. These results support **H2**, demonstrating that whole-biomass micronization is a feasible strategy for creating functional powders suitable for use in food and feed formulations. It also showed that including shells can boost calcium content and support zero-waste processing.

RQ4 – Do enzymatically hydrolyzed mussel fractions stimulate the growth of probiotic bacteria, and how does their bioactivity compare across seasonal harvests?

Hydrolysates generated via subtilisin treatment significantly stimulated growth of *Lactobacillus rhamnosus* and *Bifidobacterium animalis in vitro*, reaching up to 85% of the optical density observed in positive control (glucose-supplemented MRS). Low molecular weight peptides (less than 1 kDa) were particularly effective. These findings confirm **H3**, showing that Baltic Sea blue mussel biomass can serve as a marine-derived prebiotic source with selective microbial growth-promoting properties.

RQ5 – How do environmental and seasonal conditions affect the composition and applicability of Baltic Sea blue mussel biomass for functional food and feed formulations?

Seasonal differences were pronounced. Autumn-harvested mussels showed superior compositional metrics: **protein 52.3 ± 1.9% DW**, **EPA 18.7 ± 1.2%**, and **glycogen 3.4 ± 0.6%**, compared to lower values in spring samples. These differences translated into higher yields, improved pellet quality, and greater prebiotic activity. This validates **H4**, confirming that harvest timing is critical to achieving optimal biochemical and functional properties.

4.1 Significance of optimized processing workflows for Baltic conditions

This chapter presents biomass processing methods tailored to the physiological and morphological traits of Baltic Sea *Mytilus edulis*/*trossulus* mussels. Traditional bivalve processing technologies, designed for larger, thicker-shelled Atlantic mussels, fail to adapt to the realities of low-salinity aquaculture. These challenges include small individual size, low meat yield, fragile shell structure, and high levels of associated debris or fouling organisms. Industrial-scale shelling machinery, such as those employed in Western European mussel processors, are not economically viable or technically effective on mussels smaller than 30 mm in length (Uzcátegui et al., 2021), which comprise the vast majority of Baltic Sea farmed mussels.

Even where flesh can be separated mechanically, the extremely high shell-to-meat ratio (often greater than 85%) generates large volumes of calcium-rich waste, creating logistical, environmental, and economic burdens. These include increased transport cost for low-density shell waste, limited land-based uses for crushed mussel shell, regulatory restrictions on marine dumping and coastal shell deposition. These limitations have historically undermined the development of value chains for Baltic Sea blue mussels. As such, continued adherence to meat–shell paradigms risks perpetuating wasteful and inefficient practices and failing to unlock the full ecological and economic potential of this underutilized biomass.

Such constraints are not unique to the Baltic Sea blue mussels; other low-trophic marine resources like amphipods and small crustaceans exhibit similar biomass limitations, requiring delicate handling and often yielding only 15–30% usable material (Biandolino & Prato, 2006; Odabaşı et al., 2016). However, while crustaceans generally require artificial feed input and enrichment to reach suitable nutritional profiles, mussels can achieve high-value compositional metrics without external input, supporting their role in passive bioremediation and low-input aquaculture.

In the current thesis, the gravity-based slurry separation method, followed by low-speed centrifugation, demonstrated a practical solution to some of these constraints. With dry matter yields consistently exceeding 17% of raw biomass and protein concentrations over 50% of DW, this method far outperforms manual shelling, which remains below 8% yield and is incompatible with industrial throughput [I]. These figures validate the proposed workflow as a scalable alternative for mussel processing in marginal environments where biomass quality and quantity do not support traditional methods.

This is in contrast to other unconventional protein sources like jellyfish or starfish, where high water content (often over 90%) and complex enzymatic degradation steps pose additional processing burdens (Khong et al., 2016). Mussels offer a more straightforward pathway to protein-rich dry matter, as shown by the more than 50% protein concentrations achievable without chemical defatting or concentration steps.

Importantly, the use of non-specialized equipment, standard blenders, sedimentation vessels, centrifuges, make the method accessible to small-scale producers, supporting the decentralization of processing capacity in remote or developing aquaculture zones. In a broader blue economy context, this decentralization aligns with the principles of local circularity, minimized transportation, and co-location of value-addition infrastructure at or near farming sites (Velenturf & Purnell, 2021).

Moreover, the consistent performance across harvests from two Baltic regions (Estonia and Sweden) suggests broad regional applicability, offering practical tools for policy-linked nutrient bioextraction initiatives, such as those supported by the EU Baltic Sea Action Plan or national agri-environmental schemes.

Compared to other low trophic marine biomasses like amphipods, jellyfish, or sea cucumbers, which often require specialized collection methods or habitat management, Baltic Sea blue mussels provide a stable, sessile biomass that can be accessed with minimal ecological disturbance. For instance, amphipods like *Gammarus aequicauda* have been explored for aquafeed due to their high lipid content, yet their seasonal variability and benthic mobility complicate large-scale harvests (Biandolino & Prato, 2006). Jellyfish biomass, while abundant in certain coastal areas, has high water content (greater than 95%) and requires significant dehydration and defatting to yield useful protein fractions (Khong et al., 2016). By contrast, mussels offer a naturally nutrient-dense composition with fewer post-harvest processing constraints.

Whole-biomass micronization introduces a disruptive alternative. Rather than viewing the shell as an unavoidable contaminant or costly waste stream, this method reframes it as a functional component of a new product form. Shell-derived minerals, particularly calcium carbonate, are not only harmless but nutritionally beneficial and may act as textural agents, anti-caking agents, or natural supplements in food and feed formulations (Fritz et al., 2023).

This mineral content significantly exceeds that of many other low-trophic marine species. For instance, amphipod calcium levels generally remain below 5% DW (Biandolino & Prato, 2006), while Baltic Sea blue mussels contain 12–18% calcium, depending on harvest time and shell thickness, positioning them as a superior mineral carrier in animal and aquafeed formulations.

From a processing standpoint, the transformation of dried mussels into a fine, homogeneous powder eliminates the need for shell separation altogether. At 4000 rpm, more than 40% of biomass was reduced to particles less than 63 µm, with a texture and palatability comparable to fine cereal flours or protein powders (Dziki et al., 2024). This opens doors for incorporation into nutrient-dense snack products, protein blends or fortified baked goods, pet and aquafeed formulations where mineral supplementation is beneficial.

Comparable efforts to utilize whole macroalgae or crustaceans in food systems often encounter barriers related to strong marine odor, heavy metals, or texture incompatibility (Bonfanti et al., 2018; Suhaimi et al., 2024). Mussel powders, when appropriately micronized, bypass many of these constraints by combining mild flavor profiles with fine particle size and favorable mouthfeel.

Further, the microbiological safety and low water activity (less than 0.3) of the micronized powder indicate excellent shelf-stability and potential for ambient storage, enhancing market flexibility (U.S. Food and Drug Administration, n.d.).

The concept of zero-waste mussel processing, grounded in circular economy principles, is particularly relevant in the Baltic Sea context. Here, mussel farming serves dual roles: nutrient bioextraction and biomass production. Whole-biomass valorization ensures that environmental services are rewarded by a marketable end-product, closing both economic and ecological loops.

One of the most frequently cited barriers to small-scale aquaculture is the lack of cost-effective downstream infrastructure. The workflow presented in this study has distinct advantages in this regard:

- Low capital requirement: No need for depuration tanks, boilers, or shelling machines.
- High throughput relative to labor: One person can process more than 2 kg per hr with basic equipment.
- Minimal water and energy use: No boiling, minimal washing, air-drying possible with ambient conditions.
- Compact footprint: Equipment fits in less than 10 m² workspace, suitable for decentralized units.

Moreover, the micronization approach is modular and scalable. Full-scale systems like the one used in this study can process 1000 to 2000 kg/hour of dried biomass.

Challenges remain, particularly in terms of powder standardization, shell particle edge sharpness in coarser grades, and market familiarity with whole-biomass shellfish powders. However, the benefits, high recovery, circularity, shelf-stability, and nutritional completeness position this method as a viable processing backbone for sustainable Baltic Sea mussel farming.

To sum up, the valorization of small, low-salinity Baltic Sea blue mussels is not only possible, but potentially transformative when framed around the right technologies and value propositions. By breaking away from conventional shellfish processing assumptions and focusing on low-input, high-output workflows, this study lays the groundwork for turning ecosystem-service biomass into economically viable products. The combination of mechanical slurry processing, optimized protein recovery, flavor improvement, and whole-organism micronization provides a practical, integrated model for 21st-century low-trophic aquaculture.

4.2 Health benefits of Baltic Sea blue mussels for food and feed applications

The Baltic Sea *Mytilus edulis/trossulus* mussels offer remarkable nutritional and functional properties, despite their small size and environmental constraints. High-quality protein (46–53% DW), complete amino acid profiles, and substantial levels of omega-3 fatty acids, particularly EPA, make them a valuable source of macronutrients (Gebauer et al., 2006). Moreover, their relatively low lipid content and high digestibility align with current recommendations for balanced, cardioprotective diets.

Comparative studies have shown that while amphipods (*Gammarus komareki*) contain approximately 15–25% protein and minimal lipid reserves (Odabaşı et al., 2016), and jellyfish exhibit high moisture and limited collagen-associated protein content (Khong

et al., 2016), Baltic Sea blue mussels consistently exceed 45% protein with balanced lipid fractions enriched in EPA. This positions mussels as more nutrient-dense and functionally versatile among low-trophic marine bioresources.

From a human health perspective, Baltic Sea blue mussels provide all essential amino acids in proportions aligned with FAO/WHO guidelines, Omega-3 LC-PUFAs, especially EPA, which are associated with anti-inflammatory, neuroprotective, and cardiovascular benefits (Monteiro et al., 2024), and functional polysaccharides (e.g., glycogen) with mild prebiotic effects.

In a feed context, mussel biomass, especially in dried or micronized form, meets the nutrient demands of high-performance aquaculture species, domestic pets, and even livestock. The high protein-to-fat ratio, mineral richness (notably calcium, magnesium, and selenium), and absence of anti-nutritional factors (common in plant meals) give it an edge over soybean and fishmeal alternatives (Bjerknes et al., 2024).

Amphipods and mysids have also gained attention as live feed alternatives due to their polyunsaturated fatty acid content and fast digestibility. However, their mass culture requires enriched diets and controlled environments (Suhaimi et al., 2024). In contrast, mussels accumulate EPA and DHA directly from phytoplankton without the need for external feed input, enabling a naturally enriched profile with up to 23% of fatty acids as EPA+DHA (Gebauer et al., 2006; Paper III).

Furthermore, while thraustochytrids can achieve lipid contents of 50–70% of DW and high DHA concentrations, their cultivation is energy-intensive, often requiring fed fermentation substrates (Jaseera & Kaladharan, 2019). The ecological neutrality of mussels makes them a more passive contributor to low-carbon feed solutions.

Furthermore, the prebiotic potential demonstrated *in vitro* adds a functional layer to this nutritional profile. Gut microbiota modulation, long viewed as a key determinant of animal health, immunity, and nutrient absorption, could become an integral argument for incorporating mussel hydrolysates into advanced feed formulations.

Thus, Baltic Sea blue mussels can serve dual markets: as a novel food in protein-enriched products (e.g., soups, snacks, fortified pasta), and as a biofunctional feed ingredient for animal and aquaculture systems.

Beyond their macronutrient profile, mussel hydrolysates offer a complex mixture of low molecular weight peptides and glycogen, both of which contribute to their moderate prebiotic activity. This mirrors recent findings from broader screenings of marine bioactives, where mollusk-derived hydrolysates showed promising anti-inflammatory and immunomodulatory effects (Shahidi & Saeid, 2025). In contrast, amphipods and mysids may require enrichment or microbial fermentation to reach comparable levels of bioactivity (Suhaimi et al., 2024), further validating mussels as an intrinsically biofunctional ingredient. Emerging studies have also highlighted the techno-functional properties of mussel-derived hydrolysates and peptides, including emulsifying capacity and gel formation potential (Yousefi & Abbasi, 2022). These attributes further broaden the scope for use in restructured seafood products, high-protein spreads, and functional bakery items.

In comparison to other low-trophic marine bioresources such as seaweed, crustaceans, and fish by-products, Baltic Sea blue mussels exhibit a unique nutritional fingerprint (Table 8). While fishmeal still leads in crude protein content, mussel biomass offers a more balanced whole-nutrient package with significant calcium, glycogen, and bioactive peptide potential. Moreover, the absence of persistent organic pollutants (POPs) and lower heavy metal accumulation, confirmed in Baltic Sea blue mussels from both study

sites, further supports their use in food and feed sectors without extensive purification (Radlowska & Pempkowiak, 2002).

These safety metrics are supported by the biology of Baltic Sea blue mussels: short lifespans (typically 12–24 months), fast growth cycles, and farming in offshore areas with good water exchange collectively limit the time and exposure needed for bioaccumulation. Furthermore, their filter-feeding on smaller plankton particles reduces trophic transfer risk, differentiating them from benthic scavengers or longer-lived mollusks more prone to pollutant buildup.

In addition, mussel powders avoid several technical constraints typical of alternative biomass sources. For instance, shrimp shells and amphipod meals often contain high chitin levels that complicate digestibility and processing (Suhaimi et al., 2024), whereas thraustochytrid-based feeds, while rich in lipids, require heterotrophic cultivation and specialized fermentation facilities (Jaseera & Kaladharan, 2019). Mussel powders, by contrast, require no artificial inputs and integrate seamlessly into existing feed and food formulations of workflows.

For example, macroalgae such as *Ulva* spp. can offer notable prebiotic potential via sulfated polysaccharides, but their protein content rarely exceeds 20–25% DW, and amino acid profiles often lack essential residues like lysine or methionine (Holdt & Kraan, 2011). Baltic Sea blue mussels, by contrast, deliver a complete amino acid profile and higher protein content (more than 50% of DW), along with moderate glycogen levels that may offer synergistic benefits in feed formulations.

Similarly, starfish (*Asterias rubens*) have been evaluated for use in feed applications, particularly for their mineral content and antioxidant peptides, but their bitterness and bioaccumulation of contaminants in some regions remain challenges for food-grade utilization (Eroldoğan et al., 2022).

In terms of ecological footprint, mussels outcompete most alternatives. They require no feed, sequester nitrogen and phosphorus, and provide habitat complexity, delivering ecosystem services while generating harvestable biomass (Langdal et al., 2025). This sets them apart from resource-intensive fishmeal and energy-heavy shrimp shell processing, and from macroalgae, which often require artificial structures and intensive dewatering steps.

Table 8. *Comparative nutritional and functional characteristics of Baltic Sea blue mussels and selected marine bioresources.*

Resource	Protein (% DW)	EPA+DHA (% of FA)	Glycogen (% DW)	Calcium (% DW)	Prebiotic Potential
Baltic Sea Blue Mussels	46–53	20–23	2.6–3.4	12–18	Moderate
Fishmeal (Tacon & Metian, 2008)	60–70	25–30	<1	<2	Low
Shrimp Shells (Younes & Rinaudo, 2015)	15–25	Trace	-	20–25	Low–Moderate
Brown Seaweed (Holdt & Kraan, 2011)	10–25	Negligible	<5	5–10	High (via alginates)

Compared to conventional protein inputs such as fishmeal and soymeal, Baltic Sea blue mussels offer a substantially lower ecological footprint. Fishmeal production, while nutritionally rich in protein and long-chain omega-3 fatty acids (LC-PUFAs), relies on wild-caught forage fish, often resulting in high bycatch and energy-intensive rendering processes (Tacon & Metian, 2008). Soymeal, though plant-based, contributes to land-use change, deforestation, and freshwater depletion, factors under growing scrutiny in aquafeed sustainability assessments (FAO, 2022). In contrast, mussels require no feed, freshwater, or arable land, and actively remove excess nutrients from marine ecosystems. This results in a 60–80% lower feed-conversion footprint and positions mussels as a superior choice for low-impact protein sourcing within circular food system frameworks.

The potential of Baltic Sea blue mussels in functional food development lies not just in their nutrient density, but in their techno-functionality, versatility, and environmentally sustainable nature. From a formulation perspective, dried mussel powder or hydrolysate can be incorporated into high-protein snacks, savory spreads, or broths; used as a protein-rich binder in fish patties, burgers, or seafood mixes; and combined with fiber-rich ingredients such as oats or barley to create “complete” meal components.

From a health-positioning perspective, such products could carry claims related to high protein content, natural marine omega-3, calcium and selenium sources, and potentially gut microbiome support (pending further validation).

From a marketing perspective, Baltic Sea blue mussels offer a compelling narrative of local, sustainable, low-impact aquaculture; alignment with circular economy principles through zero-waste practices; and contributions to water quality improvement and ecosystem restoration. These attributes strongly align with growing consumer demand for climate-smart proteins and may appeal to flexitarian, pescatarian, and health-conscious segments.

However, integrating mussels into functional foods will require further R&D to address issues such as the bitterness of hydrolysates (requiring taste masking), standardization

of sensory properties, compliance with regulatory requirements and allergen labeling, and the need for consumer education and acceptance of novel marine ingredients.

Perhaps most importantly, Baltic Sea blue mussels offer a convergence point for nutritional innovation and environmental stewardship. Few biomass sources provide high-quality protein and omega-3s, require no inputs, remove nutrients from eutrophic waters, and offer bioactive and functional food potential.

This thesis shows that even small, thin-shelled mussels from low-salinity waters can meet the thresholds for nutritionally relevant, biofunctionally active, and sensorially acceptable ingredients. By valorizing them fully, we can convert ecological service providers into valuable contributors to sustainable food systems, supporting regional food security, aquaculture diversification, and the circular bioeconomy.

4.3 Contribution to low trophic aquaculture value chain development

Eutrophication remains one of the Baltic Sea's most persistent environmental challenges, with legacy nutrient loads continuing to drive hypoxia, algal blooms, and biodiversity loss (Andersen et al., 2017). Mitigation strategies have largely focused on terrestrial nutrient reduction, but in-sea solutions are increasingly recognized as essential. Mussel farming presents a biological nutrient removal strategy, wherein bivalves assimilate dissolved nitrogen and phosphorus into shell and tissue biomass that can be harvested and removed from the marine system (Kotta et al., 2020).

Data from this study and prior research (Kotta et al., 2020) confirm that Baltic Sea blue mussels efficiently filter large volumes of phytoplankton-rich water. Estimates suggest that one tonne of mussel biomass removes approximately 7–10 kg of nitrogen and 1–1.5 kg of phosphorus (Kotta et al., 2020), depending on age and nutrient saturation levels. When scaled appropriately, mussel farming can therefore contribute quantitatively to HELCOM nutrient reduction targets.

In this framework, mussels serve both as a bioextractive species and as a feedstock for valorization, creating economic incentives for farmers to engage in water-quality improvement. The challenge is not biological feasibility, but economic integration, i.e., ensuring that mussels grown for ecosystem services can be processed into marketable products (Filipelli et al., 2020).

- Baltic Sea blue mussel farming also offers a foundational component for IMTA systems, wherein species from different trophic levels are co-cultivated to mimic ecological balance and recycle nutrients. In higher-salinity regions, mussels are co-farmed with salmon, seaweed, or crustaceans. In the Baltic context, where fish farming is limited, more creative combinations are needed (Buck & Buchholz, 2004).

Conceptually viable Baltic IMTA systems could include:

- Mussels + Seaweed (e.g., *Ulva* or *Fucus*): Mussels provide biofiltration; macroalgae absorb residual nutrients.
- Mussels + Sediment-dwelling species: Harvestable deposit feeders (e.g., polychaetes) under longlines could utilize biodeposits.
- Mussels + Offshore Wind Infrastructure: Co-location on wind turbine foundations provides structural support and access to space-limited areas.

Pilot IMTA projects in Estonia, Germany, and Denmark (Horizon OLAMUR, n.d.) demonstrate technical feasibility. However, further trials are needed to assess

compatibility of farming regimes, harvesting logistics, and combined product streams. Mussels offer a low-risk entry point for IMTA due to their resilience, autonomous feeding, and known environmental benefits.

- Perhaps the most significant enabler for sustainable mussel value chain development in the Baltic is the suite of processing and valorization techniques demonstrated in this dissertation. By transitioning from meat–shell separation toward whole-biomass processing and micronization, mussel biomass can be converted into functional powders, hydrolysates, and feed ingredients with minimal waste (Uzcátegui et al., 2021).

This zero-waste, low-energy approach unlocks a number of cascading benefits:

- Reduction of shell disposal costs, historically a logistical and environmental burden (Topić Popović et al., 2023)
- Higher economic return per tonne of biomass, even for smaller mussels
- Compatibility with decentralized, low-cost processing units, increasing regional resilience.

This model creates value from a biomass stream that was previously regarded as a side-product of nutrient removal. By integrating ecosystem services, scalable harvesting, and modular processing, mussel farming in the Baltic can serve as a cornerstone of low-trophic aquaculture economies, aligned with EU Blue Growth and Farm to Fork goals.

4.4 Product design and marketing developments

The successful valorization of Baltic Sea blue mussels depends not only on biomass processing and biochemical richness, but also on the creation of viable end-products that meet market expectations, regulatory frameworks, and sustainability criteria. As demonstrated in this thesis, whole-biomass processing methods such as micronization and hydrolysis allow for the transformation of low-yield, small-sized mussels into high-value functional powders and extracts. These materials can serve as inputs for a variety of products in both food and feed sectors.

Mussels processed into fine powders (less than 63 µm) or enzymatically hydrolyzed into protein-rich extracts offer multiple advantages as functional food ingredients:

- Nutrient density: High-quality protein, marine omega-3s (especially EPA), selenium, iodine, and calcium.
- Natural umami compounds: Glutamic acid and nucleotides contribute to flavor enhancement in broths, sauces, and savory snacks.
- Techno-functionality: Water-binding, emulsification, and gelling properties make mussel powders suitable for texture improvement in mixed formulations (Yousefi & Abbasi, 2022).

Conceptual applications include:

- High-protein pasta, crackers, or flatbreads (e.g., 5–10% inclusion)
- Fortified soups and bouillons with marine flavor and calcium boost
- Protein-enriched sports nutrition blends (e.g., in combination with algae or pea protein)
- Marine “superfood” seasoning blends or table condiments.

Sensory evaluation showed that well-micronized mussel powders have neutral to mildly marine aroma and a pleasant mouthfeel when rehydrated. With minimal masking, they are acceptable to health-conscious consumers seeking sustainable animal protein alternatives.

However, despite the favorable sensory profile, consumer familiarity with marine-based powders remains limited. Strategies such as culinary integration into familiar dishes (e.g., soups, sauces, crackers) and evidence-based sensory education highlighting health and sustainability benefits may improve acceptability and perception among hesitant segments (Michel et al., 2021).

Importantly, mussel powder and hydrolysate are suitable for flexitarian, pescatarian, and religious dietary patterns (e.g., Halal if slaughter protocols are adapted), widening their market potential.

The concentration of essential micronutrients and bioactive peptides in mussel biomass opens opportunities in the nutraceutical and dietary supplement sector. Compared to fish oil capsules or krill-based supplements, mussels provide:

- A food-grade, whole-organism source of omega-3s (especially EPA)
- Naturally occurring taurine, arginine, and histidine which are important for cardiovascular and cognitive health (Santulli et al., 2023)
- Enzymatic hydrolysates that may support immune function, inflammation modulation, or gut microbiota balance (Jiang et al., 2024).

Candidate nutraceutical formats include:

- Marine protein capsules or tablets (freeze-dried hydrolysate)
- Powder sachets for mixing into beverages
- Soft chews or “functional snacks” rich in omega-3 and minerals
- Joint health blends combining mussel peptides and glucosamine.

Moreover, recent findings suggest that peptides derived from mussel hydrolysates exhibit promising techno-functional properties. These include water-binding, emulsifying, and gel-forming capacities that are useful in the formulation of restructured seafood, plant-based protein blends, and high-protein bakery applications (Yousefi & Abbasi, 2022). Such attributes enhance the versatility of mussel-based ingredients across diverse product categories.

While green-lipped mussel extracts from *Perna canaliculus* dominate the current mollusk-based supplement market (Miller et al., 2023), Baltic Sea blue mussels represent a regional, scalable, and potentially lower-cost alternative, provided that bioactivity is validated through human trials.

Development of such products requires standardized extraction protocols (e.g., for protein, lipid, or peptide fractions), accurate nutrient composition labeling verified using ISO methods, shelf-stable and taste-masked formulations, and alignment with EFSA health claim regulations or national equivalents. Efforts to integrate mussel powders into familiar culinary matrices, such as soups, sauces, or grain-based products, may ease consumer acceptance. Sensory education campaigns that highlight the product’s marine origin and health benefits could further enhance palatability and uptake.

While mussel biomass is not traditionally considered a prebiotic source, emerging evidence from marine bioresources supports their potential. Enzymatic hydrolysates from fish skin collagen, oyster proteins, and shrimp shells have shown microbiome-supportive activity (Wang et al., 2019). Glycogen and chitooligosaccharides from shellfish by-products

have demonstrated bifidogenic effects *in vitro* (Liu et al., 2023). Additionally, specific marine peptides may modulate mucosal immunity and barrier function in the gut.

Although these findings are promising, several limitations must be acknowledged. The mechanism of prebiotic action is not yet fully characterized, and future studies should include short-chain fatty acid (SCFA) production assays and 16S rRNA-based microbiome analyses (Cunningham et al., 2021). Hydrolysis protocols may require optimization for taste masking and industrial scale-up, as peptide-rich hydrolysates can have bitter off-notes. Furthermore, regulatory frameworks for health claims on marine prebiotics remain underdeveloped in many markets. Nonetheless, the study confirms that processed Baltic Sea blue mussels are not only nutrient-dense but also biofunctionally active, with relevance to both gut health and broader physiological regulation.

Animal feed remains one of the most immediately scalable and economically viable outlets for processed mussel biomass. Micronized whole mussel powder offers high-quality protein (45–50% dry weight) with a complete amino acid profile, a naturally balanced mineral content, especially calcium and phosphorus, and functional lipids and minor nutrients that support immune function and gut health.

Applications across sectors include aquafeeds, where mussel meal serves as a palatable and digestible ingredient for carnivorous species such as salmonids and seabream; pet foods, where mussel powder adds value as a marine protein source in dog and cat diets through its omega-3 content and natural calcium; and poultry and pig feeds, where inclusion rates of up to 5% have been tested in trials (Van der Spiegel et al., 2013) with no adverse effects.

Compared to conventional fishmeal, mussel powder offers a more sustainable, non-fed source, with lower trophic conversion losses and minimal contaminants. It is also more culturally acceptable in markets sensitive to overfishing or wild capture impacts.

Key barriers to broader use of mussel-based ingredients include the need for standardization of particle size and moisture content to ensure consistent product quality, achieving cost parity with conventional feed protein sources such as soybean and fishmeal, and optimizing digestibility and palatability within species-specific formulations. However, for niche markets, premium pet food, organic aquaculture, and sustainable livestock brands, Baltic Sea blue mussel meal can offer clear differentiation.

Shells make up over 70% of total mussel biomass by weight, particularly in smaller individuals. Traditionally viewed as a waste stream, shell material can be upcycled into multiple valuable forms, including fine calcium carbonate powders for food fortification (such as in bakery or baby food), pH buffers for use in aquaculture and agriculture (e.g., pond liming and soil amendment), natural abrasive agents in cosmetics or cleaning products, and bioceramic precursors for applications like bone scaffolds or biomedical fillers (Owuamanam & Cree, 2020).

In the thesis, the use of whole-biomass micronization sidestepped the need for shell disposal entirely. Instead, finely ground shell became a natural part of the powder matrix, contributing both to mineral value and techno-functional properties (e.g., anti-caking, structural integrity in pressed forms).

Successful commercial valorization of shell material requires precise control of granulometry, particularly for food applications, where particle size must be below 50 µm, along with thorough purity assessments to ensure the absence of heavy metals and toxins, and formal registration as a food or feed additive under EU or Codex standards. This represents a promising area for industrial ecology integration, turning a costly waste

problem into a scalable material stream, especially when paired with renewable energy drying and grinding systems.

The above demonstrates that processed Baltic Sea blue mussel biomass can serve as a versatile input across a range of product categories, including functional human foods, nutraceuticals and supplements, premium animal and aquafeeds, and bio-based materials derived from shell. Product development will depend on market-specific strategies, optimization of taste and texture, and regulatory alignment. With the right partnerships, these innovations can position Baltic Sea blue mussels as a flagship species in low-trophic aquaculture valorization.

4.5 Economic horizons in mussel aquaculture and valorization

The future economic viability of mussel aquaculture in the Baltic Sea lies in shifting from volume-based production models toward high-value, service-integrated valorization strategies. Due to biophysical constraints such as low salinity and short growing seasons, Baltic *Mytilus edulis/trossulus* hybrids reach marketable size slowly and remain small (Kautsky et al., 1990; Westerbom et al., 2002), making them suboptimal for traditional half-shell markets. However, their ecological role and biochemical makeup, together with innovative processing, make Baltic mussels a strong option for bioeconomic models that focus on multifunctionality, circular use, and ecosystem services (Petersen et al., 2019; Maar et al., 2023).

Nutrient bioextraction represents a foundational economic service delivered by mussels. Filter-feeding mussels incorporate nitrogen and phosphorus into biomass, and harvesting them removes these nutrients from the marine system. Empirical work from Swedish coastal farms demonstrated that one tonne of mussel biomass can extract 6.6 kg nitrogen and 0.6 kg phosphorus (Lindahl et al., 2005), with potential cost-effectiveness ranging between €22–36 per kg N removed, depending on farm design and harvest timing (Stadmark & Conley, 2011; Petersen et al., 2016). These figures are competitive with, or even lower than, engineered nutrient abatement methods such as wetland restoration or advanced wastewater treatment (Gren et al., 2009), and can become economically viable if integrated into nutrient trading schemes or marine spatial planning frameworks.

In terms of product valorization, small-sized Baltic mussels have limited direct consumer appeal but exhibit significant potential when processed into fractionated or whole-biomass products. The micronization techniques developed in this dissertation reflect a broader movement in marine biomass utilization: the transformation of low-grade biomass into functional powders, hydrolysates, or extracts for the food, feed, and health sectors (Bleakley & Hayes, 2017; Eroldoğan et al., 2022). These pathways enable entry into higher-margin markets such as aquafeeds, pet foods, and nutraceuticals – markets increasingly seeking omega-3-rich, sustainable marine proteins (Rustad et al., 2011; Shahidi & Ambigaipalan, 2015).

Baltic mussels contain favorable nutrient profiles, including more than 50% protein (DW), high levels of EPA and DHA, and bioavailable minerals such as iron and selenium (Paper III; King et al., 1990). These characteristics are comparable to, and in some cases superior to, commercial finfish and shellfish meal inputs (Tacon et al., 2009; Tigchelaar et al., 2022). When combined with their lack of reliance on feed inputs, the feed conversion efficiency and lifecycle emissions of mussel biomass are significantly lower

than terrestrial animal protein sources (SAPEA, 2017; Tsakiridis et al., 2020). As such, Baltic mussels are especially well-positioned for use in sustainable aquafeed formulations, where partial substitution of fishmeal or soybean meal is economically and environmentally desirable (Iribarren et al., 2011).

Valorization also extends to mussel shells, which are rich in biogenic $CaCO_3$ (more than 95%) and suitable for use in agriculture, construction, or polymer biocomposites (Cinelli et al., 2020). Shell reuse both offsets waste disposal costs and adds economic value through co-product development. For instance, recent studies show that substituting mussel shell powder into polylactic acid (PLA) composites can enhance mechanical strength while reducing environmental footprint (Cinelli et al., 2020; Arockiam, 2023). This type of upcycling exemplifies the circular economy logic increasingly applied in aquaculture systems.

When bundled into IMTA systems, mussels can contribute synergistically to environmental and economic performance. For example, in IMTA configurations combining fish, mussels, and macroalgae, mussels provide nutrient remediation while simultaneously generating biomass for feed or food use (Troell et al., 2009; Chopin, 2016). Such systems are under active development in Nordic countries, with techno-economic models suggesting that including low-trophic species improves farm resilience, revenue diversification, and public acceptability (Luthman et al., 2021; Kotta et al., 2023).

Despite these promising avenues, the economic scaling of Baltic Sea mussel farming remains hindered by infrastructural gaps, limited consumer awareness, and the absence of region-specific policy incentives. However, as shown by market success in the green-lipped mussel (*Perna canaliculus*) sector in New Zealand, where lipid extracts command premium prices as joint-health supplements, small mussels with bioactive potential can anchor niche, yet profitable, industries (McPhee et al., 2007; Wakimoto et al., 2011). The key lies in generating scientifically validated, differentiated products supported by robust processing technologies and clear health or environmental claims.

In conclusion, the economic horizon of mussel farming in the Baltic Sea depends not on scaling biomass volume but on scaling value. This requires simultaneous investment in processing innovation, ecological accounting, and market development. Valorization pathways based on functional ingredients, circular co-products, and nutrient offset services, as demonstrated in this thesis, can reposition Baltic mussels as a keystone resource in the transition to climate-smart aquaculture and regional bioeconomies.

4.6 Missing knowledge and future research needs

While this thesis demonstrates clear technical feasibility and strong nutritional potential for Baltic Sea blue mussel valorization, several knowledge gaps remain. Addressing these will be important to move from proof-of-concept to commercially viable, policy-aligned, and socially accepted low-trophic aquaculture systems.

Laboratory and pilot-scale processing workflows, such as mechanical blending, sedimentation-centrifugation, enzymatic hydrolysis, and micronization, showed excellent performance at volumes up to several kilograms per day. However, industrial scaling poses unresolved questions:

- Throughput consistency: Equipment must handle biomass with variable water content, shell integrity, and fouling organisms.
- Energy optimization: Drying and grinding at scale may become energy-intensive unless integrated with renewable energy or waste heat.

- Process standardization: To ensure food- or feed-grade quality, parameters such as drying temperature, particle size, and microbial safety must be rigorously controlled.
- Modularity: Systems suitable for on-site, decentralized processing (e.g., coastal farms or small cooperatives) need to be designed for low maintenance and mobility.

Pilot plants, either stand-alone or embedded in biorefineries, should be developed and tested under realistic operational conditions. Such initiatives would benefit from EU co-financing schemes like Horizon Europe, the Blue Bioeconomy ERA-NET, or regional innovation funds.

Moreover, the novel status of processed mussel biomass, particularly whole-biomass powders and hydrolysates, raises important regulatory questions that must be addressed early in commercialization.

For food applications, issues include:

- Whether whole mussel powder qualifies as a novel food under EU Regulation (EU) 2015/2283.
- Permissible health claims related to protein, omega-3, calcium, or prebiotic effects.
- Labelling requirements for allergen warnings, nutritional composition, and processing methods.

For feed applications, producers must:

- Comply with Regulation (EC) No. 767/2009 on feed marketing.
- Ensure safe levels of heavy metals, dioxins, and microbial contaminants.
- Establish digestibility and performance data for target species.

Shell-derived calcium products may also fall under feed additive regulation, requiring dossier submission for approval (EFSA Guidance on Feed Additives, 2021). Collaboration with regulatory agencies, standardization bodies (e.g., ISO, Codex), and sectoral trade associations is critical to define safe, legal, and accepted pathways for Baltic Sea blue mussel-based products.

Consumer perception also plays an important role in the success of novel aquaculture-derived food products. Although mussels are widely consumed in many European countries, products made from micronized mussel biomass, shell-inclusive powders, or hydrolysates are unfamiliar and may face skepticism.

Key questions include:

- How do consumers perceive the flavor, texture, and appearance of mussel-based functional ingredients?
- Are environmental benefits (e.g., water purification, low carbon footprint) compelling enough to offset unfamiliarity?
- What communication strategies (eco-labeling, story-based branding, endorsements) are most effective?

Initial sensory testing indicates high acceptability under controlled conditions, but quantitative consumer research, such as conjoint analysis or acceptance surveys across target groups (e.g., flexitarians, athletes, pet owners), is required to inform product development and marketing.

Importantly, despite the strong sustainability narrative of mussel farming, robust life-cycle assessments (LCAs) are still needed to quantify the actual environmental impacts and benefits of various valorization pathways. LCAs should compare whole-biomass utilization versus traditional shucking, assess different drying and processing methods such as freeze-drying versus convection drying, evaluate alternative end uses including food, feed, fertilizer, and bioplastic fillers, and consider co-location strategies such as integrating mussel farming with wind farms. In addition, valorized mussel biomass could eventually qualify for carbon or nitrogen credits under emerging nutrient trading schemes, especially when integrated with verified LCA protocols

Functional units should include both nutrient removal (g N/P) and nutritional value delivered (e.g., g protein, g EPA) to reflect the dual role of mussels as ecosystem engineers and nutrient sources (Spångberg et al., 2013). Such studies can inform policy incentives, eco-labelling schemes, and carbon/nitrogen offset markets, enabling mussel products to compete not only on nutrition and taste but also on verified environmental performance.

To enable widespread adoption of Baltic Sea blue mussel valorization, coordinated research is needed in four key areas: technical upscaling of processing solutions to ensure consistency and cost-efficiency; regulatory clarity regarding product categories and permissible claims; insights into consumer behavior to guide product development and communication strategies; and comprehensive environmental assessments to quantify sustainability impacts and potential trade-offs. These research efforts must be interdisciplinary, combining marine science, food technology, social science, and policy expertise to build an integrated Baltic Sea blue mussel innovation ecosystem.

5 CONCLUSIONS

This doctoral thesis investigated and developed valorization pathways for small-sized Baltic Sea blue mussels (*Mytilus edulis/trossulus*), with the aim of enhancing the economic viability of low trophic aquaculture (LTA) in low-salinity environments. A range of processing methods, biochemical analyses, and functional assessments were employed to optimize the use of whole mussel biomass for food and nutraceutical applications. The findings contribute to sustainable aquaculture practices by advancing zero-waste processing approaches and unlocking the nutritional and functional potential of this underutilized marine resource.

The overarching aim was addressed through four specific objectives, each of which was fulfilled through experimental trials and methodological innovations.

Objective 1: Develop and optimize mussel biomass valorization pathways for human consumption.

The research established novel workflows based on whole-biomass fractionation and micronization. These methods eliminated the need for manual shell–meat separation and yielded shelf-stable powders suitable for food and functional ingredient applications. The fine fraction (less than 63 μm) exhibited favorable textural, sensory, and compositional properties, supporting its use as a base for protein- and calcium-rich formulations.

Objective 2: Improve processing methods for small-sized mussels.

A scalable processing workflow was developed by integrating mechanical blending, sedimentation (optimal at 5 minutes), and low-speed centrifugation (4500 × g). Compared to manual shucking, which achieved only 7.4 ± 0.9% dry yield, the new approach reached up to 17.2 ± 1.1% dry matter recovery and protein concentrations exceeding 50% of DW, representing a five- to six-fold improvement in throughput. Additionally, micronization at 4000 rpm yielded more than 40% of particles smaller than 63 μm, enabling whole-biomass utilization while eliminating the need for shell removal.

Objective 3: Explore the chemical and nutritional potential of mussel biomass.

Compositional analysis revealed protein content ranging from 46.7 ± 2.1% to 52.3 ± 1.9% DW, lipid levels of 6.8–8.1% DW, and high-value marine omega-3 fatty acids, including EPA (18.7 ± 1.2%) and DHA (4.6 ± 0.9%) of total fatty acids. Glycogen content was higher in autumn (3.4 ± 0.6%) versus spring (2.1 ± 0.5%), and enzymatically hydrolyzed fractions supported up to 85% growth performance of probiotic strains compared to MRS controls. Seasonal variation strongly affected both yield and biochemical quality, confirming autumn-harvested mussels as superior for food-grade valorization.

Objective 4: Provide future perspectives for sustainable LTA development in the Baltic Sea.

The study identified multiple opportunities for product innovation and environmental services. Prebiotic potential was demonstrated through enhanced growth of probiotic strains when cultivated on mussel-derived hydrolysates. Whole-biomass processing offers a circular model for aquaculture waste minimization, while contributing to eutrophication mitigation and resource-efficient protein production. Valorization of shell material adds value in the form of dietary calcium or feed minerals.

Taken together, the findings confirm that whole-biomass processing of Baltic Sea blue mussels is not only technically feasible but aligned with emerging sustainability and circular economy goals. The methods and results presented here contribute both practical workflows for near-term industry application and a foundation for further research into functional and bioactive properties of low-trophic marine species.

Table 9 summarizes the transition of the Baltic Sea mussel sector from its initial state to the practical and scientific advancements achieved in this doctoral work, showing how key challenges were addressed through experiments, new methods, and concepts that enable sustainable, zero-waste processing and strengthen low-trophic aquaculture in the Baltic Sea.

Table 9. *Progress from initial state to achieved outcomes.*

Area / Challenge	**Initial state (before research)**	**What was done / solved in this work**
Baltic mussel size and salinity	Small-sized mussels (*Mytilus edulis/trossulus* hybrids) from low-salinity Baltic waters; existing processing methods optimized for larger, ocean-grown mussels.	Developed workflows adapted to low-salinity, small mussels, designed to sustain product quality and yield.
Manual shelling inefficiency	Manual shelling yields only 15–20% edible biomass; high labor cost; large shell waste stream.	Introduced mechanical crushing + sedimentation separation → 5–6× higher edible biomass yield, >50% protein dry weight, minimal shell contamination.
Seasonal variation	Unknown optimal harvest season for nutritional value and processing efficiency.	Analysed seasonal composition → identified spring/autumn peaks for specific applications; validated harvesting strategies.
Shell waste problem	Shell considered waste, requiring disposal; lost mineral content.	Integrated shell fraction into products via micronization → zero-waste concept with added $CaCO_3$ value.
Protein utilisation	Lack of data on protein yield and quality from small Baltic mussels; no scalable extraction process.	Developed scalable workflows for meat recovery, protein isolation, and hydrolysate production; characterised amino acid profile and functional properties.
Industry adoption barrier	No practical, scalable model linking mussel farming with processing for low-trophic aquaculture in Baltic region.	Designed and demonstrated a biorefinery concept aligning with policy goals, sustainability targets, and industry needs.

Scientific knowledge gap	Limited literature on Baltic mussel biomass valorization and biorefinery approach.	Produced four peer-reviewed articles with first-author leadership, demonstrating scalable processing of low-salinity Baltic mussels through mechanical separation, seasonal optimisation, protein recovery, and shell valorization within a zero-waste biorefinery concept.

Outlook

In conclusion, this work demonstrates that small-sized Baltic Sea blue mussels, often viewed as a by-product of ecosystem services, can be transformed into a valuable bioresource for food and functional ingredient production. The approach developed here enables scalable processing, nutrient recovery, and zero-waste utilization. Future research should focus on scaling pilot technologies, conducting consumer acceptance studies, and evaluating the life cycle benefits of mussel-based products. With appropriate investment and regulatory alignment, Baltic Sea blue mussel valorization has the potential to support regional food system resilience, blue economy growth, and marine ecosystem restoration in tandem.

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Abstract

Valorization of Blue Mussels in the Baltic Sea

The global food system is undergoing a necessary transformation to meet the demands of sustainability, climate resilience, and circularity. In this context, low trophic aquaculture (LTA) offers significant potential by enabling resource-efficient biomass production with minimal environmental impact. However, the practical application of LTA in the Baltic Sea, a brackish, eutrophication-prone region, has been hindered by the biological and economic limitations of species such as *Mytilus edulis/trossulus* mussels. These small, thin-shelled bivalves have been undervalued in traditional food and feed markets due to their limited meat yield, high shell-to-flesh ratio, and lack of scalable processing strategies.

This doctoral thesis addresses the pressing need for valorization pathways tailored specifically to Baltic Sea blue mussel biomass. The work is motivated by the dual imperative to reduce nutrient loading in the Baltic Sea and to unlock new bioeconomic value from underutilized marine resources. The novelty of this research lies in its integrative approach to whole-organism processing, its adaptation of food technology methods to marginal aquaculture conditions, and its contribution to emerging policy frameworks such as the EU Green Deal and Blue Growth.

The central problem defined in this work is the absence of scalable, sustainable, and market-relevant processing solutions for small-sized Baltic Sea blue mussels, which limits the expansion of mussel farming as both an ecological and economic activity. To address this, the study set out four key objectives: (1) to develop innovative processing workflows suited to Baltic Sea blue mussel morphology and composition, (2) to optimize biomass yield and quality through mechanical and chemical pre-treatment, (3) to evaluate the biochemical and functional properties of the processed material, and (4) to propose viable product formats and valorization models aligned with circular economy goals.

A multi-stage methodology was employed. Baltic Sea blue mussels were harvested from low-salinity farms in Estonia and Sweden and subjected to laboratory trials combining mechanical disruption, gravity sedimentation, low-speed centrifugation, and whole-biomass micronization. Pre-treatment with food-grade citric acid was tested for flavor improvement, while enzymatic hydrolysis was applied to enhance bioavailability. Analytical procedures included proximate composition, amino acid and fatty acid profiling, microbiological safety testing, and *in vitro* assessments of prebiotic activity. Sensory evaluations were conducted with trained panels to assess acceptability of micronized powders.

The results demonstrated that sedimentation-centrifugation workflows provided dry matter yields of up to 17.2% from raw biomass, with protein contents exceeding 50% of dry weight. Whole-biomass micronization, particularly at high-speed settings, produced fine powders (less than 63 µm) with excellent sensory properties, favorable particle dispersion, and high nutritional value, including marine omega-3 fatty acids (EPA 18.7%, DHA 4.6% of total FA) and natural calcium (up to 18.4% DW). Citric acid pre-treatment significantly reduced off-flavors (e.g., muddy and metallic notes by more than 50%) and enhanced overall acceptability. Enzymatic hydrolysates stimulated the growth of *Lactobacillus rhamnosus GG* and *Bifidobacterium animalis* by over 80% compared to controls, suggesting mild prebiotic effects. Seasonal comparisons indicated that autumn-harvested mussels provided superior yields and compositional quality due to post-spawning recovery.

The conclusions highlight the feasibility of using Baltic Sea blue mussel biomass for value-added, zero-waste products. The integration of micronization, sensory optimization, and bioactivity profiling offers a new paradigm for mussel valorization, especially in regions where traditional meat extraction is economically infeasible. Furthermore, the findings support the role of mussel farming as a dual-purpose strategy for nutrient bioextraction and biomass production in the Baltic Sea. This work contributes scientifically to marine food processing, functionally to sustainable aquaculture innovation, and societally to the circular bioeconomy.

The thesis offers a replicable, scalable framework for low-trophic aquaculture in brackish and marginal waters, enabling a transition from ecological service provision to economic viability. It lays the foundation for further research on consumer perception, regulatory alignment, and life-cycle assessment of mussel-based products, and provides actionable insights for industry actors, policy-makers, and sustainability-oriented entrepreneurs.

Lühikokkuvõte

Läänemere söödava rannakarbi väärindamine

Globaalse toidusüsteemi ees seisavad suured väljakutsed seoses kestlikkuse, kliimamuutustega kohanemise ja ringmajanduse eesmärkidega. Sellises olukorras pakub madala troofilise taseme vesiviljelus (LTA) olulisi võimalusi, võimaldades toota biomassi keskkonnasõbralikult ja ressursitõhusalt. Läänemere piirkonnas on LTA rakendamine seni jäänud tagasihoidlikuks, seda peamiselt bioloogiliste ja majanduslike takistuste tõttu. Meie rannikumeres elavad söödavad rannakarbid (*Mytilus edulis*/*trossulus*) on väiksemad ja õhema kestaga kui sama liigi soolasemas vees kasvatatavad liigikaaslased, mistõttu on nende väärindamine toidu- ja söödaturul olnud seni piiratud.

Käesolev doktoritöö keskendub vajadusele arendada välja sobivad töötlusviisid Läänemere karbibiomassi väärindamiseks, et muuta karbikasvatus mitte ainult keskkonnateenuseid pakkuvaks, vaid ka majanduslikult elujõuliseks tegevuseks. Uurimistöö uudsus seisneb kogu organismi hõlmava töötlusmudeli arendamises, tööstustehnoloogiate kohandamises väikese ja hapra biomassiga ning ringmajanduse põhimõtete sidumises piirkondlike vesiviljelusstrateegiatega.

Töö eesmärk oli:

1. arendada ja optimeerida töötlusprotsessid, mis sobivad väikestele ja õhukese kestaga rannakarpidele;
2. suurendada biomassi saagikust ja kvaliteeti mehaaniliste ja keemiliste eeltöötluste abil;
3. hinnata saadud toodete biokeemilist koostist ja funktsionaalseid omadusi;
4. pakkuda välja tootekontseptsioone ja väärtusahela lahendusi, mis toetaksid ringmajandust ja kestlikku vesiviljelust.

Meetoditena kasutati kombineeritult mehaanilist purustamist, gravitatsioonilist setitamist, madalapöördelist tsentrifuugimist ning kogu organismi mikropeenestamist. Maitseomaduste parandamiseks katsetati sidrunhappega töötlemist, bioaktiivsuse suurendamiseks rakendati ensümaatilist hüdrolüüsi. Analüüside hulka kuulusid keemilise koostise määramised (valkude, aminohapete, rasvhapete ja glükogeeni sisaldus), mikrobioloogiline ohutus, sensoorsed hinnangud ning prebiootilise aktiivsuse testid probiootiliste bakteritega.

Tulemused näitasid, et setitamise ja tsentrifuugimise kombinatsiooniga saavutati kuni 17,2% kuivainesaaki ning üle 50% valgu kontsentratsioon kuivaines. Mikropeenestamisel 4000 p/min juures moodustus üle 40% osakestest alla 63 µm fraktsioonis, mis andis ühtlase ja meeldiva tekstuuriga pulbri. Sidrunhappega eeltöötlemine vähendas märkimisväärselt kõrvalmaitseid (nt metalliline ja mudane maitse) ning parandas sensoorset aktsepteeritavust. Ensümaatiline hüdrolüüs soodustas *Lactobacillus rhamnosus* GG ja *Bifidobacterium animalis* kasvu 80–85% ulatuses võrreldes kontrollproovidega, kinnitades potentsiaali prebiootilise koostisosana. Sügishooaja proovid näitasid kõrgemat valgusisaldust, paremat töötlemiskvaliteeti ja suuremat glükogeenisisaldust, rõhutades hooajalisuse mõju väärindamisväärtusele.

Tulemuste põhjal võib järeldada, et kogu organismi hõlmav ja jäätmevaba töötlus on Läänemere rannakarpide puhul tehniliselt teostatav, toiteväärtuslik ning skaleeritav. See lähenemine toetab piirkondlikku ringbioökonoomiat ning loob võimaluse ühendada keskkonnateenused ja majanduslik tulu. Doktoritöö loob aluse uutele toidutoodetele, funktsionaalsetele koostisosadele ja ärimudelitele, mis väärtustavad seni alakasutatud

kohalikku merebiomassi. Töö tulemused pakuvad teaduspõhist tuge poliitikakujundajatele, tööstusele ja teadlastele ning annavad praktilise aluse kestliku LTA sektori arendamiseks Läänemeres ja mujal.

Appendix 1

Publication I

Adler, I., Kotta, J., Tuvikene, R. and Kaldre, K., 2022. Optimizing the processing of shellfish (*Mytilus edulis* and *M. trossulus* hybrid) biomass cultivated in the low salinity region of the Baltic Sea for the extraction of meat and proteins. *Applied Sciences*, 12, 5163. https://doi.org/10.3390/app12105163

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Article

Optimizing the Processing of Shellfish (*Mytilus edulis* and *M. trossulus* Hybrid) Biomass Cultivated in the Low Salinity Region of the Baltic Sea for the Extraction of Meat and Proteins

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Abstract: Mussel farming is a novel and growing aquaculture field in the Baltic Sea. Nevertheless, there is very little published evidence on the processing of shellfish biomass in the region. The aim of this study is to develop a methodology for the extraction of organic-rich fractions from small-sized blue mussels of the Baltic Sea region that is applicable and economically viable for the feed and food industry. The efficiency of mussel meat separation was evaluated using different processing, drying, and filtration techniques. The laboratory experiments have succeeded in finding a method that is operationally feasible and does not require overly complex and expensive laboratory settings. These trials also showed that the separation of meat from fresh or frozen mussels can be achieved by simple crushing and sedimentation methods and the extraction yielded a significant amount of mussel meat (7.6%) with a high protein content (3.2%, i.e., half of the total protein found in the used mussel-mass). It also appeared that the use of filtration is not practical because the protein loss was extremely high. In addition, filtration makes the process of dry-matter separation more complex, and costs are unlikely to be compensated by the energy saved in drying.

Keywords: Baltic Sea; green protein; mussel processing; mussel valorization; sustainable aquaculture

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1. Introduction

While aquaculture is often associated with water-quality degradation, the spread of invasive species, and the destruction of important coastal habitats, there is growing evidence that well-planned and managed aquaculture can provide ecosystem services, including habitats for fish and other marine organisms. Here, low trophic aquaculture sectors such as shellfish and algae farming represent novel sustainable and environmentally restorative aquaculture trends [1]. Cultivated mussels are filter feeders and during harvest, a significant amount of excess nutrients can be removed from the marine environment [2,3]. Moreover, besides their nutrient sequestration potential, mussels act as nutrient sinks by ingesting particles suspended in the water column and thereby directly improving water quality. Importantly, mussel meat is rich in proteins, omega-3 fatty acids, glycogen, magnesium, potassium, calcium, selenium, iron, and vitamin B12, just to name a few [4]; thus, its use for human consumption benefits both health and nature [4]. In highly eutrophic waters, however, where mussels may not necessarily meet standards for food safety, mussels may be also valorized for other purposes than human food.

As in other ecosystems, the Baltic Sea is characterized by its legacy nutrients that result in adverse symptoms of eutrophication [5]. To date, mussel farming is considered one of

the most promising measures to remove these excess nutrients from the Baltic Sea [3]. The most promising aquaculture species in the Baltic Sea is the blue mussel, a hybrid of the two species *Mytilus edulis* and *Mytilus trossulus* [6].

Earlier studies demonstrated that shellfish farming in the Baltic Sea is efficient, cost effective, and removes large amounts of nutrients [3,7]. Comprehensive environmental monitoring of all existing mussel farms in the Baltic Sea did not identify any significant negative environmental impacts in any aspect over a three-year period [7]. In addition to the above, Baltic Sea shellfish have very low levels of different toxins (e.g., heavy metals, PCBs, benzopyrenes, algal toxins), which means that this resource can be effectively used for human consumption and/or animal feed [8]. Despite these positive aspects, there are still many challenges that hinder the development of the mussel farming industry in the Baltic Sea region [9].

When prepared for human consumption, mussel meat yields should be >30% to have a market as a food product. Thus, mussels with lower meat yields are normally rejected due to their low commercial value and are classified as by-products. Salinity is low (below 10) in the large parts of the Baltic Sea, and therefore blue mussels are much smaller in the Baltic Sea than in the North Sea. Looking at the average size of a mussel in the Baltic Sea (2–3 cm), more than 95% of the catch is theoretically classified as by-products. For shellfish aquaculture to be successful, it is necessary to address product valorization to make this aquaculture sustainable [3]. The development of innovative products and production lines is essential for the cultivation and marketing of new species, as at present there is no effective use of shellfish collected from farms in the low-salinity parts of the Baltic Sea. The small size of mussels farmed requires viable solutions of processing mussels for feed, food, or some high-end product. To date, however, the biomass of mussels has been very poorly exploited in the Baltic Sea region.

As blue mussels in marine waters are reasonably large enough to be directly used, and fresh mussels are in high demand, there are not many studies on valorizing mussels further for human consumption. Amongst commonly used methods of extracting meat and protein fractions, acid and alkaline solubilization techniques have been used [10]. In addition, proteins can be efficiently extracted using enzymatic hydrolysis [11]. The necessity for valorization mostly comes from by-products and the biomass that is discarded from direct consumption [12]. Among very few publications on mussel valorization for human consumption, the mussel by-products can be used to produce mussel pâte [13] using the well-established technological methods of tuna pâte [14].

Besides human consumption, mussels can be used for many other applications. The inhibitor found in the liquid extracted from the mussel successfully inhibits enzymatic browning, making it a valuable “preservative” in the fruit and vegetable industry, as natural inhibitors are better absorbed by humans [15]. Mussel shells can be heat treated to effectively produce CaO powder [16] to be used in building materials, food additives, pharmaceuticals, animal feed, and plastics [17]. Further refining technology of this material results in high-purity nano-sized calcium carbonate powder that can be used for niche applications such as scaffold fabrication, bone regeneration, and as a catalyst for high-temperature reactions [18]. Moreover, mussel meal can be used for bird feed. Here, mussels are seen as a good and high-quality protein source for poultry, and may replace fish meal in organic diets for laying hens and broiler chickens [19]. Similarly, mussel meal has been effectively used as a fish-feed attractant for farming turbot, where it significantly improved the palatability of rapeseed-protein-based diets [20,21]. On the other hand, although Arctic Charr consumed the novel feed well, their growth was diminished with mussel meal compared to the traditional fish-meal-based feed [22].

The aim of this work was to develop a simple and viable methodology for the separation of the organic-rich fraction from edible mussel biomass that is applicable for the food and feed industry. A simple methodology that can be easily scaled up to meet the needs of industrial applications is a prerequisite of the development of sustainable mussel farming in the Baltic Sea region and beyond. To achieve the objective, we carried out experiments,

during which we evaluated the efficiency of separating the unprocessed mussel meat from the mussel shell and its potential for use in the production of proteins. Crushed mussels were submitted to different processing conditions (different ratios of raw mussels to water, different sedimentation times) to seek an optimal methodology for organic-rich fraction and protein-rich extract production. In addition, the impact of filtration on the overall process was assessed to see whether an additional processing step that prolongs the process could be justified. The efficiency of methods was evaluated both in terms of meat yields and pure protein content. In addition, the concentration of dissolved calcium in the suspension of mussels and water was measured as elevated calcium levels in solution directly reflect the increase in mineral part proportion apparent after drying.

2. Materials and Methods

2.1. Raw Materials Used in the Tests

The blue mussel complex (a hybrid of *Mytilus edulis* and *M. trossulus*) was used for the experiments. The *Mytilus* complex includes three incompletely isolated species of marine mussels, *Mytilus edulis* (Linné, 1758), *Mytilus trossulus* (Gould, 1850), and *Mytilus galloprovincialis* (Lamarck, 1819). The Baltic populations of *Mytilus* spp. were established after the glaciation period during the Holocene and have retained unique characteristics compared to populations from other geographic areas characterized by a high frequency of *M. trossulus* and *M. edulis* genes [6].

The material for the study was collected from a mussel farm at 0–3 m depth in Tagalaht Bay (58.45644° N, 22.05452° E), the eastern Baltic Sea on 23 September 2020. Harvesting was carried out by diving and manually removing shells from the net. The samples were collected from different depths and different locations on the net to ensure the representativeness of the samples. By the time of harvesting, the mussels had been growing on the nets for one and a half years. After harvesting mussels were immediately washed with clean seawater, packed in 300 g batches in plastic bags, labeled, and placed in a freezer, where they were stored until the day of the laboratory analyses.

2.2. Laboratory Trials

Experimental analyses were carried out at the Laboratory for Food Chemistry and Technology of the Estonian University of Life Sciences and at the Laboratory of the School of Natural Sciences and Health of Tallinn University in 2020–2021. A schematic diagram for meat and protein separation is shown in Figure 1. Before starting the experiment, mussels were defrosted and then rinsed with tap water to reduce salts. In the first set of experiments, the mussels were crushed with water in a blender (Philips HR3652, 1400 W) for 2 min at full power. In this experiment, different ratios of raw mussels (uncleaned) to water were used (1 volume of mussels and 2, 3, or 4 volumes of water). After 2 min, the top portion of the suspension (without the crushed shells) was decanted into a 1000 mL measuring cylinder (height 45 cm, internal diameter 6.3 cm) and allowed to settle for 1, 5, and 15 min.

Next, 10 mL of the mussel suspension was removed from the upper third of the cylinder by pipetting and used for the dry-matter content determination (by drying for 24 h at 60 °C). The mass of the residue was then recorded. For the determination of protein content, 50 mL of the sample was pipetted from the upper third of the cylinder, and the protein content of the sample was then determined spectrophotometrically by the Bradford assay (according to the manufacturer's instructions). To achieve this, the protein content of the sample was diluted to between 0.1 and 1.4 mg/mL. Then, 900 µL of Bradford reagent (Sigma-Aldrich B6916, Saint Louis, MO, USA) was added to 900 µL of the diluted sample and the absorbance of the solution was measured at 595 nm after 25 min. For calibration, bovine serum albumin (BSA) was used. The content of calcium ions was determined by the complexometric titration method directly from the untreated sample by titrating the test samples with ethylenediaminetetraacetic acid (EDTA) solution using Patton and Reeder's indicators.

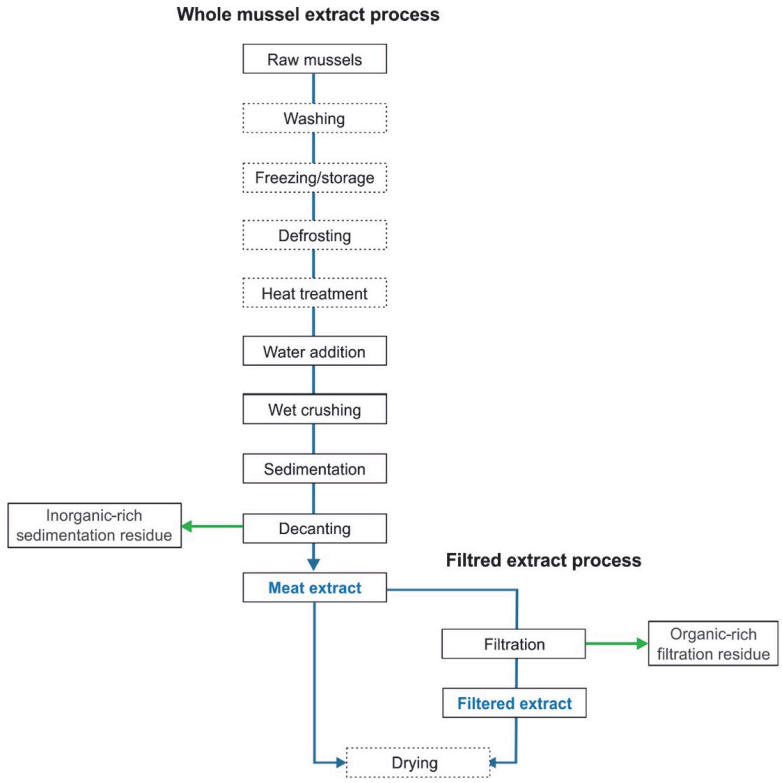

Figure 1. Schematic diagram for meat and protein separation. Dashed boxes represent optional processing steps.

In the second set of the experiment, the filtration of the liquid meat mass was tested to assess whether there could be an economic advantage in removing excess liquid prior to drying, i.e., whether the higher concentration of the filtered meat mass, and hence, lower potential drying energy input, would compensate for the dry matter that is discarded during filtration.

In this experiment, the raw mussel samples were crushed on a Grindomix GM200 homogenizer (RETSCH, Haan, Germany). The crushing speed was selected to be 6000 rpm between 7 and 9 s. From a visual assessment, 7–9 s appeared sufficient to separate the meat from the shells while leaving a fraction of the shells that were practically clean from meat particles and not too fine. Prior to the test, 250 mL of deionized water was added for every 100 g of shell mass. The water was added in three stages, each time settling the shells and decanting the liquid meat mass. The shells are heavier than the meat and settle quickly, so each sedimentation process took only a few seconds.

Half of the material was then filtered. The filtration of the liquid meat material was carried out using a 100 µm cloth mesh. The mesh was placed on a Bunsen flask and excess water was removed by vacuum. The filtered mass was placed on a tray and weighed. In order to heat dry the filtered and unfiltered samples, the liquid meat mass was poured into porcelain tubes and dried in a thermal oven until the liquid was completely removed. Then, the content of dry matter was found. The total protein content of the samples was determined by the Kjeldahl method [23] by heating the sample with concentrated sulfuric acid at 360–410 °C.

In addition, control samples were taken to measure the dry weight and protein content in the clean mussel meat before the experiments started. For this purpose, three batches of mussels were separated, 10 g per batch. The mussel flesh was extracted from the shells using tweezers. The dry matter and protein contents of the mussel meat were then measured. The measured values were used as reference values for the evaluation of the dry matter and protein content of the mechanically separated mussel meat and the efficiency of the separation methodology in a later phase (Figure 1).

2.3. Statistical Analyses

The effect of different treatments was tested by the analysis of variance. In the first experiment, the content of dry matter, protein, and calcium ions were dependent variables and the ratio of raw mussels to water (3 levels) and sedimentation time (3 levels) were used as factors. In the second experiment, the content of dry matter, protein, and calcium ions were dependent variables and filtration (2 levels) was used as a factor. Tukey's post-test was used to compare the effect of pairwise factor levels. The significance level was set at 0.05. Data analysis was performed in the statistical software R [24].

3. Results

One liter of wet mussel mass contains about 695 g of wet matter and 250 g of dry matter. From this amount of dry matter, it is possible to extract around 40 g pure meat in dry weight, the remainder being mainly minerals.

The first experiment showed that adding less water to the mussels increases the solids content of the sample. Moreover, the shorter the settling time, the more solids the sample contained. If the dry weight was calculated on the basis of the mussel volume, its content was mainly determined by the settling time, with a shorter settling time having higher amounts of solid material (Figure 2).

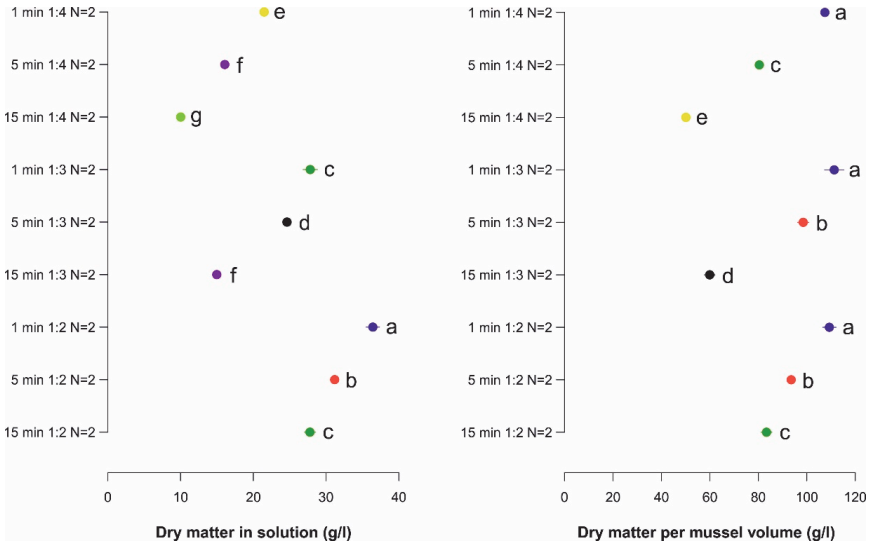

Figure 2. Average dry-matter content with 95% CI (g per liter suspension or g per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

As expected, the higher the concentration of mussels in the solution, the more protein was present in the suspension. The solution containing one part mussels and two parts water contained about two times more protein than the solution containing one part mussels

and four parts of water. The longer the sample was allowed to settle, the less protein was present in the solution, i.e., some of the protein settled to the bottom of the cylinder with the mussel shells. Similar to solid material, on the basis of the mussel volume, the content of proteins was mainly determined by the settling time, with a shorter settling time having higher amounts of solid material (Figure 3).

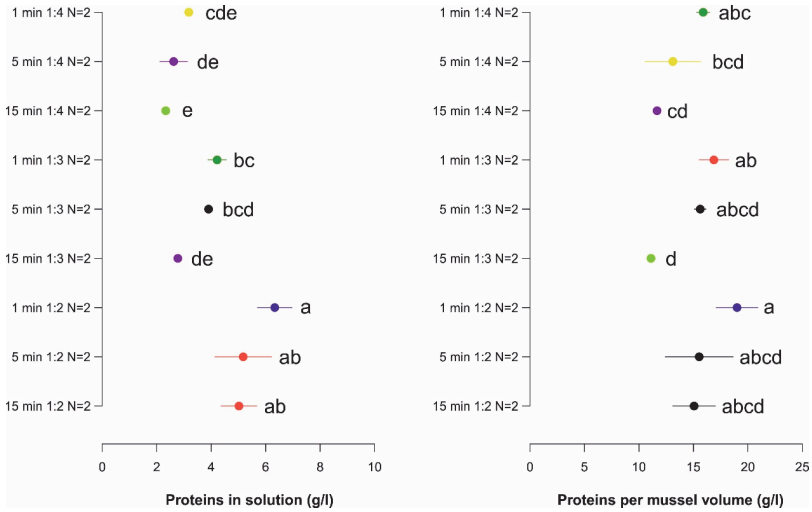

Figure 3. Average protein content with 95% CI (g per liter suspension or g per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

The concentrations of calcium ions were not significantly affected by the ratio of mussels to water. However, samples that settled longer contained less calcium (Figure 4).

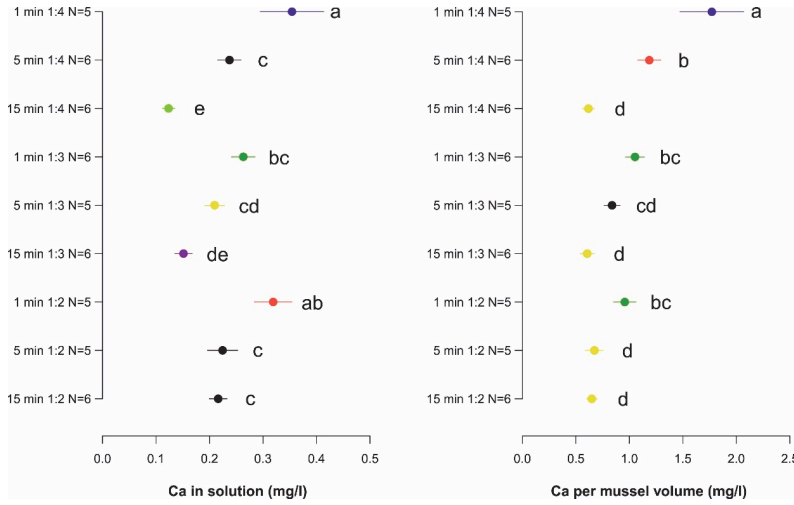

Figure 4. Average calcium ion content with 95% CI (mg per liter suspension or mg per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

The second experiment showed that as a result of filtration, dry-matter yields were significantly reduced at $p < 0.05$. Although filtration had no effect on the percentage share of protein in the dry matter, the filtered sample contained only 30% of the protein yield of the unfiltered sample (Figure 5).

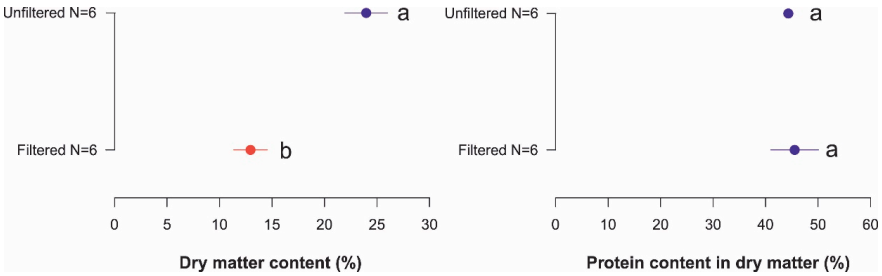

Figure 5. Average content of dry matter and the content of proteins in dry mass with 95% CI (%) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

4. Discussion

Our experiment showed that using conventional methods, it is possible to obtain about 40 g of pure meat from 1 L (290 g) of fresh mussel-mass cultivated in the low-salinity conditions of the Baltic Sea. This is about 2–3 times lower amounts than observed in fully oceanic waters [25]. Moreover, the shells of the Baltic Sea mussels are smaller, and they are much thinner compared to their counterparts in oceans [26,27]; thus, the traditional processes that are used to effectively separate meat from intact mussels in other seas cannot be used in the Baltic Sea region.

Our study also revealed that the separation of meat from fresh or frozen mussels can be achieved by simple means without requiring overly complex and expensive machinery. Simple and industrially scalable technology (i.e., crushing and sedimentation) allowed us to extract a significant amount of meat with a high protein content (i.e., half of the total protein found in the used mussel-mass).

Importantly, the mussels need to be crushed with water. Optionally, the biomass could first be heat treated to minimize foam formation during the homogenization process. The meat is light and sinks slowly in the water column, while the shells sink quickly. This helps to separate a significant amount of the mussel meat through simple sedimentation. However, the technology can be further improved, and thereby the dry matter separation performance can be optimized even further.

The experiments showed that both the ratio of raw mussels to water and the subsequent sedimentation time significantly affect the protein and dry-matter contents of the separated suspension. A 15 min exposure time allows significantly lower calcium concentrations to be achieved compared to 1 and 5 min exposure times. Therefore, 15 min is the preferred time for lower-calcium yields. Lower-calcium yield is often a desired outcome when extracting meat and protein mass, as it increases the quality of extracted products. However, a longer sedimentation time will also reduce the protein content, with an estimated 1.5-fold difference observed between 1 min and 15 min sedimentation times.

Nevertheless, the calcium content of the Baltic Sea mussel shell estimated in the current study was about two times lower than estimated in oceanic waters [25] and this difference is caused by low salinity [28]. Reduced salinity is coupled with lower availability of calcium and inorganic carbon in seawater, which often results in thin, small, and fragile shells of mussels inhabiting the Baltic Sea [29]. When Baltic mussels are moved to more saline environments, they grow larger, indicating that the rate of calcification and maximum shell size depends on the environment [30]. Moreover, one of the most striking features of the Baltic Sea is low predatory pressure on mussels, and this is another reason why

mussels do not have to invest in thicker shells and their morphology differs between the Baltic and higher-salinity seas [31]. Lower calcium content and more fragile shells make the Baltic Sea mussels better material for meat and protein extraction compared to their oceanic counterparts. Even if higher calcium content was measured at shorter exposure times, the concentrations are not too high to cause a significant deterioration in product quality during the industrial extraction of meat and proteins.

It also became clear that the filtration resulted in high losses of the dry matter and protein content of suspension. Averaged over all samples, only 20% of the proteins that were present in the manually separated meat were recovered after filtration. One-third of the protein originated from the settled shell material that partly contains meat. In addition, the residual water from the filtration contained a quarter of the total protein of the sample, with the protein content of the residual water being significantly higher than that of the filtered meat mass. Thus, in the context of the present experiment, filtration makes the process of dry-matter separation more complex, the losses during filtration were high, and the potentially lower energy input during subsequent drying does not compensate for the lost protein. In addition, the filtration process prolongs the protein-extraction process, making it more economically costly. Nevertheless, in the current experiment, we only used one mesh size, and it may be possible that other mesh sizes result in better dry matter and protein yields. However, we believe that even if other mesh sizes are used, filtration still involves considerable losses of dry matter and proteins, along with high energy-consumption and challenges related to mesh clogging.

The amount of protein in the residual water suggests that the solids in the suspension are of a very fine fraction and that a more efficient method of filtration is needed. It is possible to improve the efficiency of the decanting process and thereby increase the number of solids and protein that can be extracted from the crushed meat mass.

To our knowledge, there are no similar valorization experiments in the Baltic Sea region, and therefore we cannot compare our results with other experimental trials. However, the BONUS Optimus project [32] investigated the efficiency of a Super Heat Steam Dryer System as well as grinding and winnowing to separate meat from the mussel mass; but all their experiments resulted in a very poor separation of shells and mussel meat (actual data were not reported). Outside of the Baltic Sea region Naik et al. [25] reported very similar protein content in the processed mussel meat (58.7%) as obtained in the current study (54.8%). Joyner and Spinelli [33] achieved a very high protein yield in their experiment (13.5% of meat wet weight opposing to 6.3% obtained in this study). However, their separation process was overly complex and thereby costs are expected to be very high. Moreover, the extraction of meat using their preprocessing method is difficult to conduct with the Baltic Sea small-sized mussels.

The reason why we had lower protein levels was that the share of free proteins in the raw mussel homogenate was relatively low, and most of the proteins were present in the meat particles. In order to increase the proportion of soluble proteins, chemical and/or enzymatic digestion, optionally combined with ultrasonication or microwave digestion of the meat particles, is necessary [34]. Due to increased/faster digestibility, insulinotropic effect and flavor-enhancing properties, such protein hydrolysates, could be effectively used in specialized food and feed applications. Peptide-rich fractions have been shown to act as prebiotics and exert potential biological activities (e.g., antioxidant, antihypertensive properties), also making them valuable ingredients for cosmetic and pharmaceutical industries [11,35]. In order to obtain high-quality protein hydrolysates, the optimal hydrolysis techniques and conditions for this particular raw material have yet to be determined. Nevertheless, the production of meat hydrolysates by enzyme technology is generally well established and scalable in practice.

It is rewarding to develop new uses of separated mussel meat and the remaining residual material in order to better valorize the Baltic Sea mussel biomass. To date, employing residual meat as an additive for fishmeal and animal feed [36] and separated shell fractions with some protein as a source to produce poultry feed are a few of the most common

industrial valorizations [19,37]. Even though the required industrial technologies are well established, other sustainable options such as emulsions for human consumption or as food-flavoring agents are not quite extended in an industrial context [38]. Moreover, the current processing chains need to be further explored to find commercially feasible solutions. An additional exciting feature of mussel meat is its anti-inflammatory properties, which make mussel protein a suitable component for both fitness and dietary supplements [39,40]. Further research is needed to develop commercial processing solutions from mussel-meat mass to a purified protein powder. Moreover, mussels also contain many components that could be used in the pharmaceutical industry and, if successfully extracted, would add even more value than food components [41].

Shellfish aquaculture is a blue aquaculture with no significant adverse environmental impact [9]. Mussel farming has the potential to remove nutrients that have already accumulated in the Baltic Sea and beyond, as well as to compensate for the pollution emitted by, for example, fish farming [9,42]. In the coming years, an increase in the demand for alternative protein is expected, which mussels will fulfill perfectly. Protein from mussels is a sustainable, blue protein that does not pollute the environment, but improves it. Consumers are becoming more environmentally conscious and food producers are under pressure to use greener technologies and alternative biomass. In order to develop the mussel farming industry in the Baltic Sea region, however, the products need to be valorized, as at present, due to the small size of the Baltic Sea mussels, there is no effective use of shellfish collected from the Baltic mussel farms [3]. In the present work, we developed an extraction method that would be cost effective and also industrially applicable without the use of too-complex processing chains. It is expected that by visually assessing the shell fraction after decanting, even larger amounts of dry matter can be extracted. Importantly, drying techniques also need to be further explored. In the present experiment, the drying time was not quantified, but obviously there are still possibilities for optimization of the process. The method needs to be further developed for upscaling and use; however, due to the simplicity of the method, it is easy to scale it up to meet the needs of industrial applications.

5. Conclusions

The experiments of this study showed that the separation of meat from fresh or frozen small Baltic Sea mussels is feasible by simple means. Simple crushing and sedimentation succeeded in extracting a significant amount of dry matter with high protein content. It also became clear that the use of filtration was not feasible because of the exceptionally high protein loss. In addition, filtration makes the process of dry-matter separation more complex and costly, which is unlikely to be compensated by the energy saved in drying. To confirm this, it would be necessary to determine the exact energy-consumption of the respective processes in the future. The study also suggested that further valorization of both the residual material and the extracted dry matter is needed, e.g., through enzymatic digestion. In order to achieve this goal, it is necessary to identify optimal enzymes or enzyme combinations, hydrolysis durations and processing temperatures to break down this mussel meat and residual material.

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Appendix 2

Publication II

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Unlocking the potential of shellfish biomass: refining protein extraction from Baltic blue mussels for sustainable food applications

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ABSTRACT

Exploiting the underutilised biomass of the Baltic Blue Mussel, *Mytilus edulis* and *Mytilus trossulus* hybrid offers a promising avenue for strengthening the economic potential of aquaculture in the Baltic Sea region. This study describes the optimisation of a novel meat extraction technique to address the unique low salinity challenges of the region. The method involves a sequence of procedures, initiated by thawing and desalting via freshwater rinsing to reduce salt content, followed by mechanical disruption. The decantation stages were carefully designed, including the use of citric acid to neutralise off-flavours, thereby improving the sensory profile of the product—a critical determinant of consumer acceptance. Process refinement resulted in a significant increase in meat extraction from an initial 7.62 to 12.06%, with autumn harvests proving superior in both quantity and sensory quality. Further processing steps, including stirring, boiling, centrifugation, and iterative drying, calibrated the moisture content and produced a highly pure, fine, and homogeneous mussel powder. This comprehensive approach highlights the potential for a scalable, efficient, and economically viable extraction method that could make a significant contribution to the regional seafood industry.

PRACTICAL APPLICATION

The new method developed will allow us to get more meat out of small Baltic blue mussels and reduce the muddy taste. It could make mussel farming more profitable in places where it has been difficult before.

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1. Introduction

Marine ecosystems are facing unprecedented degradation due to multiple stressors, including overfishing (Sumaila et al., 2023), pollution, and climate change. These stressors have led to a decline in the abundance and diversity of species and habitats, threatening the resilience of marine ecosystems and the provision of valuable ecosystem services (Jones et al., 2022; Ojaveer et al., 2010; Viitasalo & Bonsdorff, 2022). To promote the restoration of these habitats, policy initiatives, such as the European Green Deal have identified the promotion of sustainable aquaculture as a key component (Campbell et al., 2021; Overton et al., 2023). In this context, extractive aquaculture, particularly mussel farming, is emerging as a worthwhile approach to achieving these policy objectives. In addition to providing an important source of protein, shellfish aquaculture enhances ecosystem services, such as water quality improvement, sediment stabilisation, and biodiversity conservation, while reducing the impact of other extractive activities, such as commercial fishing. The promotion of sustainable shellfish aquaculture can therefore contribute to the restoration of valuable marine habitats and the achievement of wider policy objectives for the conservation and sustainable use of marine resources (Gren, 2019).

The valorisation of shellfish biomass is intimately linked with environmental sustainability and economic viability, particularly in regions like the Baltic Sea, where eutrophication poses a significant threat to marine ecosystems. The mussel farming initiatives, targeting nutrient remediation, underscore a dual

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benefit—environmental restoration and provision of raw materials for the aquaculture industry (Kotta et al., 2020a, 2020b; Petersen et al., 2014; Suplicy, 2020). Mussels, through biofiltration, sequester nutrients, thus serving as a natural solution to mitigate eutrophication while simultaneously producing biomass for potential economic use (Lindahl, 2011, 2012; Elmgren et al., 1985). However, the reduced size of mussels in the Baltic Sea, attributed to low salinity, necessitates further processing to make them suitable for consumption or other uses. This reduced size primarily affects their marketability, as consumers generally prefer larger, meatier mussels. Additionally, the smaller mussels require more extensive processing to meet market standards, which involves greater effort in cleaning and preparation, thereby reducing their economic viability. While taste and safety are not explicitly mentioned in the literature, the smaller size may also result in different textural qualities that could be perceived as less desirable. Overall, the unsuitability for consumption is mainly due to the reduced size impacting consumer appeal and increasing processing requirements, rather than issues of taste or safety (Bøhle, 1972; Bongiorno et al., 2015; Kautsky, 1982; Maar et al., 2015; Riisgård et al., 2014).

Based on this premise, the necessity of valorisation is not only environmental, but also economic. The current state of underutilisation of shellfish biomass (Cangiotti et al., 2022; Hellen et al., 2019) reflects the urgency to devise methods that can convert this biomass into high-value products in a cost-effective and scalable manner. While some advancements have been made (Adler et al., 2022; Cangiotti et al., 2022; Cunha et al., 2021; Iribarren et al., 2010; Jeong et al., 2021; Schulbach et al., 2013), the gap in upscaling these processes to industrial levels persists. This gap is particularly evident when considering the complex regulatory environment in the EU, which affects the commercialization of technologies for by-product valorisation.

To realise the full economic potential of mussel biomass, the industry must overcome the challenge of integrating scalable, cost-effective technologies with regulatory compliance. Although biotechnological methods, such as enzymatic hydrolysis and pH shift processing show promise in the laboratory for recovering valuable proteins from e.g. crab by-products and bivalves (Mao et al., 2017; Pezeshk et al., 2022; Vareltzis & Undeland, 2012; Zou et al., 2023), their commercial viability remains limited. Bridging the gap between innovation and industrial application could accelerate the transition to a sustainable bioeconomy, ensuring that the mussel farming practices that contribute to the restoration of the Baltic Sea can also support economic growth through the creation of value-added products. This would mark a significant step towards circularity in the aquaculture sector, turning a regional environmental challenge into an opportunity for sustainable development.

Extracting meat from mussel shells is a labour- and energy-intensive process, which has hindered the development of the mussel industry in the Baltic Sea region. In recent years, efforts have been made to address this problem, and various methods have been proposed to facilitate the extraction process (Adler et al., 2022; Cunha et al., 2021). However, these methods have often been limited by economic feasibility as well as their inability to effectively address the problem of shell debris and muddy taste, which can reduce the quality and desirability of the extracted meat.

Traditional mechanical shucking, enzymatic methods, and the integration of automation and robotics represent various strategies for separating shellfish meat from its shell. Mechanical shucking, the conventional approach, relies on physical tools and manual labor. This labor-intensive process can be inefficient, variable, and often leads to high rates of meat damage and wastage (Alizadeh et al., 2007). Additionally, the physical exertion required poses ergonomic risks to workers and results in inconsistencies.

Enzymatic methods offer an alternative by using proteolytic enzymes to weaken the adductor muscles that hold the shell closed, easing meat extraction (Bhat et al., 2018). These enzymes can selectively degrade specific tissue components without affecting meat quality. However, enzymes must be chosen carefully to avoid unwanted breakdown of the meat, and there is a need to optimize reaction conditions and enzyme formulations for each shellfish species (Bhat, Morton, Mason, et al., 2020; Bhat, Morton, Zhang, et al., 2020).

The emergence of automation and robotics in shellfish processing represents a significant advancement, potentially overcoming the drawbacks of mechanical and enzymatic methods. Robotic systems can adapt to different sizes and shapes of shellfish, providing precise and consistent shucking with less meat damage. They also increase processing speed and improve worker safety by reducing manual handling (Rong et al., 2018). Machine learning and computer vision enable these systems to improve continuously, adjusting the shucking mechanism to maximize yield and quality (Singh et al., 2015a, 2015b).

Despite these advances, each method must be evaluated for technical efficacy and economic viability.

High initial investment costs for automated systems can be a barrier for smaller operations, while enzymatic methods add complexity to processing. Integration of these techniques must be tailored to each facility's capabilities and needs, balancing cost, efficiency, and product quality (Banerjee & Maheswarappa, 2019; Singh et al., 2014).

In mussel production, managing and valorizing by-products is crucial economically and environmentally. As of 2016, blue mussels (*Mytilus edulis*) made up 9% of the 2 million tonnes of global mussel production, primarily in Europe (FAO, 2022). Notably, 27% of harvested blue mussels are by-products, including undersized or barnacle-fouled mussels, byssal threads, toxin-laden mussels, and broken shells (Vareltzis & Undeland, 2012). The Baltic blue mussel, often smaller, is considered a by-product due to its low flesh yield, below the commercial threshold of 30% cooked meat yield (Bongiorno et al., 2015).

The perishable nature of mussel by-products necessitates rapid post-harvest processing within 72 h to prevent degradation due to high moisture content, neutral pH, and enzymatic activity (Bhunia et al., 2017; Ovissipour et al., 2013). Studies, like Zhou et al. (2019), highlight significant lipid oxidation within three days at refrigerated temperatures. To preserve the integrity of mussel by-products for high-value applications, careful characterization before processing is essential, using indicators, such as peroxide value (PV), thiobarbituric acid reactive substances (TBARS), and total oxidation (TOTOX) values to assess quality.

Effective utilisation of *M. edulis* by-products requires a comprehensive understanding of their composition and potential for use in various applications. This highlights the need for innovative strategies that not only minimise waste but also add economic value to the by-products of mussel harvesting, thereby contributing to the sustainability of the aquaculture industry.

The current paper builds upon the results presented in a recent study (Adler et al., 2022) and aims to further refine the protein extraction technique from blue mussels grown in the brackish waters of the Baltic Sea. The primary focus was to investigate methods to improve the yield of meat extraction while eliminating the gritty and muddy taste commonly associated with blue mussels. Ultimately, this material could be a potential ingredient for the food industry. Our hypotheses were as follows: (1) Mussels harvested in autumn yield higher meat quality and better sensory attributes compared to those harvested in spring; (2) The optimized processing method significantly increases the yield and quality of mussel powder; (3) The developed method is scalable for industrial applications. To improve scalability, the method needs to use widely available industrial equipment and cost-effective approaches. By presenting a refined meat extraction technique, this work provides an opportunity to add value to blue mussel biomass and ultimately contributes to the sustainable use of this valuable natural resource. Moreover, in contrast to farming the mussels for animal feed or fertilizer (Baltic Blue Growth, 2019; Carlberg et al., 2018; Jönsson & Elwinger, 2009; Nagel et al., 2014; Weiß & Buck, 2017) this study shows promising application for human consumption, that significantly boosts the value of the mussel mass extracted.

2. Materials and methods

2.1. Raw materials used in experimental trials

The blue mussel complex (a hybrid of *M. edulis* and *Mytilus trossulus*) was used for the experiments. The *Mytilus* complex includes three incompletely isolated species of marine mussels, *M. edulis* (Linné, 1758), *M. trossulus* (Gould, 1850), and *Mytilus galloprovincialis* (Lamarck, 1819). The Baltic populations of *Mytilus* spp. were established after the glaciation period during the Holocene and have retained unique characteristics compared to populations from other geographic areas characterized by a high frequency of *M. trossulus* and *M. edulis* genes (Wenne et al., 2020). Baltic blue mussels exhibit several phenotypic adaptations congruent with their brackish habitat, such as reduced size and notably delicate shells. These mussels are characterized by a thinner and more fragile exoskeleton, a trait that has been observed to influence both their survival and utility as a raw material in laboratory trials (Khaitov et al., 2021).

The material for the study was collected from a mussel farm at 0–3 m depth in Tagalaht Bay (58.45644° N, 22.05452° E), the eastern Baltic Sea in May and October 2022. Harvesting was carried out by diving and manual removal of shellfish from the net. Samples were taken from different depths and different parts of the cultivation net to ensure that the samples were representative. At the time of harvest, the mussels had been growing on the nets for one and a half to two years. After harvesting, the mussels were immediately washed with clean seawater, packed in 1 kg batches in plastic bags, labelled, and placed in a freezer at −20 °C where they were stored until the day of laboratory analysis.

2.2. Mussel mass processing

Experimental analyses were carried out at the Laboratory for Food of the Estonian Maritime Academy, Tallinn University of Technology, and at the Laboratory of the School of Natural Sciences and Health of Tallinn University in the autumn of 2022. Mussels harvested in both spring and autumn have undergone a variety of processing methods. The general description of meat separation is shown in Figure 1 and Table 1.

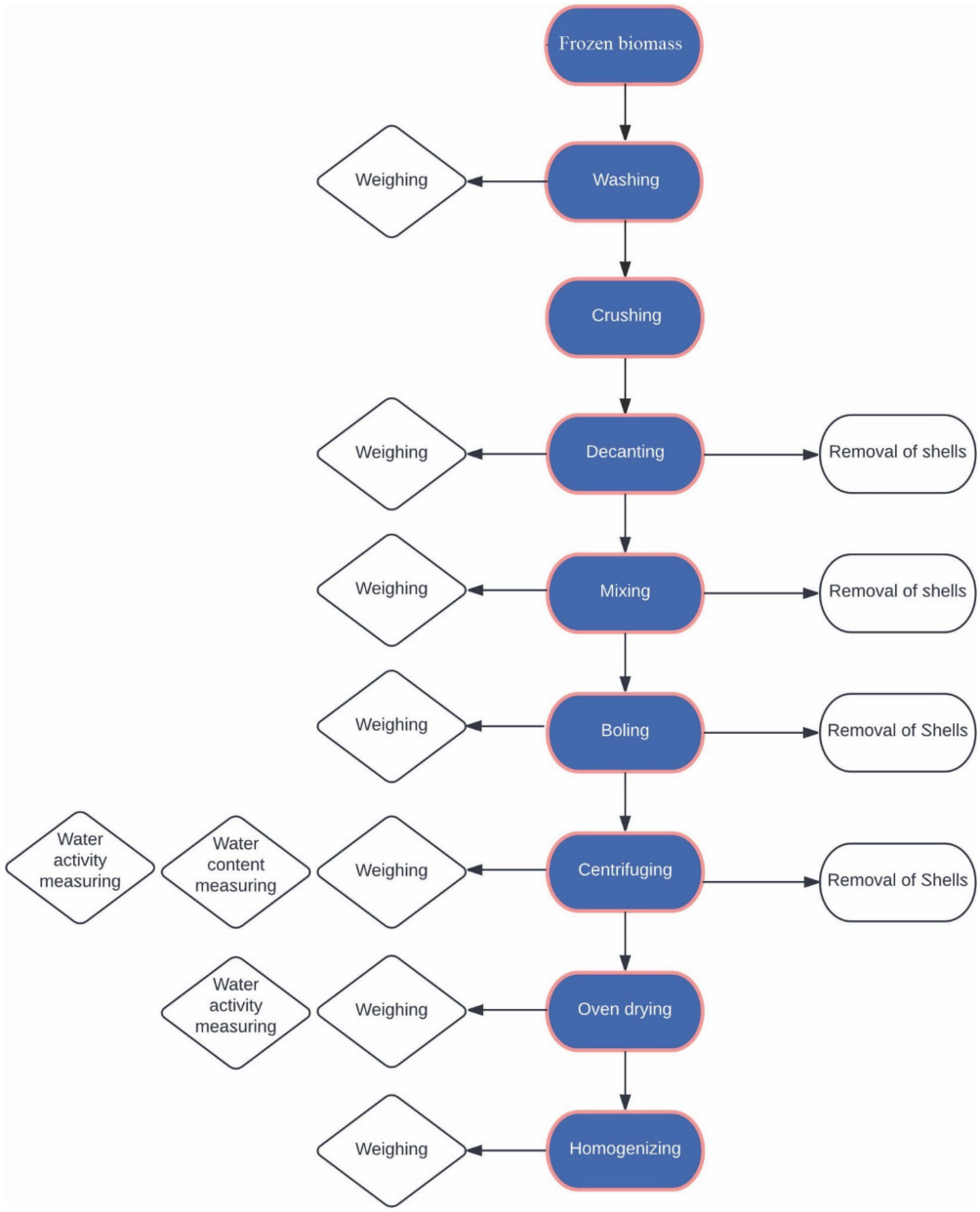

Figure 1. Schematic diagram of the meat extraction and refinement process.

Table 1. General description of processing methods.

Processes	I	II	III
Mixing	Kitchen aid 1 h	Kitchen aid 1 h	Kitchen aid 1 h
Boiling	15 min	15 min	15 min
Centrifugation	4500 rpm, 10 min	4500 rpm, 10 min	4500 rpm, 10 min
First drying	100 °C, 30 min	100 °C, 60 min	100 °C, 105 min
Second drying	100 °C, 45 min	100 °C, 30 min	None

In preparation for the experimental protocol, mussel samples were first thawed and then rinsed with fresh water to reduce the salt content. Subsequent mechanical crushing was facilitated by the use of a KitchenAid Artisan meat grinder operating at 1200 W, fitted with a coarse grinding plate to process the mussels within 2 min. The decantation process began with the gradual addition of deionised water at a rate of 250 ml per 100 g of shell mass, carried out in three incremental stages to allow the sedimentation of shell fragments and the collection of the liquid meat fraction.

A preliminary sensory evaluation (see next subsection for more details) revealed an undesirable earthy taste in the product, necessitating the use of citric acid, which is recognised in the literature for its efficacy in neutralising off-flavours in aquaculture products, such as catfish (Forrester et al., 2002). In the second decantation phase, a 4% citric acid solution was added in two sequences to reach a final concentration of 2% in the meat suspension. Due to the difference in density, the shells precipitated rapidly and the sedimentation intervals were reduced to a few seconds.

After sedimentation, the liquid meat mixture was mechanically stirred for 60 min using a KitchenAid blender to achieve homogeneity and facilitate further detritus separation, giving the resulting product a visibly lighter colour compared to non-agitated samples. Subsequent thermal treatment at boiling temperatures for 15 min was essential to coagulate the proteinaceous particles and optimise yield.

Centrifugal separation was then applied at 4500 rpm for 10 min. The supernatant was discarded, and the precipitate was distributed onto baking paper and subjected to convective heat in Rational CombiMaster Plus Mod. CMP 101 at 100 °C for durations of 30, 60, or 105 min, with select samples undergoing a secondary drying phase. These iterative drying conditions aimed to calibrate the moisture content of the dried mass to an optimal level of 10%. The dehydrated mass underwent a final comminution in a standard coffee grinder for 15 s, resulting in a fine, homogenous powder.

To increase the purity of the final product, additional cycles of mixing, boiling, and decanting were incorporated to remove residual shell fragments, thus refining the final product for sensory analysis (see next subsection for more details).

2.3. Evaluation of smell and taste

Specific odor and taste parameters were assessed by sensory analysis (Oliveira et al., 2015) using six independent panelists, all of whom had the ability to taste and smell. These panelists had undergone thorough general descriptive analysis panel training with a variety of food products, ensuring that they could adequately describe the products. The following parameters were assessed: sea, shellfish, mud odor and sea, shellfish, mud, sweet, sour, bitter, and umami taste.

To assess the sea flavor of the mussel powder, 1 g of mussel powder was mixed and soaked in 50 ml of cold water for 24 h. This method was used to efficiently extract the unique flavor and aroma of mussels specifically for sensory evaluation. The resulting mussel powder infusion was then served in a sniffing glass by adding 15 ml of the infusion with a measuring spoon.

The muddy odor and taste were benchmarked using beetroot cubes. For this, 2 g of beetroot cubes cut from a raw, unpeeled, and uncooked beetroot were placed in a sniffing glass. This unconventional approach of using beetroot cubes as a standard reference allowed the panelists to accurately assess the presence and intensity of the muddy odor and taste in the mussel powder samples.

For the other parameters, the samples were prepared by dissolving 1 g of dried mussel powder in 100 ml of water at room temperature. To ensure proper flavor development, the resulting mixture was seasoned and refrigerated for 24 h. Care was taken to ensure that each evaluator received both a ground chew and a liquid sample for evaluation. The samples were then poured into 30 ml sealable tubes and coded with three-digit codes to maintain blinding during the tasting sessions and ensure objective evaluations. Each tasting session took ~1 h to complete.

During the sensory analysis, tap water, Carr's biscuits, and Conference pear slices were made available to the panelists. The purpose of these palate cleansers was to neutralize the taste buds between samples of different mussel powder products, to ensure a consistent sensory experience, and to avoid any flavor carry-over that might affect the panelists' scores.

A scoring scale of 0–5 was used to rate the samples, with 0 representing the absence of the

characteristic and 5 representing a very strong characteristic. The evaluators scored the samples according to the following criteria: 0 = no characteristic, 1 = very weak characteristic, 2 = weak characteristic, 3 = medium characteristic, 4 = strong characteristic, and 5 = very strong characteristic. The scale also allowed for 0.5 increments to increase the accuracy of the measurements. The ratings were made in six repetitions and in a random order to avoid bias, with the intensity of a trait being rated by the trained panelists. The small panel of only six members was justified as these panelists were highly trained.

2.4. Mussel powder characterisation

For the determination of total protein in mussel powder, the dry sample was hydrolyzed with 6 M HCl solution in sealed pressure tubes at 120 °C for 15 h. The hydrolyzed sample was then dried at 95 °C under a nitrogen atmosphere, dissolved in water, and the total protein content was estimated using the Bradford reagent (Sigma, B6916) according to the manufacturer's instructions.

The lipid content was determined in freeze-dried and cryogenically ground samples of mussel flesh or flesh extracts using a chloroform:methanol (2:1, v/v) mixture for extraction. Approximately 0.3 g of homogenized sample was placed in a glass test tube to which 1 mL of methanol and 2 mL of chloroform were added. The suspension was shaken vigorously for 90 s and then incubated in an ultrasonic bath at 40 °C for 30 min. Then 1.25 mL of aqueous 2% NaCl solution and 1.25 mL of chloroform were added and shaken vigorously. The sample was centrifuged at 1700 × *g* for 20 min and the chloroform layer was transferred to a pre-weighed glass extraction tube using a glass Pasteur pipette. Organic solvents were removed by drying in a stream of nitrogen, and the remaining mass of the sample (mussel oil) was expressed as the percentage of lipids relative to the original freeze-dried material.

Glycogen content was measured colorimetrically by determining the total sugars using the phenol-sulphuric acid method. This involved hydrolysing the sample in a hot 30% KOH solution, following the method described by Rasouli et al. (2015).

2.5. Statistical analysis

The results of different processing methods to extract mussel meat mass were analyzed using analysis of variance. Two separate analyses were used one to test the effect of processing method on meat yield, shell residues, and water content, and another to test the effect of harvest season on meat yield, shell residues, and water content. Tukey's post-test was used to compare the effect of pairwise factor levels. The significance level was set at 0.05.

We analyzed the rankings of the sensory analyses (which were on a sufficiently fine scale) as continuous variables using linear mixed models. The rater was included as a random factor. Data analysis was performed using the statistical software R (R Foundation for Statistical Computing, 2023).

This study does not require ethical approval. This study was conducted in strict accordance with the ARRIVE guidelines. The ARRIVE guidelines were developed to improve the reporting of research involving animals, ensuring that the data is comprehensive, transparent, and reproducible.

3. Results

3.1. Mussel mass processing

Different processing methods to extract mussel powder resulted in statistically significant mass ($p = 0.008$) and shell residue yields (ANOVA, $p = 0.022$), whereas water content in mussel powder did not vary significantly between processing methods ($p > 0.05$). Processing methods with longer first drying resulted in a significantly higher mussel powder yield of 12%. Shell residue was measured at 22–39% with significantly lower yields when the first drying time was moderate (Figure 2).

There was no significant seasonal difference in mussel powder and shell residue yields (ANOVA, $p > 0.05$), but the water content in mussel powder was significantly higher in spring ($p < 0.001$), estimated at 19% (Figure 3).

3.2. Evaluation of smell and taste

When the different processing methods were compared using samples taken in the autumn, there were no statistically significant differences in the tasting results (Linear Mixed-Effects Model, $p > 0.05$). All odor and taste characteristics were found to be favorable for the food processing industry, with most scores of 1 or less. Only the smell and taste of the sea and shells achieved a higher score of 2–3.

Sea and mud odor and shellfish, mud, and sour taste were significantly different between seasons, with higher scores (i.e. unfavorable for the food industry) in spring (Linear Mixed-Effects Model, $p < 0.001$) (Figures 4 and 5). This variation is

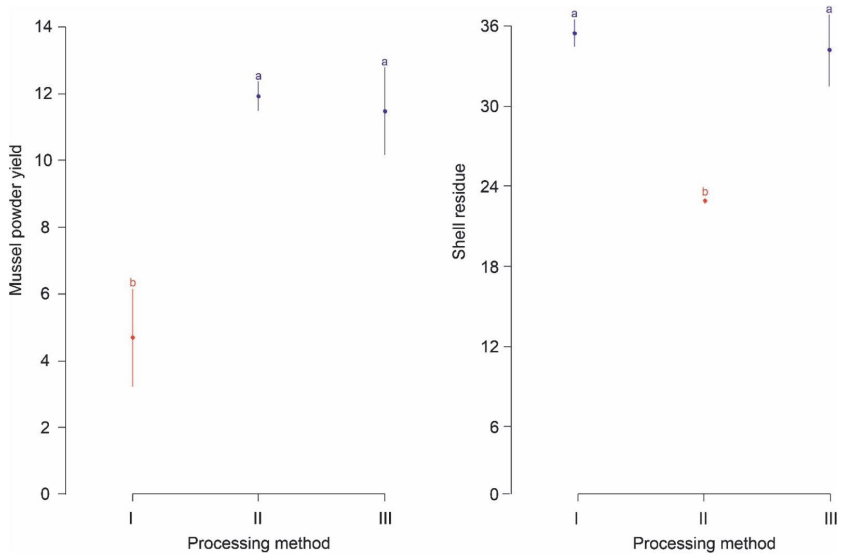

Figure 2. Average mussel powder yield and shell residue with SE (%, wet mass) between different processing methods. Means with different letters are significantly different at $p < 0.05$. The number of replicates was three. The different processing methods are detailed in Table 1.

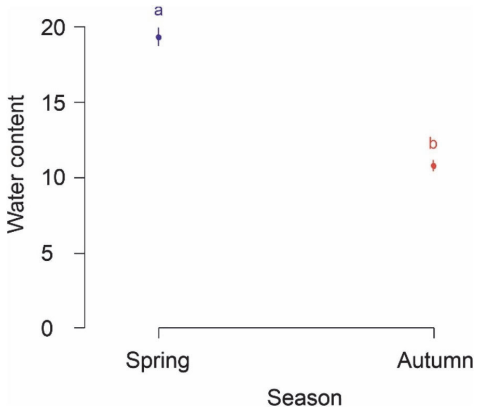

Figure 3. Average water content in mussel powder with SE (%) between different seasons. Means with different letters are significantly different at $p < 0.05$. The number of replicates was three.

independent of the product's shelf life, which was the same for all samples. The difference in taste is attributed to seasonal factors.

3.3. Mussel powder characterisation

The extracted mussel powder was analyzed for its nutritional content, revealing significant differences between autumn and spring harvests. The protein content was found to be 47% in autumn and 49% in spring. Lipid content varied more significantly, with 7% in autumn and 22% in spring. Additionally, glycogen content was 16% in autumn and 22% in spring. We also examined the properties of mussel powder, including solubility, emulsifying capacity, water-holding capacity, and gel formation ability. Preliminary tests indicated that the powder has good solubility and emulsifying properties, which are critical for its incorporation into various food products.

4. Discussion

This study presents an innovative approach to the valorization of mussel biomass using simple equipment that can be easily set up and scaled up for industrial production. Through a series of experiments and analyses, the results demonstrate the feasibility of this approach and its potential for commercialization. The processes and equipment used in this study are readily available, making them an attractive option for industries looking to add value to their mussel waste streams. This suggests that this study provides a promising pathway for the sustainable valorization of mussel biomass that can benefit both the environment and the economy. Although we did not quantify costs in the current study, as it is beyond the scope, the significance of this process lies in its likely cost-effectiveness and its

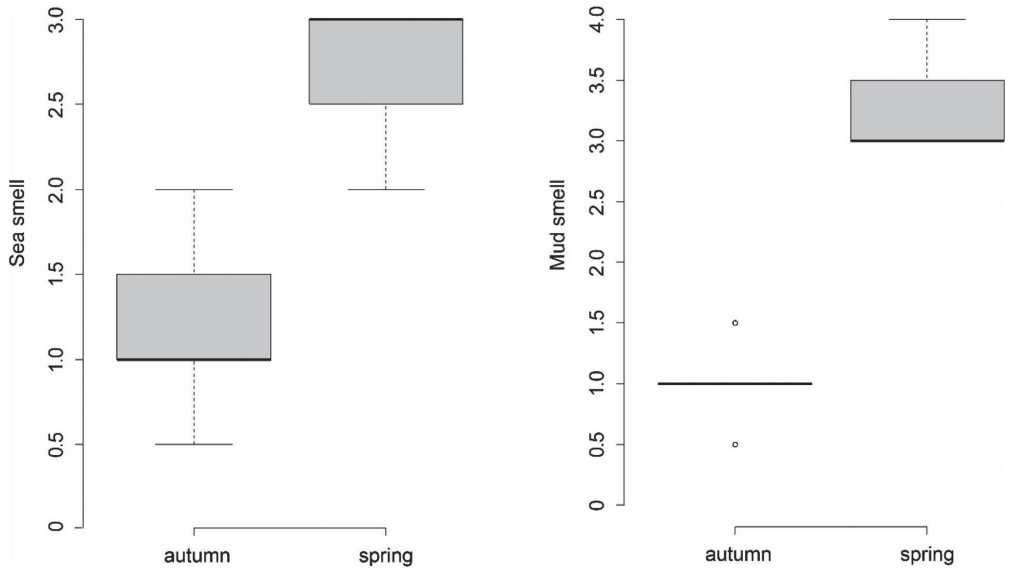

Figure 4. Average score in sea and mud odor with quartiles, minimum, and maximum between different seasons.

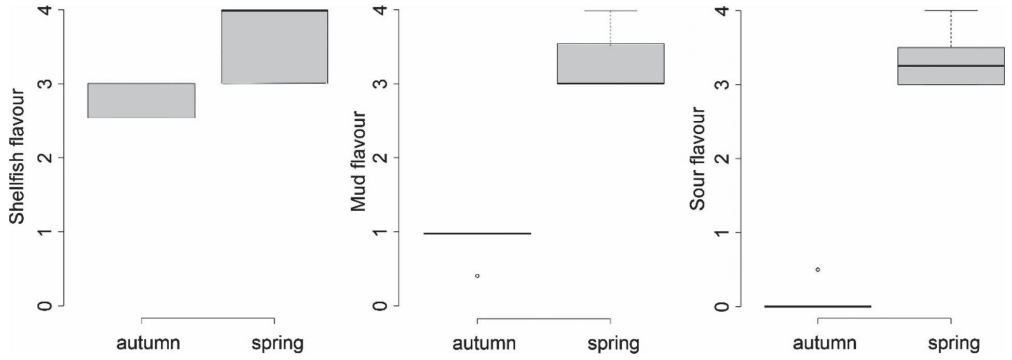

Figure 5. Average score of the taste of the shellfish, mud, and sour with quartiles, minimum, and maximum between the different seasons.

use of widely available industrial equipment. Moreover, our approach is simpler and uses significantly less energy compared to existing processes that use steam to extract meat from the shell.

Our experiment showed that using conventional methods, it is possible to obtain about 49 g of pure meat from 100 g of fresh mussels grown in the low salinity conditions of the Baltic Sea. In addition, the process extracted 44 g of wet shell. We used 0.5 liter of water for every 100 g of fresh mussels and ended up with 357 g of water at the end of the process. Therefore, 49% of the wet mass is meat, 44% is shell and the remaining 7% of the wet mass remains in the excess water. In addition, an average of 9.5 g of dried and homogenized meat powder was obtained, giving an average yield of 9.5% of the fresh mussel wet weight.

The biological condition and behaviour of mussels, like many other organisms, are influenced by seasonal effects (Krešic et al., 2020). Similarly, in the Baltic Sea region, blue mussels are typically in better condition in autumn than in spring, when they may have experienced periods of starvation due to limited food resources during the winter (Bongiorno et al., 2015; Hirabayasi et al., 2021). Depending on the region and local climatic conditions, spring may

be a time when mussels are hungry or nutrient deficient, especially after a long winter. This can affect their growth, reproduction, and vitality. Interestingly, seasonal variability also played a significant role in the odor and taste characteristics of mussel products, with significantly better products obtained from the autumn harvest. This suggests that it may be advisable to harvest mussels in autumn for better results. During autumn, blue mussels may be in better condition as they have had sufficient time during the summer to feed, grow, and accumulate energy reserves. Autumn may also be a period when blue mussels are more active, with increased movement, which can affect the development of their muscles and consequently their scent and flavor attributes (Hirabayasi et al., 2021).

However, it is important to note that the scent and flavor attributes of blue mussels may depend on various factors, including their habitat, diet, harvesting methods, storage, and cooking techniques (e.g. Gallardi et al., 2014; Xin et al., 2022). While blue mussels harvested in autumn may be in better condition, blue mussels collected during other seasons can also possess good scent and flavor attributes if properly handled and prepared. Therefore, seasonal variability may impact the scent and flavor attributes of blue mussel products, but it is important to consider other factors as well and follow proper handling and cooking practices to ensure high-quality blue mussel products regardless of the season.

Similar to mussel powder yield, the scent, flavor, and the content of protein, lipid, and glycogen in mussels exhibited strong seasonal variability. These variations highlight the influence of seasonal factors on the nutritional profile of mussel powder. To ensure the viability of the extracted mussel powder for human consumption, it is essential to evaluate its physical and functional properties. The analyses showed that the powder has good solubility and emulsifying properties, which are critical for its incorporation into various food products. Taking these results into consideration, mussel protein can be used in human food products. The high protein content, combined with favorable lipid and glycogen levels, makes mussel powder an excellent ingredient for nutritional supplements. For example, incorporating mussel protein into protein bars provides a marine-based substance that enhances the nutritional value of the product. These bars offer a unique source of protein and other nutrients essential for athletic performance and recovery.

While the novel extraction method for Baltic blue mussels is promising, the limitations of the current manuscript in characterising the extracted powder need to be addressed. Detailed analysis of protein, lipid, and glycogen content, together with evaluation of physical and functional properties, is required to fully validate the method. In addition, demonstration of the practical application of mussel protein in human food products, such as protein bars, may significantly enhance the credibility of the method and its potential for commercialisation.

Several studies have investigated the impact of environmental factors, such as water temperature and wet storage, on the quality and biochemical composition of blue mussels. Warmer water temperatures during winter negatively affect the soft body mass of Baltic blue mussels (Waldeck & Larsson, 2013), while seasonal variations in yield, composition, and sensory quality of steamed blue mussel meats are influenced by the populations examined and the time of harvest (Slabyj et al., 1978). Wet storage has also been shown to affect the biochemical composition of mussels, resulting in changes in lipid and glycogen content over the holding period during fall and spring seasons, with a progressive loss of dry tissue weight and an increase in water content (Gallardi et al., 2014). Nevertheless, sensory evaluation did not reveal a significant difference in palatability between held and freshly harvested mussels, indicating strong regional differences.

In comparison, the current study was focused on optimizing a processing method for the extraction of mussel meat from shells using small mussels cultivated in the low salinity region of the Baltic Sea. The results demonstrated that the yield of meat was significantly higher for mussels harvested in the autumn and by adding additional steps to the processing method, the yield was significantly increased. However, a moderate yield of protein was observed in the final powdered product. While the process produces high-quality mussel powder, commercial viability is challenged by the need for specialized equipment, high labor and energy costs, and strict quality control measures. Optimization and streamlining of the process, coupled with a thorough market analysis and cost-benefit assessment, are essential for commercial application. These results highlight the complex, multifaceted nature of mussel quality and the importance of site-specific approaches to optimise harvesting and processing methods. Such optimisation is essential to maximise mussel meat yield and quality, thereby supporting its potential cost-effectiveness and scalability for industrial use.

5. Conclusion

The study aimed to refine protein extraction techniques for mussels in the low salinity conditions of the Baltic Sea, producing smaller mussels that require additional processing to be suitable for consumption and to meet market standards. In this study, we have developed a novel approach to extracting mussel meat from shells in an industrially scalable manner that effectively addresses the issue of shell debris and muddy taste. Our work confirmed all the initial hypotheses. The results showed that mussels harvested in the autumn produced higher quality meat and better sensory characteristics. This suggests that the mussels have had more time to feed and build up energy reserves by autumn, resulting in a better overall condition. The results also showed that an optimised processing method significantly increased the yield and quality of mussel powder. The iterative decantation and sedimentation processes, although time-consuming, effectively separated the liquid meat fraction from the shell fragments. The use of citric acid for flavour correction improved product quality, although it increased material costs. Finally, the developed process is potentially cost-effective and scalable for industrial applications. However, several challenges need to be addressed. The process involves high labour and energy costs due to steps, such as mechanical stirring, boiling, centrifugation, and multiple drying cycles. These steps require specialised equipment and careful monitoring to ensure consistent product quality, which could affect the economics and scalability of the process. In conclusion, while the process produces high quality mussel powder, its commercial viability is challenged by the need for specialised equipment, high labour and energy costs, and strict quality control measures. Optimisation and streamlining of the process, coupled with a thorough market analysis and cost-benefit assessment, are essential for commercial application.

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Author contributions

Substantial contributions to the conception or design of the work, I.A. and J.K.; the acquisition, analysis, or interpretation of data for the work, I.A., J.K., R.T., H.O.-K.; drafting the work and reviewing it critically for important intellectual content, I.A., J.K., R.T., H.O.-K.; visualization, J.K., I.A.; supervision, J.K., R.T.; project administration, J.K.; funding acquisition, J.K. All authors have read and agreed to the published version of the manuscript. All authors also agree to take responsibility for all aspects of the work to ensure that questions about the accuracy or integrity of any part of the work are properly investigated and resolved.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data supporting the findings of this study are available in the paper.

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Appendix 3

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Valorization of Baltic Sea farmed blue mussels: Chemical profiling and prebiotic potential for nutraceutical and functional food development

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ABSTRACT

The severe eutrophication of the Baltic Sea requires mussel (*Mytilus* spp.) farming to remove nutrients, but farming in a low salinity environment results in smaller mussels that require value enhancement to be economically viable. This study evaluates the biomass valorisation of smaller Baltic mussels, focusing on the extraction of oil, protein and glycogen. It analyses the amino acid profiles, oil and fatty acid contents and glycogen levels of the mussels, as well as their prebiotic properties on beneficial gut bacteria. In addition, the study improves the extraction of bioactive compounds through enzymatic hydrolysis. Results indicate significant seasonal differences, with summer mussels having higher meat and lower ash content, and a rich content of essential fatty acids, particularly omega-3, and amino acids, underscoring the mussels' sustainability as a food source. The enzymatically treated biomass exhibited notable prebiotic activity, proposing health-promoting benefits. The study underscores the valorization of Baltic mussel biomass, highlighting its role in health, nutrition, and environmental sustainability.

1. Introduction

Marine ecosystems boast a vast diversity of species, many of which are currently used as sources of protein and industrial raw materials. However, <10% of marine bioresources are utilized for human food and other purposes (Pauly, 2007). The exploration of marine bioresources as sources of functional food, feed, cosmetics, pharmaceuticals, and biomedical research is on the rise (Rotter et al., 2021).

Mussels are no exception; they are not only valuable for human consumption but also serve as a promising source of essential nutrients for shrimp and possess excellent chemo-attractant properties for fish. Furthermore, mussels have been identified as commercially significant, new, and potential biomaterial resources. Their polysaccharides, enzymes, peptides, lipids, and biominerals have various applications in the biomedical field, including hard and soft tissue engineering, bioadhesives, dental biomaterials, and drug and cell delivery systems (Eroldoğan et al., 2023; Rotter et al., 2021).

In the Baltic Sea, the cultivation of mussels, in particular *Mytilus edulis*, *Mytilus trossulus* and their hybrids, presents a unique combination of challenges and opportunities. Despite years of attempts to control nutrient inputs, the Baltic Sea continues to suffer from severe eutrophication. This persistent problem is attributed to the accumulation of legacy nutrients (Andersen et al., 2017). In this context, low trophic aquaculture is emerging as a promising solution to effectively remove these excess nutrients from the ecosystem (Kotta et al., 2020). However, due to the low salinity of the region, these bivalves are smaller than their oceanic counterparts and therefore require a thorough biomass valorization strategy for sustainable business viability (Adler et al., 2022; Petersen and Stybel, 2022). The smaller size of these mussels, while a challenge, also presents an opportunity to develop innovative processing methods that can fully exploit the entire biomass for food, feed, and other purposes, thereby contributing to the economic viability and ecological sustainability of mussel farming in this region (Kotta et al., 2020; Maar et al., 2023). However, this must be done alongside efforts to change human attitudes. Currently, mussels are relatively unpopular as a mass-market food compared to other meat items, limiting our ability to

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achieve their potential environmental and health benefits. Increased publicity that takes into account regional and cultural differences in attitudes, emphasizing the health and environmental benefits of mussel meat, is essential. Additionally, industry engagement to develop a diverse range of appealing, affordable, and convenient mussel products is crucial for driving growth in the bivalve sector in the Baltic Sea region and beyond (Gawel et al., 2023). Furthermore, economic incentives, such as government subsidies and grants for sustainable aquaculture, are likely to encourage Baltic farmers to adopt sustainable practices (Sokołowski et al., 2022).

Previous research has shown that these mussels have a diverse and rich nutrient profile in the Baltic Sea and beyond (Azpeitia et al., 2016; Kube et al., 2007; Oliveira et al., 2015). They are rich in essential fatty acids, high-quality proteins, and glycogen, which provide a significant nutritional advantage. Notably, they contain omega-3 fatty acids, including EPA and DHA, which are widely recognized for their health benefits, such as reducing inflammation and providing cardioprotective effects (Calder, 2015; Jeromson et al., 2015; Zanetti et al., 2015). Additionally, the protein content in these mussels includes all essential amino acids, making them an even more valuable source of sustainable and premium nutrition that is vital for human health (De Swaan & Wijsman, 1976; Wu, 2009; Şereflişan & Altun, 2018).

In addition to their historical use in various culinary traditions, mussels are now being explored as a sustainable source of nutraceuticals and functional foods. This shift goes beyond basic nutrition and highlights their potential in the nutraceutical industry (FAO, 2018; Granato et al., 2023). This tendency has been significantly influenced by technological advancements in bioprocessing, particularly enzymatic hydrolysis. Techniques such as the use of specific enzymes, like subtilisin, have been essential in improving the extraction and refinement of bioactive compounds from shellfish biomass. This process has led to the breakdown of proteins into bioactive peptides and amino acids. Mussel-derived bioactive components are recognized for their various health-promoting effects, such as antihypertensive, antioxidative, and antimicrobial properties (Naik et al., 2020). This expands the potential of mussel-derived products in health and nutrition (Gupta et al., 2002; Harnedy & FitzGerald, 2012; Jin et al., 2016; Kim & Wijesekara, 2010; Nag et al., 2022).

Intestinal microbiota, particularly *Lactobacillus* and *Bifidobacterium* species, enhance gut health by producing short-chain fatty acids (van den Berg et al., 2021) and inhibiting the growth of pathogenic bacteria such as *Escherichia*, *Shigella*, and *Streptococcus* species (Dominguez-Bello et al., 2019). Prebiotics, including various polysaccharides like inulin, remain undigested in the small intestine and promote gut health by stimulating the growth of probiotic microbes. They also suppress the growth of pathogenic bacteria through antagonistic activity and modulate both pro-inflammatory and anti-inflammatory responses (Saltzman et al., 2017). Extracts from various mussel species, including proteins, peptides, amino acids, lipids, and polysaccharides, have demonstrated antibacterial and prebiotic activities, as well as antioxidant, antihypertensive, and anticoagulant properties (Saltzman et al., 2017). Peptides may also influence the growth and diversity of beneficial gut microbes, functioning as prebiotics (Maury, 2018). The novel prebiotic effect of the mussel *Perna canaliculus* has been reported, potentially related to glucosamine or similar compounds (Coulson et al., 2013; Siriarchavatana, 2021). Similarly, cysteine-rich antimicrobial peptides isolated from the blue mussel have shown strong bactericidal activity against both Gram-positive and Gram-negative bacteria (Charlet et al., 1996). Thus, advances in this field not only improve the quality of extracts, but also open up new avenues for their use in various health-promoting products (Durazzo et al., 2022).

Based on Adler et al. (2022), the current study aims to delineate the chemical composition of Baltic blue mussel biomass, focusing on the extraction, characterization, and prebiotic potential evaluation of oils, proteins, and glycogen. It further assesses the prebiotic efficacy of these mussel-derived fractions against other marine biomass, such as fish and algae, noted for their bioactive properties (Holdt & Kraan, 2011; Lordan et al., 2011; Venugopal, 2011). This research integrates advanced biotechnological methods with traditional fractionation techniques to underscore the value of the Baltic blue mussel as a sustainable health industry ingredient, contributing to novel health-promoting product development. Through a comprehensive analysis, encompassing the extraction, characterization, and evaluation of various bioactive compounds from mussel biomass, the study aims to advance our understanding of mussel biomass valorization, potentially revolutionizing nutraceutical and functional food innovations and highlighting the sustainability and versatility of blue mussels as a significant resource in health-related industries.

When designing the study, we had the following expectations: (1) We anticipate significant seasonal differences in the meat, fatty acids, and amino acids of harvested mussels; (2) Enzymatic hydrolysis with subtilisin increases the extraction yield of essential amino acids from Baltic mussel biomass, thereby enhancing its prebiotic effects and nutritional value; (3) The valorization process of Baltic mussel biomass through specific biochemical techniques can significantly enhance its nutritional, health-promoting and commercial value.

2. Materials and methods

2.1. Origin of mussels

The mussel biomass for this study was sourced from a mussel farm located in Tagalaht (58.456° N, 22.055° E) in the Baltic Sea, harvested at depths ranging from 0 to 3 m. The mussels were collected on four occasions: 23 September 2020, 23 November 2021, 30 July 2022 and 26 October 2022. Mussels were not harvested during the spring months, as this season is known for their poor condition. This is evidenced by their protein content, which peaks during autumn and winter and declines in the spring (Wołowicz et al., 2006). The age of the mussels at the time of collection was 1–1.5 years. The collected biomass was wet packed in 1 L portions in plastic bags and stored at −80 °C until laboratory analysis.

2.2. Biomass processing and fractionation

2.2.1. General processing schemes

The methods used to obtain different fractions from the mussel biomass are illustrated in Fig. 1, while Table 1 shows the fractions obtained, each characterized by its unique chemical composition. The processes involved soaking the frozen biomass in equal mass of water until completely thawed, removing most of the liquid part by draining, resulting in the sample labeled ‘M-LIQ’ after freeze-drying. Subsequently, an equal mass of water was added to the solid part, and the mixture was homogenized using a Philips ProBlend 6 3D blender at maximum speed for 3 min. This yielded the homogenized mussel slurry, utilized in further steps, with the exception of the meat separation process.

2.2.2. Meat separation process

Freeze-drying makes it easy to separate the mussel flesh from the shell, unlike high temperature drying, which makes it difficult to separate the flesh. During the meat separation process, barnacles, algae and empty shells were removed from the freeze-dried biomass to ensure a selection of pure mussels. Opened mussels were also excluded to avoid loss of dry matter from the meat during the freeze-drying process. After drying, approximately 70 mussels were carefully opened using a thin spatula and the dried flesh adhering to the shell was removed using the same instrument. Both the meat and the shell were then ground independently in a water-cooled laboratory mill (IKA A10 basic, Germany) to avoid heating the sample. This flesh separation technique was similarly applied to approximately 140 barnacles. This study included data on barnacles, as these organisms grow on mussel shells and inevitably become part of the mussel biomass to some extent, thereby affecting the

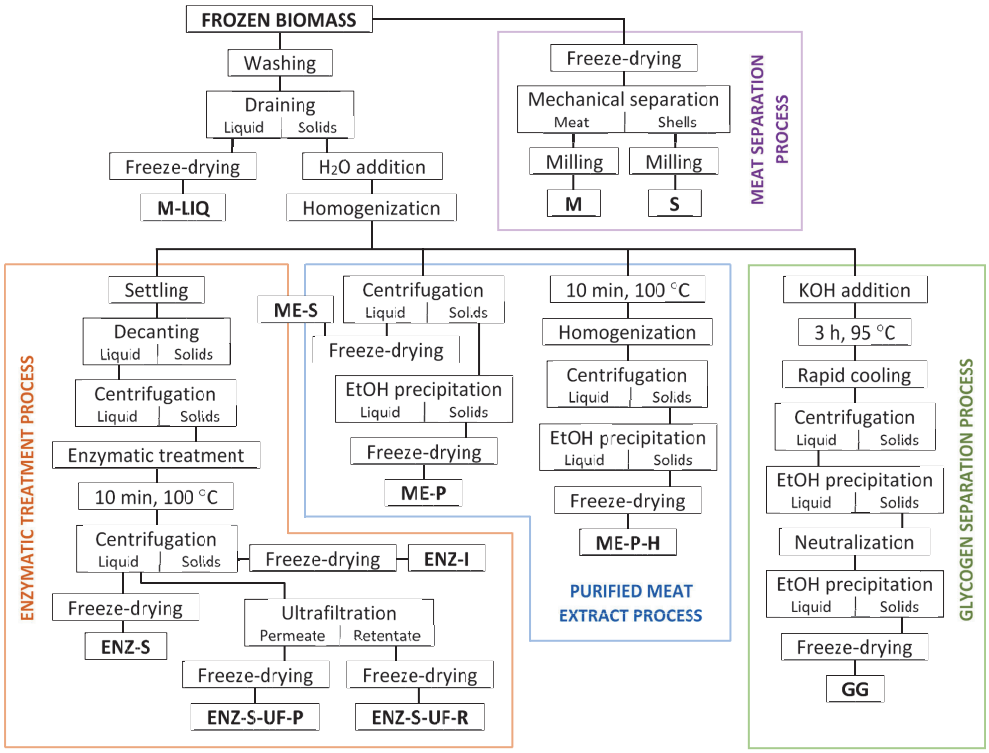

Fig. 1. Schematic overview of the methods employed to derive diverse fractions from blue mussel biomass. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Fractions derived from processing the biomass of the blue mussel farm, with numbers following letters indicating the timing of biomass collection.

Abbreviation(s)	Description	Mussel collection time
M-1, M-2, M-3, M-4	Mechanically separated mussel meat	Sept 2020, Nov 2021, July 2022, Oct 2022
B-2, B-3	Mechanically separated barnacle meat	Nov 2021, July 2022
S-1, S-2, S-3, S-4	Mechanically separated mussel shells	Sept 2020, Nov 2021, July 2022, Oct 2022
M-LIQ	Substances from thawed water released from mussel biomass	July 2022
ME-S	Soluble part of homogenized mussel slurry	July 2022
ME-P	Purified mussel meat extract using a room-temperature process	July 2022
ME-P-H	Purified mussel meat extract using a high-temperature process	July 2022
GG	Glycogen-rich fraction from mussel biomass	July 2022
ENZ-S	Soluble part of subtilisin-treated mussel meat	July 2022
ENZ-I	Insoluble part of subtilisin-treated mussel meat	July 2022
ENZ-S-UF-P	Ultrafiltration permeate of soluble part of subtilisin-treated mussel meat	July 2022
ENZ-S-UF-R	Ultrafiltration retentate of soluble part of subtilisin-treated mussel meat	July 2022

chemical composition of the resulting products.

2.2.3. *Purified meat extract process*

The purification of the mussel flesh fractions by the removal of less hydrophilic compounds and low molecular weight substances was achieved by an alcohol precipitation process. For this the homogenized mussel slurry was centrifuged at 12000*g* for 10 min at 4 °C to separate the supernatant (sample after freeze-drying as 'ME-S') from the residue. An equal volume of 96% ethanol was added to the residue, mixed and kept for 24 h at 4 °C. This precipitate was then separated and freeze-dried to obtain fraction 'ME-P'.

Similar procedure was employed to obtain the heat-treated purified meat extract. This was achieved by initially treating the homogenized mussel slurry for 10 min in a boiling water bath, followed by additional homogenization. The sequential centrifugation and ethanol precipitation steps were as previously described, resulting in the fraction 'ME-P-H' after freeze-drying.

2.2.4. *Enzymatic treatment process*

The homogenized mussel slurry was allowed to settle briefly, after which the upper flesh-rich part was decanted and the crushed shells were discarded. The decanted part was then centrifuged at 4500*g* for 5 min at 20 °C, the obtained precipitate was diluted with equal mass of water. The enzyme subtilisin (2.4 U/g) from *Bacillus licheniformis* (Sigma, P4860) was added to the diluted residue (pH = 7.9) to attain a final concentration of 1% in the mixture. The mixture was enzymatically treated for 2 h at 60 °C in a water bath equipped with a magnetic stirrer.

Subsequently, the enzyme was inactivated by heating the solution in boiling water for 10 min. The inactivated and cooled sample was then centrifuged twice at 20 °C for 10 min at 12000*g*, resulting in separate collections of centrifuged residue and supernatant. The insoluble residue, representing to enzyme-resistant part of the sample, was subjected to freeze-drying, resulting in the sample named 'ENZ-I'. Simultaneously, a portion of the supernatant containing solubilized proteins, peptides, and amino acids was also freeze-dried to produce the sample labeled 'ENZ-S'. Another portion of the supernatant was fractionated by ultrafiltration technique using VivaFlow 200 PES membrane (Sartorius, Germany) with molecular weight cut-off of 10 kDa. The process involved reducing the sample volume by 15 times, followed by washing the retentate with water, using a volume equivalent to 13 times that of the original sample. This procedure produced two fractions: the low-molecular weight permeate (ENZ-S-UF-P) and the ultrafiltration retentate (ENZ-S-UF-R).

2.2.5. Glycogen separation process

The homogenized mussel slurry was subjected to alkali treatment procedure by adding 100 g KOH per 500 g (~800 mL) of the starting raw biomass. This mixture was then heated in a water bath at 95 °C for 30 min. After heating, the treated material was rapidly cooled to room temperature in a cold water bath. It was then centrifuged at 12000*g* for 10 min at 20 °C. After centrifugation, 1.5 times the volume of 96% ethanol was added to the supernatant. The precipitate obtained was separated by centrifugation. To reduce the alkaline residue, this ethanol precipitation step was repeated three times. A small amount of water was then added to the final precipitate, which was carefully neutralized with an aqueous 1 M HCl solution until an acidic reaction was achieved. Soluble biopolymers were then separated from the solution by adding ethanol again and centrifuging. This precipitation step was repeated to ensure a thorough separation. The final precipitated glycogen-rich fraction was freeze-dried, resulting in the production of sample named 'GG'.

2.3. Dry matter and ash content determination

The dry matter and ash contents of the samples were determined gravimetrically. Freeze-drying was used to dry the samples. Prior to ashing, all samples were freeze-drying, then initially ashed in a muffle furnace at 550 °C for 6 h, followed by cooling in a desiccator, weighing and further ashing at 950 °C for 3 h.

2.4. Chemical analyses

2.4.1. Protein and amino acid analysis

The amino acid content of freeze-dried and cryogenically ground mussel flesh or flesh extracts was determined by gas chromatography coupled to mass spectrometry (GC–MS). For the determination of total (proteinaceous and free) amino acids in biomass (including protein composition), 0.02 g of dry sample was hydrolyzed with 2 mL of 6 M HCl solution in hermetically sealed glass tubes at 120 °C for 15 h. The hydrolyzed sample was dried at 95 °C under a stream of nitrogen and dissolved in 2 mL of water. To determine the free amino acids, 0.05 g of the wet sample (mussel extract) was added to 1.5 mL of 0.1 M HCl and shaken vigorously at 1400 rpm for 5 min at room temperature. The mixture was then centrifuged at 4 °C for 15 min at 21000*g* and the supernatant was stored at −80 °C until analysis.

For gas chromatographic analysis, to 100 μL of the amino acid solution obtained in the previous steps, 250 μL of acetonitrile was added, shaken and centrifuged at 21000*g* for 3 min. 100 μL of the supernatant was pipetted into a heat-resistant, capped Eppendorf tube and 100 μL of internal standard solution (5 μg/mL DL-norleucine) was added. The sample was evaporated under a stream of nitrogen, 50 μL of dichloromethane was added, gently vortexed and evaporated again under a stream of nitrogen. To the dried sample, 100 μL MTBSTFA (Supelco, 77,626) and 100 μL acetonitrile were added and thoroughly mixed. The mixture was heated at 100 °C for 1 h, then centrifuged at 4 °C for 15 min at 21000*g* and transferred to a 200 μL sample vial. The vial was centrifuged again at 2000 rpm for 5 min and then analyzed by GC–MS. Amino acid concentrations were determined using a Shimadzu GCMS-QP2010 Ultra gas chromatograph system equipped with a mass detector (MS) and a Phenomenex Zebron ZB-5MS silicon-filled capillary column (30 m × 0.25 mm, 0.25 μm layer thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The sample injector operated at 280 °C using a 2 mm diameter straight liner. The MS detector operated at 325 °C and the ion source at 300 °C. Scans ranged from $m/z = 25$–500, sample injection was in split mode (distribution flow 100) and the sample injection volume was 0.5 μL. In the analysis program the column was held at 100 °C for 2 min, then heated to 298 °C at 5 °C/min and held for 25 min. Quantification was performed using analytical standards (Supelco A6407, A6282).

2.4.2. Lipid and fatty acid analysis

The lipid content was determined in freeze-dried and cryogenically ground samples of mussel flesh or flesh extracts using a chloroform: methanol (2:1, *v*/v) mixture for extraction. Approximately 0.3 g of homogenized sample was placed in a glass test tube to which 1 mL of methanol and 2 mL of chloroform were added. The suspension was shaken vigorously for 90 s and then incubated in an ultrasonic bath at 40 °C for 30 min. Then 1.25 mL of aqueous 2% NaCl solution and 1.25 mL of chloroform were added and shaken vigorously. The sample was centrifuged at 1700*g* for 20 min and the chloroform layer was transferred to a pre-weighed glass extraction tube using a glass Pasteur pipette. Organic solvents were removed by drying in a stream of nitrogen, and the remaining mass of the sample (mussel oil) was expressed as the percentage of lipids relative to the original freeze-dried material.

Fatty acid methyl esters (FAME) were prepared, and their content quantified by gas chromatography. Approximately 0.05 g of the previously obtained mussel oil was weighed into a glass test tube and 1.5 mL of 5% sulphuric acid solution in methanol was added. The mixture was heated in a water bath at 50 °C for 1 h, shaking gently for 30 s every 15 min. The tubes were then cooled in a mixture of ice and water, and 1 mL of water and 1.5 mL of hexane were added to the samples. The mixture was shaken vigorously and allowed to stratify. The top layer was transferred to a new glass tube using a glass Pasteur pipette, dried under a nitrogen stream and 500 μL of hexane was added. Samples were stored at −20 °C until GC analysis.

The fatty acid methyl esters obtained in the previous step were quantified using a Shimadzu GCMS-QP2010 Ultra gas chromatograph system with a mass spectrometric detector and a Phenomenex Zebron ZB-5MS silica-filled capillary column (30 m × 0.25 mm, 0.25 μm layer thickness). Helium was used as the carrier gas at a flow rate of 30 cm/s. The injection apparatus operated at 280 °C. The MS detector operated at 325 °C and the ion source at 300 °C. The scan range was $m/z = 25$–500 and the sample was injected in split mode (split flow 100) with a sample volume of 1 μL. In the analysis program the column temperature was raised from 160 °C to 260 °C at 2.5 °C/min, then to 298 °C at 5 °C/min and held for 15 min. The results were expressed as mass percentage of the total fatty acids identified. The standard mixtures PUFA-2 (Sigma, 47,015 U) and 38 FAME Mix (Supelco CRM47885) were used for fatty acid identification/quantification.

2.4.3. Glycogen content analysis

Glycogen content was determined in freeze-dried and cryogenically ground (Retsch cryomill, Germany) samples of mussel flesh or flesh extracts using alkaline extraction and spectrophotometric detection with phenol-sulphuric acid reagent. In order to prepare samples, 0.001–0.005 g of freeze-dried mussel flesh was placed in screw-capped Eppendorf tubes to which 100 μL of 30% KOH aqueous solution was added. The mixture was carefully stirred and heated in a thermoshaker at 99 °C for 20 min (1000 rpm). It was then cooled in an ice bath, 150 μL

of 96% ethanol was added and mixed vigorously at 2000 rpm. The samples were placed in a thermoshaker at 99 °C for 15 min (1000 rpm), then cooled and analyzed spectrophotometrically.

To the previously prepared samples, 1250 μL of demineralized water was added and mixed vigorously at 2000 rpm. 60 μL of the resulting solution was transferred to a new 2 mL Eppendorf tube to which 180 μL of water was added and then mixed vigorously at 2000 rpm. Then 20 μL of 80% aqueous phenol solution was added, followed by the rapid addition of 1200 μL of concentrated sulphuric acid using an automatic pipette (directing the acid stream directly into the centre of the solution layer). The sample was vortexed vigorously, allowed to stand for 30 min at room temperature, then transferred to a poly(methyl methacrylate) (PMMA) semi-micro cuvette and the absorbance measured at 490 nm. Measurements were performed triplicate, with pure water in the reference cuvette. Glycogen was quantified using an oyster glycogen standard (Sigma, G8751).

2.5. Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy technique was employed for the analysis of freeze-dried samples that were thoroughly homogenized prior to the measurement. Spectra were acquired using a Thermo Scientific Nicolet iS50 FTIR spectrometer (64 scans per spectrum, nominal resolution of 4 cm^{-1}) quipped with a diamond attenuated total reflectance (ATR) accessory. The ATR-FTIR spectra were recorded in the 4000–400 cm^{-1} region.

2.6. Molecular weight determination

Molecular weight profiles of the glycogen-containing samples were determined by high-performance size exclusion chromatography (HP-SEC) (Tuvikene et al., 2015). The 0.05% sample in 0.1 M $NaNO_3$ solution was prepared after solubilisation in a boiling water bath, filtered through a 0.22 μm RC membrane and 100 μL was injected into the HP-SEC system. The analysis was performed using a Shimadzu chromatograph (Kyoto, Japan) equipped with a DGU-20A5R degasser, Nexera X2 LC-30 CE pump, Nexera X2 SIL-30 AC autosampler, CTO-20 AC column oven, RID-10 A refractive index detector, Shodex OHpak SB-G (6.0 × 50 mm) guard column and two consecutive Shodex OHpak SB-806 M HQ (300 × 8 mm) columns (Tokyo, Japan) maintained at 60 °C. The mobile phase was 0.1 M $NaNO_3$, flow rate 0.8 mL/min, analysis time 45 min. Pullulan standards (PSS GmbH, Germany) ranging from 0.342 to 2400 kDa were used to calibrate the system for determining the weight average molecular weights (Mw) of the samples by the LabSolutions software version 5.97 (Shimadzu, Kyoto, Japan).

2.7. Prebiotic effect determination

Bifidobacterium animalis subsp. *lactis* and *Cutibacterium acnes* subsp. *acnes* were grown in BSM and nutrient broth at 37 °C in anaerobic condition for 72 h. Microbial cells were seeded on 96-well plate in 50 μL amounts after adjusting the OD to 0.5, treated with 50 μL of mussel sample dissolved in water to obtain the final 0.5% concentration and incubated for 48 h at 37 °C. After 48 h, 10 μL of WST/ECS solution per well was added and the plate was incubated for 4 h in dark condition. Absorbance was measured at 460 nm by microplate reader FLUOstar OPTIMA (BMG LABTECH, San Diego, USA). Prebiotic effect was estimated as percentage change in viability relative to the control (50 μL water added instead of the sample) for the both microorganisms studied.

3. Results and discussion

3.1. Biomass characteristics and variations

On average, the dry matter content of the farmed mussels was 36%. Nearly 81% of the dry weight consisted of shell, while 19% was meat. The expectation that harvested mussels would exhibit significant seasonal variations in meat held true. The mean dry weight of mussels was significantly higher in summer compared to other seasons, and the proportion of meat in the dried biomass also peaked during this period. This coincides with the lowest ash content observed in both mussel meat and shells in summer (Table 2). Such variations in the dry matter content of mussel meat are well documented (Thompson and Bayne, 1974; Fernández et al., 2015; El Oudiani et al., 2016; Grkovic et al., 2023) and provide valuable insights into its potential applications. In particular, the meat-to-shell ratio, together with the average mass and dimensions of the mussels, play a crucial role in determining the yield and quality of biomass for commercial purposes. Higher average mussel mass and favorable meat-to-shell ratios indicate more efficient harvesting, with implications for both economic and nutritional value (Thompson and Bayne, 1974; Fernández et al., 2015; El Oudiani et al., 2016; Grkovic et al., 2023). In addition, meat colour varied seasonally, with darker meat observed in spring and lighter meat in autumn. This colour variability, possibly due to differences in pigment concentrations influenced by diet, environmental factors or genetics (Saraiva et al., 2011), could affect the visual appeal and perceived quality of the mussels, with implications for marketability (Saraiva et al., 2011). Ash content at different temperatures reveals significant information about the composition of the biomass. At 550 °C, organic material is burned off, leaving behind primarily inorganic constituents such as carbonates, which make up a major component of shell material. At 950 °C, the decomposition of carbonates and the release of carbon dioxide suggest the presence of significant carbonate content in the samples. The variation in ash content at two different temperatures could guide processing and utilization strategies for mussel and barnacle shells, possibly for mineral extraction or incorporation into other products. In addition, this information supports discussions on increasing the value of shellfish biomass, contributing to circular economy approaches in marine industries, as seen in literature focusing on the sustainable use of marine resources for food and nutraceutical applications (Granato et al., 2023; Lordan et al., 2011). On the other hand, the calcium carbonate from shells can be repurposed (Elegbede et al., 2023), complementing the nutritional analysis of the biomass itself as explored by Maar et al. (2023) and Kotta et al. (2020), who have highlighted the role of mussel farming in nutrient cycling and potential eutrophication control. The ability to extract value from all parts of the biomass, whether for nutritional content or mineral composition, aligns with global sustainability goals and the efficient use of natural resources.

3.2. Biomass processing

In this study, we employed a systematic and diverse fractionation strategy for blue mussel biomass, using different extraction, purification and fractionation techniques tailored to the characteristics of the

Table 2
Characteristics of the mussel and barnacle biomasses used in the study. The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, M-3: Mechanically separated mussel meat collected in July 2022, M-4: Mechanically separated mussel meat collected in October 2022, B-2: Mechanically separated barnacle meat collected in November 2021, B-3: Mechanically separated barnacle meat collected in July 2022.

Sample	Average dry weight of one organism, g	Meat in dried biomass, %	Ash in dried meat, %		Ash in dried shells, %	
			550 °C	950 °C	550 °C	950 °C
M-1	0.30	13	7.6	5.7	93.6	53.5
M-2	0.27	16	5.6	5.1	93.8	53.8
M-3	0.39	30	5.6	5.0	92.9	53.6
M-4	0.27	16	9.4	6.9	94.0	53.7
B-2	0.08	10	19.7	15.0	92.9	54.1
B-3	0.13	12	18.0	14.6	92.3	54.1

biomass, including its small size and the inseparable but easily crushable nature of its soft shells (Fig. 1). This approach facilitated the isolation of bioactive and nutritionally relevant components such as proteins, peptides and glycogen.

The enzymatic treatment phase exploited the specificity of subtilisin to hydrolyze proteins into peptides and free amino acids, thereby enhancing the release of bioactive compounds. This step was essential not only for the inherent bioactivity of peptides and amino acids but also for the release of additional bioactive compounds from the biomass matrix (Gupta et al., 2002). Such enzymatic processes, by increasing protein digestibility and the solubility of the resulting peptides and amino acids, are critical for the production of bioavailable and functional nutraceutical ingredients (Daliri et al., 2018).

The processes of homogenization, centrifugation, drying, and alcohol precipitation were carefully controlled, taking into account the effect of heat treatment on ME-P and ME-P-H fractions. The variation in treatment conditions between these two fractions played a crucial role in elucidating the thermal stability, mobility, and bioactivity retention of different biomass components (Jin et al., 2016).

Ultrafiltration was employed to refine the fractions further, segregating compounds based on molecular weight, which is particularly relevant for the isolation of bioactive peptides with prebiotic properties. These compounds have been shown to have significant implications for gut health, as they can selectively stimulate the growth of beneficial gut flora (Gibson et al., 2017).

Glycogen extraction from blue mussel biomass used precise methods to maintain its structural integrity, essential for its role as an energy reserve and prebiotic potential (Ze et al., 2012). Controlled ethanol precipitation techniques preserved the molecular structure of glycogen, which is critical for its biological activity and fermentability by gut microbiota. This preservation ensures that intact glycogen molecules are preferentially metabolized by gut bacteria, facilitating the production of beneficial short-chain fatty acids (Morrison & Preston, 2016) highlighting the potential of glycogen in functional foods and nutraceuticals, supporting health through diet and sustainable use of marine resources.

The fractionation process developed in this study is adaptable, highlighted by the range of fractions produced, each with unique functional and prebiotic properties. Importantly, this method extends beyond mussels and is adaptable to various marine organisms, indicating the wider potential of marine biomasses as sources of bioactive compounds for diverse applications. The developed process outlines an optimized fractionation sequence tuned to the intrinsic properties of the biomass and the specifications of the target products. Future studies should investigate the bioactivity of peptides and amino acids in comparable marine biomasses, taking into account the effects of environmental conditions and seasonal variations on the composition and bioactivity of the derived fractions (El Oudiani et al., 2016; Thompson & Bayne, 2004; Grkovic et al., 2023; Fernández et al., 2015).

3.3. Protein content and amino acid profiles

Fig. 2 illustrates the amino acid profile of different fractions derived from blue mussel biomass, detailing both the total and free amino acid composition. No statistically significant differences in total amino acid content were observed in mechanically separated mussel flesh, with total amino acid levels approaching 50%, indicating a substantial presence of proteinaceous substances. Total amino acid concentrations were high in the thawed water of the mussel biomass and in the soluble fraction of the homogenized mussel slurry, but significantly lower in the separated mussel flesh. This suggests that mechanical separation and homogenization may affect amino acid retention. Notably, ENZ-S shows a comparable total amino acid content (44.4%) to the mechanically separated meat fractions, which aligns with previous research indicating that enzymatic treatment can preserve or even enhance the availability of amino acids in marine biomass preparations (Adler et al., 2022).

Analysis of free amino acid content showed that mussels harvested in summer have the highest levels, suggesting a higher initial presence of free amino acids in the biomass collected during this period. The expectation that enzymatic hydrolysis with subtilisin increases the extraction yield of essential amino acids from Baltic mussel biomass, thereby enhancing its prebiotic effects and nutritional value held true. The data for the ENZ-S fraction indicated an amino acid content of 42.5%, demonstrating the effectiveness of the enzymatic treatment in liberating amino acids into the solution. This high percentage suggests that subtilisin acted efficiently to break down proteins into amino acids that then dissolved in the surrounding medium. Unlike the minimal free amino acids observed in the ENZ-I fraction, the substantial total amino acids in ENZ-S reflect the enzyme's capacity to facilitate the transition of amino acids from bound to free states, which can be essential for enhanced bioavailability and potential bioactivity in subsequent applications. This aligns with the known efficiency of enzymatic hydrolysis in generating bioactive peptides and amino acids from protein-rich biomasses, offering valuable insights into the utility of such treatments in maximizing the nutritional yield from blue mussel biomass.

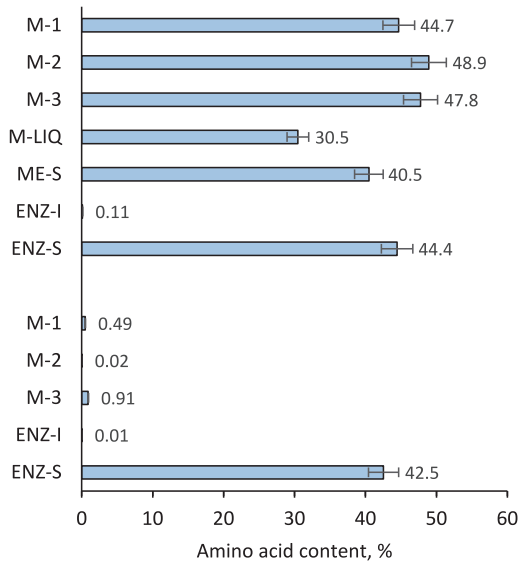

Fig. 2. Total amino acid contents (top seven samples) and the quantities of free amino acids (bottom five samples) in the fractions obtained from the blue mussel biomass. Data shown as mean ± SD, $n = 3$. The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, M-3: Mechanically separated mussel meat collected in July 2022, M-LIQ: Substances from thawed water released from mussel biomass collected in July 2022, ME-S: Soluble part of homogenized mussel slurry collected in July 2022, ENZ-I: Insoluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Comparing these results with previous studies reveals that the amino acid profiles of marine organisms are highly influenced by environmental factors, including diet, water temperature, and seasonal cycles, which can affect both the composition and total content of amino acids in the biomass (Cretton et al., 2023; Smaal & Haas, 1997). The observed variations in the free amino acid content across different fractions also underscore the complexity of enzymatic processes and their outcomes on the bioavailability of nutrients (Jin et al., 2016). Despite the enzymatic treatment, the ENZ-S fraction retains a high total amino acid

content, indicating the effectiveness of enzymatic hydrolysis in releasing amino acids from protein complexes for subsequent utilization or analysis. This efficiency is important for the production of high quality nutraceuticals and functional foods where amino acid bioavailability is important (Gupta et al., 2002; Kim & Wijesekara, 2010).

Amino acid analysis of blue mussel biomass did not show significant seasonal variations in the overall profiles of the mechanically separated fractions (M-1, M-2, M-3) (Fig. 3). The M-2 treatment showed the highest total amino acid content at 48.9%, while the M-3 treatment exhibited a notable lysine content of 7.08%. However, these variations were not sufficient to indicate a significant seasonal difference ($p < 0.05$). The consistency across different harvest times suggests that the amino acid composition of mussel meat is relatively stable regardless of season, underlining the potential reliability of mussel biomass as a source of essential amino acids for nutritional applications. This finding contrasts with the common assumption that significant environmental factors such as temperature and food availability can influence the nutritional composition of marine organisms between seasons. It highlights the robust nature of mussel meat composition, providing a reliable nutritional profile for consumers and processors alike. This analysis

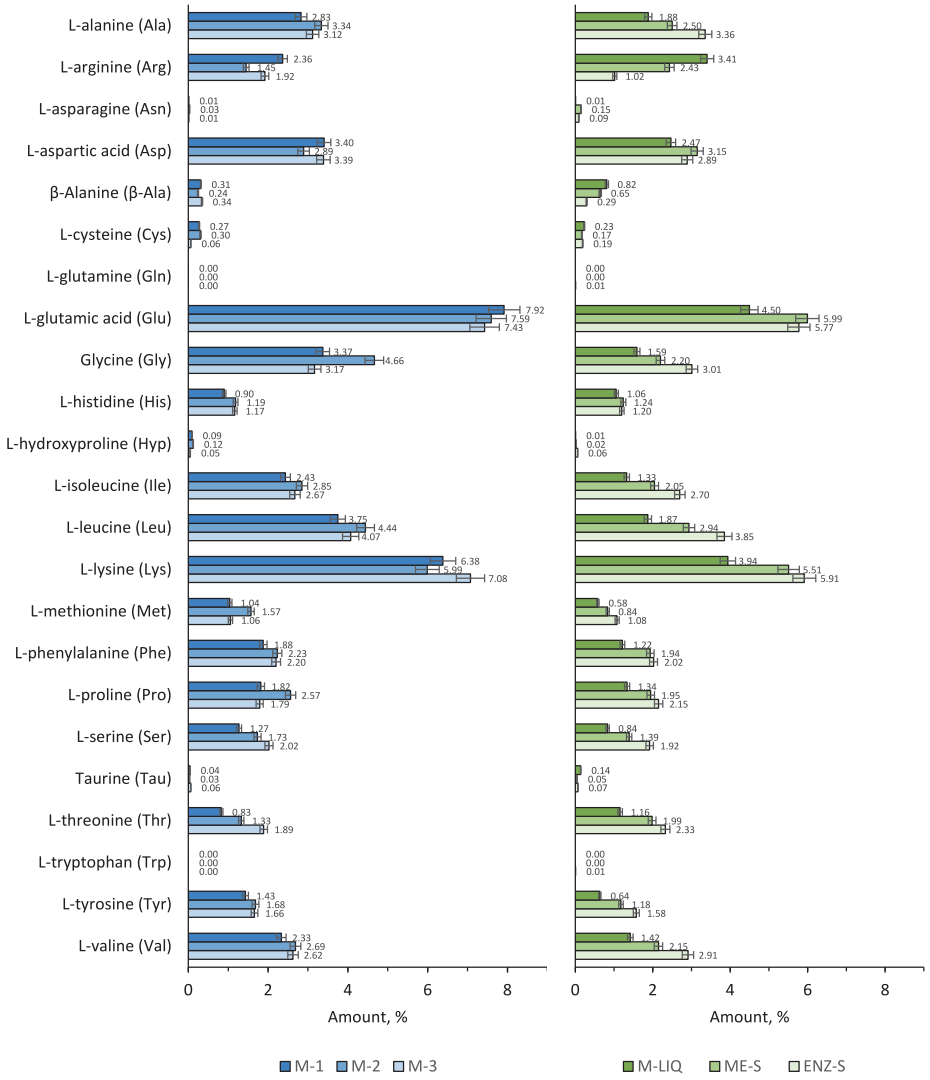

Fig. 3. Amino acid profiles of the fractions obtained from the blue mussel biomass. SD < 5% for the analysis method. The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, M-3: Mechanically separated mussel meat collected in July 2022, M-LIQ: Substances from thawed water released from mussel biomass collected in July 2022, ME-S: Soluble part of homogenized mussel slurry collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed a significant presence of glutamic acid in all fractions, with the soluble portion of the subtilisin-treated shellfish meat fraction containing 6.0%. This is consistent with observations in marine bivalve mollusks, which are known to contain high levels of glutamic acid, a critical component in protein synthesis (Dall & Moriarty, 1983). In addition, essential amino acids, including leucine and lysine, are prominent, highlighting their potential role in meeting nutritional requirements (Wu, 2009). The expectation that the valorization process of Baltic mussel biomass through specific biochemical techniques can significantly enhance its nutritional, health-promoting and commercial value held true. Directly extracted mussel meat fractions showed superior levels of these essential amino acids compared to those released from

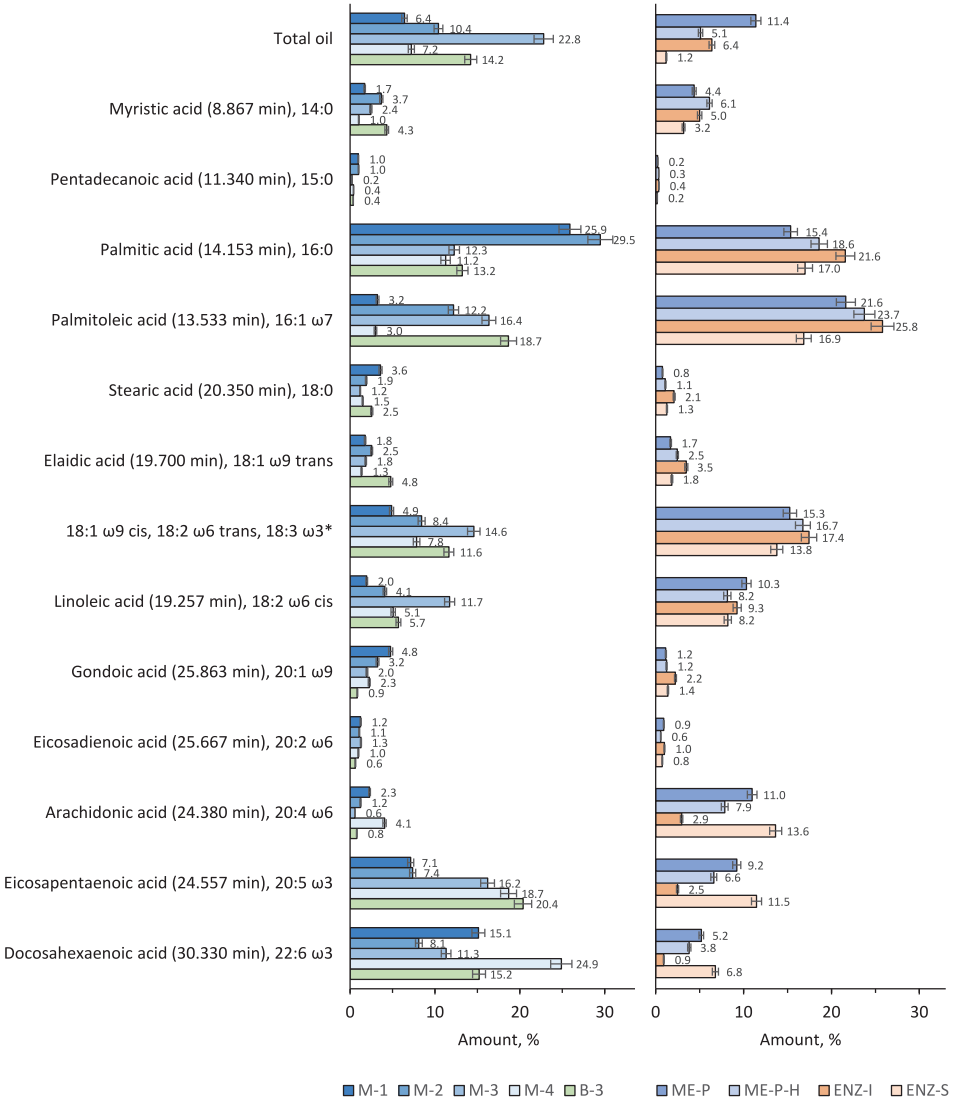

Fig. 4. Fatty acid profiles of the fractions obtained from the blue mussel and barnacle biomasses. Values in brackets represent the GC retention times corresponding to the methyl ester derivatives of the respective fatty acids. * Sum of oleic acid (18:1 ω9 *cis*), linoelaidic acid (18:2 ω6 *trans*), α-linolenic acid (18:3 ω3), whose methyl derivatives at 19.493 min could not be separated by the used GC method. SD < 5% for the analysis method. The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, M-3: Mechanically separated mussel meat collected in July 2022, M-4: Mechanically separated mussel meat collected in October 2022, B-3: Mechanically separated barnacle meat collected in July 2022, ME-P: Purified mussel meat extract using a room-temperature process collected in July 2022, ME-P-H: Purified mussel meat extract using a high-temperature process collected in July 2022, ENZ-I: Insoluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thawed mussel biomass and the soluble portion of homogenized mussel slurry. The variation in the amino acid content of different mussel fractions underlines the key influence of the processing techniques on the preservation and solubility of these nutrients, which is of crucial importance for their use in the food industry (Sikorski, 1990). This emphasizes the importance of optimizing processing methods to preserve essential amino acids, thereby enhancing the nutritional value of foods derived from the marine environment (Tacon et al., 2010).

3.4. Oil content and fatty acid profiles

The analytical data from mussel biomass revealed significant variation in oil content, with total oil percentages in the autumn harvests (M-2 and D-B-3) demonstrating notably higher values, peaking at 10.4% in M-2, as compared to the summer harvests (M-1 and D-M-4) where M-1 contained 6.4% total oil (Fig. 4). This finding illustrates the influence of seasonal variation and species-specific feeding behaviour on the accumulation of lipid reserves (Cretton et al., 2023; Ismail et al., 2016). The oil content data reported in this study are expressed relative to the dry weight of mussel meat, which presents a different context compared to many studies in literature that often report lipid content as a percentage of wet weight. When adjusted for moisture content, our findings show that the oil content in dried mussel meat fractions, with autumnal samples like M-2 reaching as high as 10.4%, could correspond to a lower percentage on a wet weight basis, potentially aligning with earlier studies. Lipid content in marine organisms such as *Mytilus edulis* typically ranges from 1% to 2% by wet weight, with variation dependent on environmental factors such as season and diet (Ackman, 1989; Smaal & Haas, 1997). This implies that when considering the water content in fresh mussel meat, our observed oil percentages in dry mass may parallel these established ranges, underlining the need for consistent measurement standards to enable accurate comparisons across studies. These findings highlight the potential of mussels and crustaceans as a viable alternative to traditional fish oils, especially when considering the fatty acid profiles identified in this study.

Focusing on the fatty acid profiles, Palmitic acid (16:0) predominated in autumn. This is in line with previous research identifying palmitic acid as a common fatty acid in marine species (Cretton et al., 2023). In addition, the fractions harvested in both autumn and summer had significant concentrations of polyunsaturated fatty acids (PUFAs), including particularly high levels of EPA and DHA. Such profiles highlight the nutritional importance of these fractions, particularly their potential contribution to anti-inflammatory and cardiovascular health benefits, paralleling the recognized benefits of EPA and DHA in human nutrition (Calder, 2015; Zanetti et al., 2015). Taking into account the similarity in oil content and fatty acid compositions between the mussel fractions and fish oil, it becomes evident that mussels, particularly those harvested in autumn and summer, could act as an equivalent source to the widely acclaimed fish oil, renowned for its rich ω-3 content. When considering barnacles in mussel biomass, it is important to be aware of the impact they can have on the composition of fatty acids. Barnacles have a unique lipid composition that could alter the fatty acid distribution in the biomass with a significant presence of these crustaceans, potentially increasing omega-6 fatty acid levels relative to the omega-3 rich profile characteristic of mussels. Consequently, samples with a high barnacle content may have an altered oil profile characterized by an increased ratio of omega-6 to omega-3 fatty acids.

Converting the dry weight oil content of mussel flesh to a wet weight basis allows more direct comparisons with the lipid content of typical shellfish and fish. Taking the highest dry weight oil content observed in our study, 10.4% for the M-2 fraction, and taking into account the usual water content of mussels, the recalculated oil content as a percentage of wet weight would fall within a lower range, potentially comparable to the 1–2% wet weight lipid content commonly reported for mussels, including in studies like those by Ackman (1989). This recalibrated value facilitates a better comparison with existing literature, where oily fish species are noted for their high oil content, which can reach 10–15% of the wet weight, not to mention the nearly pure oil content of fish oil supplements.

3.5. Glycogen separation and content

Seasonal fluctuations are a significant factor in determining the glycogen content in mussel biomass (Fig. 5). During colder months, when metabolic rates slow and food is scarcer, mussels tend to exhibit reduced glycogen levels as they utilize their stored energy. Contrarily, the levels generally increase when environmental conditions are less stressful and food is more abundant, usually in the warmer seasons. This cycle is evident in the current data, which shows a tendency for glycogen to decrease as the year progresses into winter, aligning with observations in similar marine organisms (Lemaire et al., 2006).

The current analysis of glycogen content reveals a notable variance across different fractions. The GG fraction is particularly rich in glycogen, with a content of 98%, suggesting the efficiency of the extraction process tailored for glycogen isolation. Meanwhile, the ME-P-H fraction, which has undergone heat treatment, displays a high glycogen content of 82%, indicating that controlled processing conditions can effectively preserve glycogen integrity and minimize thermal decomposition. These results underscore the impact of both

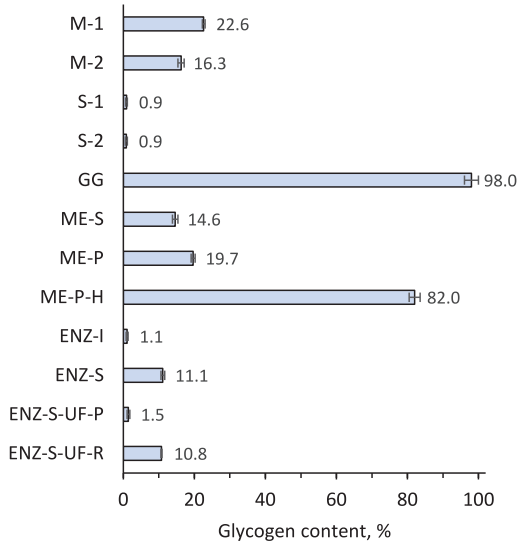

Fig. 5. Glycogen contents of the fractions obtained from the blue mussel biomass. Data shown as mean ± SD, $n = 3$. The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, S-1: Mechanically separated mussel shells collected in September 2020, S-2: Mechanically separated mussel shells collected in November 2021, GG: Glycogen-rich fraction from mussel biomass collected in July 2022, ME-S: Soluble part of homogenized mussel slurry collected in July 2022, ME-P: Purified mussel meat extract using a room-temperature process collected in July 2022, ME-P-H: Purified mussel meat extract using a high-temperature process collected in July 2022, ENZ-I: Insoluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-P: Ultrafiltration permeate of soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-R: Ultrafiltration retentate of soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

environmental factors and processing methods on the glycogen content in mussel biomasses.

Contrastingly, the enzymatically treated fractions, ENZ-I and ENZ-S-UF-P, show markedly lower glycogen levels. Given that subtilisin targets proteins and not polysaccharides like glycogen, it is evident that the observed reduction in glycogen content is not a direct consequence of subtilisin action but may result from the separation processes post-enzymatic treatment. The ENZ-S fraction, with a glycogen content of 11.1%, and the ultrafiltration retentate, ENZ-S-UF-R, at 10.8%, further demonstrate the impact of molecular separation techniques on the distribution of glycogen.

The glycogen levels in the GG and ME-P-H fractions are substantially higher than those typically found in marine mollusks, which are reported to have glycogen contents ranging from 0.1% to 2% of dry weight (De Zwaan & Wijsman, 1976). The elevated glycogen levels achieved by the extraction and purification methods in this study underscore their efficacy and the potential of mussel-derived glycogen for high-energy applications, including sports and medical nutrition. These results highlight the significant effect of processing on glycogen yield and suggest the feasibility of developing glycogen-enriched products from mussel biomass for targeted nutritional and pharmaceutical applications.

3.6. Prebiotic effect of the fractions

Blue mussel-derived fractions show the potential prebiotic activity of *Bifidobacterium animalis* subsp. lactis (Fig. 6). The ME-P-H fraction, which was subject to high-temperature processing, notably promoted the growth of *B. animalis*. This suggests that certain processing conditions can preserve or enhance components such as prebiotic peptides and glycogen that benefit probiotic bacteria growth (Gibson & Roberfroid, 1995). However, regarding antimicrobial activity, none of the fractions in the presented data showed a negative impact on the growth of *C. acnes*. Instead, they all either had an insignificant or positive influence on bacterial growth.

Enzymatically treated fractions, especially the subtilisin-treated ENZ-S and ENZ-S-UF-P samples, demonstrated significant antimicrobial and prebiotic activity. This is likely due to their peptide size and solubility. Specifically, these samples reduced the growth of *C. acnes* by 12–18% and increased the survival of *B. animalis* by 37–40%, indicating the release of bioactive peptides following enzymatic processing (Corzo & Gilliland, 1999). These findings highlight the potential of mussel extract for applications in the food, pharmaceutical, and cosmeceutical industries. This enzymatic efficacy, however, appears to be contingent on molecular weight. The larger molecular weight fraction, ENZ-S-UF-R, did not demonstrate prebiotic activity, suggesting that smaller molecular weight peptides, often below 1 kDa, are preferentially utilized by probiotic bacteria and are more effective in prebiotic functions (Walsh et al., 2013).

Analysis showed that the GG fraction had a relatively uniform weight average molecular weight (Mw) distribution with an average of 310 kDa (Fig. 7). The Mw of ME-P-H was slightly higher at 416 kDa, the largest of the samples analyzed. The enzymatically treated samples contained predominantly components with Mw below 1 kDa, whereas ultrafiltration of these samples produced a retentate (ENZ-S-UF-R) with Mw of the primary component being approximately 5.4 kDa. These results indicate that preparations with Mw below 0.7 kDa have the most pronounced prebiotic effects. Prebiotic activity in preparations with the Mw below 0.7 kDa correlates with findings that probiotic bacteria use smaller peptides and oligosaccharides more efficiently, thereby enhancing their

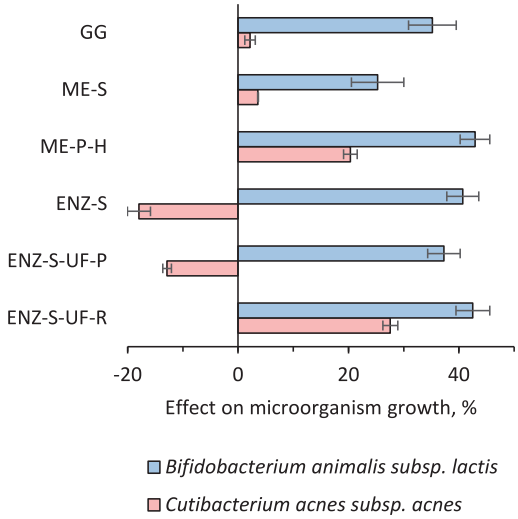

Fig. 6. Effect of the fractions obtained from the blue mussel (at 0.5% concentration) on the growth of *Bifidobacterium animalis* subsp. *lactis* and *Cutibacterium acnes* subsp. *acnes*. Data shown as mean ± SD, $n = 3$. The sample abbreviations are GG: Glycogen-rich fraction from mussel biomass collected in July 2022, ME-S: Soluble part of homogenized mussel slurry collected in July 2022, ME-P-H: Purified mussel meat extract using a high-temperature process collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-P: Ultrafiltration permeate of soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-R: Ultrafiltration retentate of soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

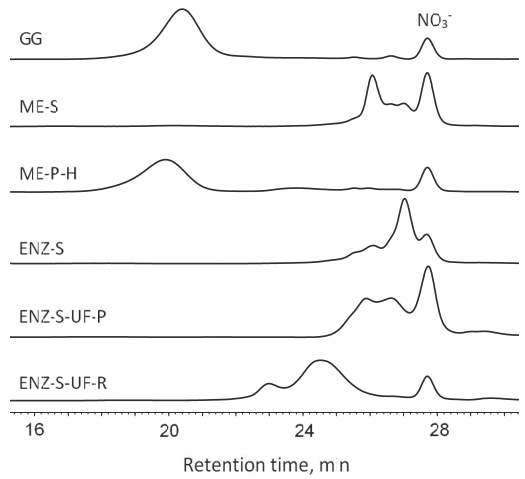

Fig. 7. Size exclusion chromatography profiles of the fractions obtained from the blue mussel biomass. The nitrate peak designated by NO_3^- is systemic and comes from the eluent used in the chromatography system. The sample abbreviations are GG: Glycogen-rich fraction from mussel biomass collected in July 2022, ME-S: Soluble part of homogenized mussel slurry collected in July 2022, ME-P-H: Purified mussel meat extract using a high-temperature process collected in July 2022, ENZ-S: Soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-P: Ultrafiltration permeate of soluble part of subtilisin-treated mussel meat collected in July 2022, ENZ-S-UF-R: Ultrafiltration retentate of soluble part of subtilisin-treated mussel meat collected in July 2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

growth and activity (Gibson et al., 2017). This observation highlights the important role of molecular size in fermentation processes and suggests that lower molecular weight fractions may specifically influence the composition of the gut microbiota.

The Mw of glycogen exhibits considerable variability depending on its source and structural characteristics, with a wide range reported in the literature. For example, mammalian liver and muscle glycogen ranges from several hundred kDa to several thousand kDa (Roach et al., 2012). The documented Mw values of 310 kDa for the GG fraction and 416 kDa for the high-temperature processed mussel extract are at the lower end of this spectrum. This suggests that glycogen derived from blue mussel biomass may have a more branched structure or shorter chain lengths compared to mammalian glycogen.

For enzymatically treated preparations, the reported Mw below 1 kDa indicates the production of low molecular weight peptides. These findings are in line with studies demonstrating that enzymatic hydrolysis can effectively reduce protein size, resulting in the formation of oligopeptides and amino acids that can have various biological activities, including prebiotic effects (Daliri et al., 2018). The retentate from ultrafiltration showing the Mw around 5.4 kDa is consistent with the retention capabilities of ultrafiltration membranes, which are often used to separate peptides based on size (Toldrá et al., 2018).

The results of this study are consistent with the principle that the efficacy of prebiotics is determined by their physicochemical properties, which influence how they are fermented by the gut microbiota, resulting in health benefits (Gibson et al., 2017). Size exclusion chromatography clarifies this by showing that probiotic bacteria use lower molecular weight fractions more efficiently, resulting in enhanced prebiotic effects (Walsh et al., 2013). The results pave the way for the incorporation of mussel-derived fractions into functional foods and skin care products, exploiting their ability to selectively enhance beneficial microorganisms. This highlights the importance of marine-derived bioactives and their functional properties, opening up new opportunities for the development of health-promoting products.

3.7. Spectroscopic characteristics

The ATR-FTIR spectra for the freeze-dried products/fractions derived from blue mussels (or barnacles) provide a valuable tool for rapid insight into their proximate chemical composition (Fig. 8). The meat fractions exhibit characteristic signals commonly associated with animal-derived samples rich in proteins, fatty acids, and carbohydrates.

Despite their complexity, the spectra allow differentiation of the main characteristic bands associated with proteins. Broad bands attributed to NH-stretching for amide A (~3282 cm^{-1}) and amide B (~3070 cm^{-1}), along with signals from CH_3 symmetric stretching (2872 cm^{-1}) and those from amide I (80% C═O stretching, 10% N—H bending, 10% C—N stretching) at 1640 cm^{-1}, amide II (60% N—H bending, 40% C—N stretching) at 1536 cm^{-1} and amide III at 1307 cm^{-1}, were observed, all predominantly arising from proteins (Bozkurt et al., 2012).

The olefinic ═CH stretching vibration at 3011 cm^{-1} was assigned to

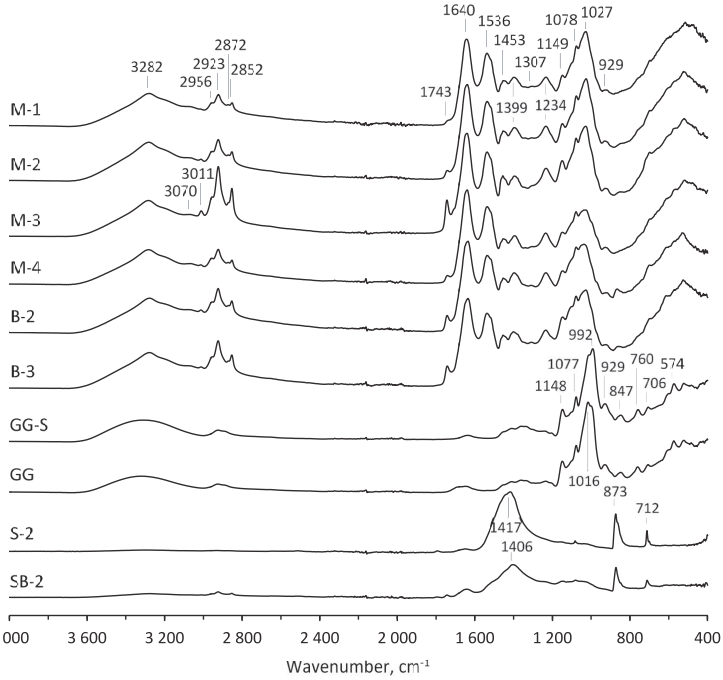

Fig. 8. ATR-FTIR spectra of blue mussel meat (M-1, M-2, M-3, M-4), barnacle meat (B-2, B-3), glycogen from blue mussel (GG) and from oyster (GG-S, commercial sample from Sigma), and shells of blue mussel (S-2) and barnacle (SB-2). The sample abbreviations are M-1: Mechanically separated mussel meat collected in September 2020, M-2: Mechanically separated mussel meat collected in November 2021, M-3: Mechanically separated mussel meat collected in July 2022, M-4: Mechanically separated mussel meat collected in October 2022, B-2: Mechanically separated barnacle meat collected in November 2021, B-3: Mechanically separated barnacle meat collected in July 2022, GG-S: Glycogen-rich soluble fraction from mussel biomass collected in July 2022, GG: Glycogen-rich fraction from mussel biomass collected in July 2022, S-2: Mechanically separated mussel shells collected in November 2021, SB-2: Soluble part of barnacle homogenate collected in November 2021. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

unsaturated lipids and cholesterol esters (Zupančič et al., 2022). Other lipid-specific signals, including CH_2 asymmetric stretching (2923 cm^{-1}) and CH_2 symmetric stretching (2852 cm^{-1}), were also observed (Lozano et al., 2017). Furthermore, a distinctive band at 1743 cm^{-1}, corresponding to saturated ester C═O stretching in phospholipids and cholesterol esters, was also present (Bozkurt et al., 2012). Signals characteristic of both lipids and proteins included CH_3 asymmetric stretching at 2956 cm^{-1}, CH_2 bending at 1453 cm^{-1}, and the band at 1399 cm^{-1} from fatty and amino acids due to COO^- symmetric stretching (Lozano et al., 2017). Additionally, the spectra of all meat samples exhibited bands from PO_2^- asymmetric stretching (1234 cm^{-1}) and symmetric stretching (1078 cm^{-1}), inherent to phospholipids and nucleic acids (Zupančič et al., 2022).

Several carbohydrate-specific signals, such as a strong broad band at ~1027 cm^{-1} from C—O bending in polysaccharides, and weaker vibrations at 1149 cm^{-1} (C—O—C asymmetric stretching) and 929 cm^{-1} (C—O—C stretching) of glycosidic linkages, were detected in the mussel and barnacle meat preparations, indicating the presence of glycogen (Bozkurt et al., 2012; Zupančič et al., 2022). The FTIR spectra of the glycogen isolated from blue mussel (sample GG) and the commercial glycogen sample from oyster were very similar, indicating similar purity, and revealed additional signals (847, 760, 706, 574 cm^{-1}) in the fingerprint region associated with carbohydrates, not clearly observed for the meat samples.

The shells of mussel and barnacle exhibited three fundamental bands corresponding to C—O from carbonates: asymmetric stretching (1417–1406 cm^{-1}), out-of-plane bending (873 cm^{-1}) and in-plane bending (712 cm^{-1}) (Ferraz et al., 2019). Additionally, several minor signals were observed in the spectrum of barnacle shells, suggesting the presence of residual meat particles. The finding is not unusual, as in the case of barnacles, the mechanical separation of meat and shells proved to be challenging. Contamination of meat by residues from shells could be detected by the sharp band at 873 cm^{-1}, as this signal does not overlap with any signals from pure meat.

The consistent features in the FTIR spectra enable the rapid chemical profiling of blue mussel meat as well as that of barnacles, with the latter showing substantial similarities to those of mussels in their spectra. In such meats, the relative proportion of glycogen could be estimated based on the signal area in the range of 1186–877 cm^{-1}, while lipids could be assessed using the integral of the bands at 2995–2800 cm^{-1}. For lipids, this method demonstrated a very high linear correlation ($R^2 = 0.988$) with oil contents measured gravimetrically. However, integration of the band in the region of 1774–1724 cm^{-1} arising from phospholipids and cholesterol esters, on the other hand, showed a slightly lower correlation ($R^2 = 0.965$) with oil levels.

4. Conclusions

This study quantified the nutritional composition of mussel meat and demonstrated how strategic harvesting and processing can optimize nutrient profiles. The dry mass of mussel biomass varied, with meat content ranging from 13% to 30% and ash content ranging from 5.0% to 9.4% in dried meat. Amino acid analysis revealed the prominence of glutamic acid, leucine and lysine, up to 48.9% in certain fractions, while free amino acids were present in lower percentages. The oil content showed a remarkable range from 6.4% to 22.8%, with palmitic, myristic and oleic acids as the main fatty acids, and EPA and DHA levels varying significantly, indicating the health benefits of the mussels. Glycogen content was exceptionally high in some extracts, reaching up to 98%, illustrating the potential of these fractions for energy applications. In addition, enzymatically treated fractions showed significant prebiotic activity, supporting their potential use in various health-promoting applications. The results of the study on the bioactive compounds of blue mussel biomass offer new avenues for innovation in food and cosmetics, confirming the value of marine resources in advancing health and nutrition.

CRediT authorship contribution statement

Indrek Adler: Writing – review & editing, Writing – original draft, Project administration, Data curation. **Jonne Kotta:** Writing – review & editing, Writing – original draft, Supervision. **Marju Robal:** Visualization, Investigation. **Sanjida Humayun:** Investigation, Formal analysis. **Kristel Vene:** Writing – review & editing, Validation. **Rando Tuvikene:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

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

Rando Tuvikene reports financial support was provided by Estonian Research Council. Jonne Kotta reports financial support was provided by Horizon Europe. Jonne Kotta reports financial support was provided by European Maritime and Fisheries Fund. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix 4

Publication IV

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Micronization of Low-Salinity Baltic Sea Blue Mussels: Enhancing Whole-Biomass Utilization and Nutritional Viability

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Abstract: The micronization of low-salinity Baltic Sea blue mussels (*Mytilus edulis/trossulus*) was investigated as a novel valorisation pathway to eliminate the need for labor-intensive meat–shell separation. The small size of Baltic mussels poses a challenge for traditional meat–shell separation. This study investigates micronization as an alternative processing approach to enhance biomass utilization while preserving functional and nutritional properties. This study assessed the feasibility of whole-mussel micronization, focusing on its impact on particle size distribution, grittiness, and the potential separation of meat and shell fractions post-processing. The results demonstrated that micronization at 4000 rpm resulted in a fine powder (<63 µm), significantly reducing grittiness. However, mild chalkiness was observed at higher concentrations (4% solution), highlighting the need for formulation adjustments. While it was expected to facilitate the separation of soft tissue from shell material, the results indicated that this remained impractical due to structural or compositional similarities at finer scales. A sensory evaluation of the whole-mussel powder assessed its texture and palatability, revealing its potential suitability for functional food applications. The findings highlight the potential of micronization as a resource-efficient and scalable processing method, enhancing the economic and environmental value of Baltic mussels in the food industry.

Keywords: Baltic blue mussels; micronization; dried seafood powders; sensory evaluation; microbiological safety; functional food ingredients; sustainable aquaculture products; marine bioresources

Key Contribution: This study demonstrates that whole Baltic Sea mussels (hybrids of *Mytilus edulis* Linnaeus, 1758/*Mytilus trossulus* Gould, 1850) can be successfully micronised into a fine powder suitable for food applications, providing a sustainable solution to enhance biomass utilization and reduce aquaculture waste.

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1. Introduction

The Baltic Sea, characterized by its low salinity, presents unique challenges for blue mussel (hybrids of *Mytilus edulis Linnaeus, 1758/trossulus Gould, 1850*) aquaculture. Baltic Sea blue mussels (*Mytilus edulis/trossulus*) differ from their higher salinity counterparts due to their small size and nearly equal meat-to-shell ratio. This unique composition makes conventional meat–shell separation labor-intensive and inefficient, necessitating innovative

processing approaches that rely on efficient meat–shell separation for commercial viability [1,2]. Despite these limitations, Baltic mussels have gained attention for their nutritional composition and potential applications in sustainable food production, making innovative processing solutions essential [3,4].

One of the main advantages of Baltic mussel farming is its potential for nutrient bioextraction, which helps mitigate eutrophication by removing excess nitrogen and phosphorus from the marine environment [5,6]. Research suggests that mussel farming could serve as a nature-based solution for reducing coastal nutrient enrichment while simultaneously offering a protein-rich biomass that can be integrated into food and feed applications [7–10]. However, the small size of Baltic mussels and their strong shell adhesion present processing challenges, particularly when aiming for cost-effective and high-value end products [4,11].

A promising solution for valorizing Baltic mussels is micronization, a process that reduces biomass to fine particles, thereby eliminating the need for labor-intensive separation of meat and shell fractions [12]. Here, the high shell-to-meat ratio of Baltic Sea mussels is actually an advantage for this processing technique. Micronization has been successfully applied to marine bioresources such as macroalgae and shellfish byproducts, where it enhances bioavailability and functional properties [13]. However, its application to whole Baltic mussels remains underexplored, particularly regarding its impact on sensory attributes and bioactive retention [14]. The feasibility of whole-mussel micronization remains underexplored, particularly in terms of its impact on particle size distribution, grittiness, and the potential for post-processing fractionation of soft tissue from shell material [15].

Recent advances in Baltic mussel processing have demonstrated the potential for high-value ingredient production. Adler et al. [16] optimized meat extraction techniques for Baltic mussels, achieving high protein recovery through mechanical and enzymatic methods. Additionally, Adler et al. [17] highlighted the prebiotic potential of micronized Baltic mussels, identifying bioactive compounds that could be incorporated into nutraceuticals and functional foods. These findings highlight the importance of alternative processing strategies in increasing the economic feasibility of mussel farming in the Baltic region.

Beyond traditional mussel processing, there is growing interest in utilizing shell-derived ingredients in food applications, particularly as a natural calcium source and as a strategy for reducing food processing waste. The seafood industry generates significant amounts of shell waste, which, if not properly managed, can lead to environmental concerns and economic inefficiencies. Repurposing mussel shells for food applications aligns with sustainable food production goals by promoting circular economy practices and minimizing waste.

Eggshell powder, a widely studied shell-based ingredient, has been investigated for its nutritional potential and bioavailability in human diets. Several studies have demonstrated that micronized eggshell powder can serve as an effective calcium supplement, with absorption rates comparable to or even exceeding those of commercial calcium carbonate supplements [18,19]. Furthermore, eggshell-derived calcium has been successfully incorporated into fortified foods, enhancing bone health and reducing the risk of osteoporosis [20,21]. Studies have also explored the use of eggshell membranes for their bioactive compounds, which may offer anti-inflammatory and regenerative properties [22,23].

Given the structural similarities between eggshells and mussel shells, particularly their calcium carbonate composition, there is potential for adapting similar food applications for mussel shell-derived ingredients. Micronization has been widely explored in the food industry to enhance the functional, nutritional, and bioavailability properties of various materials, such as grains, legumes, and calcium-rich shell materials. This process reduces particle size and improves solubility, dispersibility, and absorption in the human digestive system, thereby enhancing the overall nutritional efficacy of the ingredient [13]. For

example, micronized eggshell powder has been successfully incorporated into fortified foods as a bioavailable calcium source. The valorization of mussel shells could contribute to reducing waste streams from mussel aquaculture and processing, converting a byproduct into a valuable nutritional ingredient. The development of micronized mussel shell powder for human consumption represents a novel pathway to enhance the economic viability of mussel farming while providing sustainable food fortification options.

While the current study does not focus on direct product development, it provides a foundation for future research by evaluating the feasibility of whole-mussel micronization and its impact on particle size, grittiness, and sensory attributes. Understanding these properties is essential for determining the functional potential of mussel-derived ingredients in food applications. These findings will contribute to the development of a scalable, resource-efficient approach for utilizing Baltic mussels in the food industry, thereby enhancing their economic and environmental value [8,24].

2. Materials and Methods

2.1. Raw Material and Preprocessing

Baltic blue mussels (*Mytilus edulis/trossulus*) were harvested from Sankt Anna farm, Sweden, on 20 September 2023. Following standard seafood processing protocols, the mussels were immediately frozen at −18 °C to preserve their nutritional and biochemical integrity. The mussels were then stored for 11 months prior to micronization. Freezing is a widely recognized method for preventing protein denaturation and lipid oxidation in shellfish, ensuring that essential bioactive compounds remain intact during storage and processing [25]. Extended freezing may affect biochemical composition and requires further analysis [13].

To prepare the mussels for further processing, a KitchenAid meat grinder (KitchenAid, Benton Harbor, MI, USA) was used to crush the frozen mussels. Mechanical crushing enhances surface area, thereby optimizing moisture removal and improving subsequent drying efficiency [26]. The crushed material was then evenly spread onto baking paper-lined oven trays in 1–1.5 cm layers to allow for uniform dehydration.

2.2. Drying Process and Moisture Content Analysis

The crushed mussel material was dried in a convection oven set to 70 °C for 10 h in drying mode. Convection drying is a commonly used method for processing shellfish due to its efficiency in reducing moisture content while minimizing nutrient degradation [13].

Post-drying, the moisture content was measured using a KERN DAB 100-3 moisture analyzer (KERN & SOHN Gmbh, Balingen, Germany, yielding a final moisture level of 3%. A low moisture content prevents microbial growth and ensures extended storage stability [27]. For dried seafood products, the moisture content should remain below 5% to ensure microbial safety and biochemical stability [27].

2.3. Storage, Transportation, and Microbiological Analysis

The dried mussel powder was stored in a thermally insulated box at +6 °C for 10 days before being transported to the micronization facility. Maintaining a controlled storage temperature prevents oxidative changes and enzymatic degradation, thereby preserving the sensory and nutritional attributes of dried seafood products [28].

The microbiological quality of mussel-derived food products needs to be evaluated to ensure safety and compliance with health standards [8]. To assess food safety, a microbiological analysis was performed on the dried mussel powder before further processing. The microbiological assessment included the following criteria:

- Total aerobic mesophilic bacteria count (CFU/g);

- Enterobacteriaceae (CFU/g);
- Yeast and mold count (CFU/g);
- *Salmonella* spp. detection;
- *Listeria monocytogenes* detection.

Microbiological tests were conducted at the National Centre for Laboratory Research and Risk Assessment (Riigi Laboriuuringute ja Riskihindamise Keskus, Tartu, Estonia) under ISO 4833-1:2013 [29], ISO 21528-2:2017 [30], ISO 21527:2008 [31], ISO 6579-1:2017 [32], and ISO 11290-1:2017 [33] standards.

2.4. Chemical Analysis

The chemical composition of the micronized mussel powder was determined using standardized analytical methodologies to ensure accurate quantification of key macronutrients, minerals, and energy content. The total fat content was analyzed using a solvent extraction method (KE-TJ-5, var. 2 SBR), following the guidelines of NMKL 131:1989 (Fat Determination according to SBR in Meat and Meat Products) [34] and AOAC 948.15 (Fat in Seafood) [35]. The total carbohydrate content was determined through enzymatic hydrolysis (KE-TJ-89, var. 2), in compliance with EÜ 1169/2011 [36], which regulates the presentation of food information to consumers. The protein content was quantified using a nitrogen-based method, converting the total nitrogen content to protein via an established conversion factor, thereby ensuring an accurate estimation of the protein fraction. The analysis was conducted using ISO 937:1978 (Meat and Meat Products—Determination of Nitrogen Content) [37], EVS EN ISO 8968-1:2014 (Milk and Milk Products—Nitrogen Determination by the Kjeldahl Method) [38], ASN 3406 (Nitrogen Determination in Fish Meal by the Kjeldahl Method) [39], and AOAC 920.87 (Total Protein in Flour [40], also referenced by AOAC 979.09 for grains [41] and AOAC 950.36 for bread) [42].

The ash content, indicative of the total mineral content, was measured using high-temperature incineration, during which the organic matter was combusted, leaving behind inorganic residues for quantification. Sodium concentration, expressed as salt content (Na $\times$ 2.5), was assessed using atomic absorption spectrometry (AAS) following ISO 9964:1993 [43]. Energy content was calculated using KE-TJ-87, var. 3, in accordance with EÜ 1169/2011 [36], ensuring alignment with European regulations on nutritional labeling and consumer information.

The chemical analysis was conducted at the National Centre for Laboratory Research and Risk Assessment (Riigi Laboriuuringute ja Riskihindamise Keskus, Tartu, Estonia), which is an accredited institution under the Estonian Accreditation Centre (EAK). This accreditation ensures that the analytical procedures meet international laboratory quality standards, guaranteeing reliable and precise results for food safety and nutritional assessments. All procedures followed internationally recognized ISO, AOAC, and NMKL methodologies to ensure accuracy and compliance with regulatory standards. Mineral and trace element analysis was performed according to EVS-EN 13804:2013 [44], EVS EN 13805:2014 [45], and EVS EN 15505:2008 [46].

2.5. Micronization Process

Industrial-scale micronization was performed using a Librixer industrial micronizer (Librixer AB, Mölndal, Sweden). Since the industrial system does not allow for direct particle fraction selection, the milling parameters were adjusted by varying the rotational speed of the mill as follows:

- 1500 rpm (clockwise);
- 2500 rpm (clockwise);
- 4000 rpm (anticlockwise, to achieve finer particle size distribution).

The micronization process significantly influences powder morphology, surface area, and sensory perception—factors that should be considered when designing food formulation applications [13]. The resulting powders were subsequently visually inspected to assess particle fineness and suitability for food applications. The particle size distribution of the micronized mussel powder was determined using a mechanical sieving method with a Retsch analytical sieve shaker (Retsch, Haan, Germany). This method allows for the precise fractionation of particles based on size, ensuring accurate characterization of the powder's fineness and uniformity.

2.6. Particle Size Determination

The particle size distribution of the micronized mussel powder was determined using a Retsch AS 200 analytical sieve shaker (Retsch, Haan, Germany) equipped with a series of standard stainless-steel mesh sieves. The procedure was designed to evaluate the fineness of the powder produced at different micronization speeds. A representative 100 g sample from each powder batch was placed on the top sieve of a stack arranged in descending mesh size (e.g., 250 μm, 150 μm, 100 μm, 63 μm, and bottom pan). The sieve stack was secured and subjected to vibration for 10 min at an amplitude of 1.5 mm.

Following sieving, the mass retained on each mesh was collected and weighed. The particle size distribution was expressed as the percentage of the total sample mass retained on each mesh, allowing for the quantification of coarse versus fine fractions. The finest particles—those passing through the 63 μm mesh—were considered the most suitable for food applications due to their reduced grittiness. This method provided a reproducible means to compare the impact of different micronization speeds (1500, 2500, and 4000 rpm) on the final powder fineness and supported the visual and sensory evaluation results.

2.7. Sensory Evaluation

2.7.1. Panel and Training

Sensory evaluation was performed by eight trained assessors (mean age 32 ± 8 years) selected from TFTAK's (TFTAK AS, Tallinn, Estonia) pool of assessors. The panel was selected based on ISO 8566:2023 and had prior sensory training according to the same standard, along with previous experience in sensory analysis. In addition to their previous training, assessors underwent an additional training session with the specific sample to familiarize themselves with the product and refine the evaluation protocol. During the session, assessors became acquainted with the expected flavor profile and intensity ranges. The training was conducted as a quantitative descriptive test, mirroring the later evaluation. PanelCheck (version 1.4.2, Nofima, Tromsø, Norway) software was used to evaluate the performance of the panel and assessors [47]. The final sensory analysis method was established based on scientific literature, in-house protocols, and panel discussions [48].

2.7.2. Sample Preparation and Presentation

Preliminary testing of the powders in dry form with a small group of selected assessors revealed challenges such as overpowering flavor intensity and excessive dryness. To address these issues, preliminary testing was also conducted using aqueous solutions at varying concentrations (1%, 1.5%, 2%, 4%, 5%). Although the powder is expected to be used at low levels in the final products, the aim was not to replicate the final product concentration. Rather, the goal was to identify a solution concentration that was sufficiently dilute to prevent overwhelming sensory intensity and powder precipitation, yet concentrated enough to enable the characterization of key flavor attributes. Preliminary testing indicated that 2–4% w/v solutions were suitable for these purposes, as the samples exhibited a perceivable overall intensity in odor and taste without being too bitter, astringent, or chalky, and with minimal precipitation during evaluation.

In the sensory evaluation, mussel powders were dissolved in room temperature water at concentrations of 2% w/v and 4% w/v. The samples were homogenized and served in 40 mL sensory glasses equipped with glass lids to preserve volatiles. To eliminate bias and carryover effects, the order of sample presentation was randomized using Williams' Latin Square design [48]. Each sample was assigned a three-digit code, and palate cleansing between samples was facilitated with water and unflavored crackers.

2.7.3. Sensory Attributes and Data Collection

Sensory evaluation followed ISO 8589:2007 guidelines for controlled sensory environments to minimize external influences [49]. Quantitative Descriptive Analysis (QDA) was applied, using a 10-point scale, as follows:

- 0 = None;
- 1 = Very weak;
- 5 = Moderate;
- 9 = Very strong.

The following sensory attributes were assessed:

- Odor (O.): Overall intensity, fishy, seaweed, metallic, sweet, sour, and off odor;
- Taste (T.): Overall intensity, salty, umami, bitterness, astringency, and off taste.

Due to the high precipitation of the mussel powder, panelists were instructed to thoroughly shake and mix the samples before tasting. Sensory evaluation was conducted individually in a dedicated sensory room, ensuring compliance with ISO 8589:2007 standards to minimize environmental distractions [49].

2.7.4. Data Collection and Statistical Analysis

Sensory responses were recorded digitally using RedJade Sensory Software version: 6.7.3 (RedJade Sensory Solutions LLC, Martinez, CA, USA). Data visualization and statistical analysis were performed in Microsoft Excel version: 2411 (Microsoft, Redmond, WA, USA). Mean intensity values, along with standard deviations, were calculated for each sensory attribute to assess the impact of concentration levels on sensory perception.

3. Results

3.1. Drying Yield

The drying process was conducted in three independent batches, each consisting of 1 kg of raw mussel material. The final dried weights were recorded as 520 g, 489 g, and 532 g, respectively. The average drying yield was calculated at 51.4% of the initial wet mass. The moisture content of the dried material was measured in each batch, and the results consistently indicated a residual moisture level of 3%. This aligns with previous studies highlighting that seafood products dried to a moisture content below 5% exhibit enhanced shelf stability and reduced potential for microbial growth [26].

3.2. Chemical Composition Analysis

The micronized mussel powder had a high mineral content, with ash comprising 58.51 g/100 g, indicating a significant inorganic fraction primarily derived from calcium carbonate present in mussel shells. The protein content was measured at 17.78 g/100 g, reinforcing its potential as a marine-derived protein source suitable for dietary applications. The total carbohydrate content was 19.4 g/100 g, contributing to the overall macronutrient balance, while the fat content remained low at 2.47 g/100 g, highlighting the lean nutritional composition of the product (Table 1).

Table 1. Chemical composition of dried mussel powder.

Component	Result (g/100 g)
Fat	2.47
Carbohydrates	19.4
Protein	17.78
Ash	58.51
Salt (Na × 2.5)	1.38

In addition, sodium content, expressed as salt (Na × 2.5), was quantified at 1.38 g/100 g, aligning with the naturally occurring salt levels in marine-based ingredients. The relatively low fat content and high ash concentration further emphasize the potential functional applications of micronized mussel powder, particularly in fortified food formulations where mineral and protein enrichment is desired.

These results confirm that micronized mussel powder is rich in minerals and protein while maintaining low fat levels, making it a promising ingredient for functional foods and nutraceutical applications.

3.3. Microbiological Analysis

Microbiological analysis was conducted to evaluate the food safety of the dried mussel powder and to ensure compliance with EU food safety standards. The results are summarized in Table 2.

Table 2. Microbiological analysis of dried mussel powder.

Microbiological Parameter	Result	Regulatory Limit (EU Standards)
Total aerobic mesophilic bacteria	<10 CFU/g	$\leq 10^4$ CFU/g
Enterobacteriaceae	<10 CFU/g	≤100 CFU/g
Yeast and mold count	<10 CFU/g	$\leq 10^3$ CFU/g
Salmonella spp.	Not detected	Absent in 25 g
Listeria monocytogenes	Not detected	Absent in 25 g

All microbiological parameters remained below detection limits, confirming that the drying and storage conditions effectively prevented microbial contamination. The absence of pathogenic bacteria, such as *Salmonella* spp. and *Listeria monocytogenes*, supports the microbiological safety of the product, ensuring that it meets European Union regulatory standards.

The microbiological assessments were conducted in accordance with ISO standards, including ISO 4833-1:2013 (enumeration of microorganisms) [29], ISO 21528-2:2017 (detection of Enterobacteriaceae), ISO 21527:2008 (enumeration of yeasts and molds), ISO 6579-1:2017 (detection of *Salmonella* spp.) [32], and ISO 11290-1:2017 (detection of *Listeria monocytogenes*) [33,50,51].

These findings confirm that micronized mussel powder is microbiologically safe and suitable for human consumption, reinforcing its potential as a sustainable and nutritionally valuable food ingredient.

3.4. Micronization Performance

Each micronization test was conducted using 1 kg of dried mussel material. The material recovery was 98.3%, with minor losses attributed to adherence within the milling chamber, a common phenomenon in micronization. The particle size distribution obtained from the different milling speeds was as follows:

- 1500 rpm (clockwise rotation) → 150 μm (average particle size);

- 2500 rpm (clockwise rotation) → 100 µm (average particle size);
- 4000 rpm (anticlockwise rotation) → below 63 µm (limited by the finest mesh available).

These results are consistent with literature reports indicating that higher rotational speeds enhance particle fragmentation due to increased impact forces and shear stress [34]. Micronization at 4000 rpm (anticlockwise) produced the finest particle size and was subsequently selected for further sensory evaluation, as coarser fractions (150 µm and 100 µm) exhibited noticeable grittiness when tasted.

3.5. Sensory Analysis

The 4000 rpm micronized powder underwent sensory evaluation at 2% and 4% solution concentrations to assess its odor and taste profile. Material recovery was 98.3%, with minor losses attributed to adherence within the milling chamber, a common phenomenon in micronization [52].

A statistical comparison (paired *t*-tests) between the 2% and 4% concentrations showed that only the umami taste differed significantly ($p = 0.02$) [28], while all other sensory attributes exhibited no significant differences ($p > 0.05$). This suggests that increasing concentration had a minimal impact on most sensory characteristics.

The taste profile was balanced, with moderate umami intensity (T.Umami: 1.6 ± 0.6 at 2% concentration, 2.8 ± 0.9 at 4% concentration) and mild saltiness (T.Salty: 0.8 ± 0.9 at 4%). No significant off odors or off tastes were detected, suggesting high sensory acceptability of the micronized product. Texture observations indicated a grainy, chalky mouthfeel, which was slightly more pronounced in the 4% concentration solution, according to the assessors' comments. Additionally, the higher concentration exhibited a purple undertone in the precipitated layers, as noted by the assessors.

3.6. Summary of Key Findings

- Drying efficiency resulted in a yield of 51.4% with a final moisture content of 3%, ensuring product stability and microbial safety [28].
- Micronization at 4000 rpm (anticlockwise) produced the finest powder (<63 µm), which was the only fraction suitable for food applications due to its reduced grittiness.
- Sensory evaluation indicated that the product had a balanced taste profile, with dominant seaweed odor notes, mild umami, and no perceivable off flavors.
- Higher concentration (4%) resulted in a slightly chalkier texture, but no significant differences in overall odor and taste intensity were observed.

These findings demonstrate that micronized Baltic blue mussel powder has potential for functional food applications, particularly when incorporated into formulations where a fine particle size is critical for mouthfeel and sensory acceptability.

4. Discussion

4.1. Drying Yield and Moisture Content

The drying process resulted in a 51.4% yield, with moisture content stabilized at 3%, ensuring a low-water activity product with enhanced shelf stability. This yield aligns with previously reported values for dried bivalve powders, where reducing moisture below 5% is essential for microbial safety and biochemical stability [28].

The observed minor variations in final dried weight between batches (489–532 g) are likely attributable to slight differences in initial mussel composition and variability in drying efficiency. The achieved moisture level meets industrial requirements for dried seafood powders, which typically require <5% moisture to prevent lipid oxidation and protein degradation during storage.

In addition, the absence of pathogenic bacteria confirms product safety at the initial processing stages, supporting the effectiveness of the drying process in ensuring microbial safety. The low moisture content likely contributed to microbial inhibition, reducing the risk of spoilage and pathogenic contamination [28]. However, further studies are needed to assess long-term microbial stability under different storage conditions [53] in the final product.

4.2. Micronization Efficiency and Particle Size Reduction

The micronization process demonstrated a high material recovery rate of 98.3%, indicating that very little mass loss occurred during processing. This finding is consistent with previous research on micronization, which shows that material losses of <5% are common in industrial-scale milling systems due to particle adhesion and airflow losses.

The particle size distribution was significantly influenced by rotational speed, with finer fractions achieved at higher speeds. The 4000 rpm anticlockwise setting resulted in a powder fraction below 63 μm, compared to 100 μm at 2500 rpm and 150 μm at 1500 rpm. These findings are in accordance with earlier studies showing that increasing rotational speed enhances fragmentation through higher impact and shear forces.

The finest fraction (<63 μm) was deemed most suitable for food applications, as coarser fractions (100–150 μm) exhibited noticeable grittiness when tasted, a sensory limitation that has also been reported for other micronized seafood powders.

Microbiological evaluation confirmed that the micronization process did not introduce microbial contamination, with all tested parameters remaining within food safety limits. The absence of bacterial growth post-processing suggests that the combination of drying and micronization effectively prevents microbial proliferation [54].

4.3. Sensory Evaluation and Suitability for Food Applications

The 4000 rpm micronized powder was selected for sensory evaluation because of its superior texture. The quantitative descriptive analysis (QDA) revealed a dominant seaweed-like odor (O.Seaweed: 6.3 ± 0.6–0.9) and moderate umami intensity, particularly in the 4% solution (T.Umami: 2.8 ± 0.9) (Table 3).

Notably, off odors and off tastes were not detected, indicating a high sensory acceptability of the product. The absence of significant differences in odor and taste intensity between the 2% and 4% solutions suggests that the flavor profile remains relatively stable across different concentrations (Table 3).

However, umami intensity exhibited a statistically significant increase at higher concentration levels, which is consistent with previous research on marine protein hydrolysates, where increased solubilized amino acids and nucleotides enhance umami perception [54]. The slightly chalkier texture and visible precipitation in the 4% solution could be attributed to protein aggregation, a common occurrence in micronized seafood powders when reconstituted in aqueous solutions [54].

Importantly, microbiological testing confirmed the absence of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli*, reinforcing the product's suitability for food applications. These findings suggest that the drying and micronization processes effectively reduce microbial risks while maintaining sensory quality.

Table 3. Sensory profile of shellfish powder in different concentrations (average and standard deviation). Abbreviations: "O"—odor, "T"—taste.

Sample	2%	4%
O.Overall intensity	6.9 ± 0.9	6.8 ± 0.7
O.Fishy	2.9 ± 1.0	2.9 ± 1.0
O.Seaweed	6.3 ± 0.9	6.3 ± 0.6
O.Earthy	2.8 ± 0.9	2.2 ± 0.5
O.Metallic	2.3 ± 0.4	2.3 ± 0.7
O.Sweet	2.3 ± 0.5	2.3 ± 0.4
O.Sour	0	0
O.Off odor	0	0
T.Overall intensity	5.6 ± 0.7	6.4 ± 1.0
T.Fishy	2.4 ± 0.8	2.4 ± 0.6
T.Seaweed	3.8 ± 0.9	4.0 ± 1.0
T.Earthy	2.4 ± 0.8	3.1 ± 1.0
T.Metallic	2.4 ± 0.5	2.4 ± 0.8
T.Sweet	1.4 ± 0.7	2.3 ± 0.8
T.Sour	0	0
T.Salty	0	0.8 ± 0.9
T.Umami	1.6 ± 0.6	2.8 ± 0.9
T.Bitter	1.4 ± 0.7	1.8 ± 0.9
T.Astringent	3.1 ± 0.9	3.6 ± 0.9
T.Off taste	0	0
Additional comments (optional)	Grainy, chalky mouthfeel	Even more chalky, cement, has a purple undertone

4.4. Implications for Future Applications

The findings suggest that micronized Baltic mussel powder can be effectively used in food formulations, particularly where fine particle size is critical for mouthfeel and sensory acceptability. The absence of strong fishy or off flavors, combined with a moderate umami profile, makes the powder a viable candidate for incorporation into functional foods, soups, and seasoning blends.

However, the textural limitations observed at higher concentrations indicate that further optimization of the formulation may be required. Techniques such as protein solubilization through enzymatic hydrolysis or the addition of stabilizers could be explored to improve dispersion and reduce precipitation issues in liquid applications.

From a safety perspective, the microbiological analysis confirmed that the product meets food safety standards, with no detectable levels of harmful bacteria. This suggests that micronized mussel powder can be considered microbiologically stable, provided that appropriate storage conditions are maintained.

4.5. Limitations and Future Research

While the study provides valuable insights into the micronization and sensory characteristics of Baltic mussel powder, the following limitations should be acknowledged:

- Regulatory classification under the "Novel Food" regulation: As whole-shell micronization is not a conventional food processing method, regulatory classification under the EU "Novel Food" framework must be considered. Prior approvals for shell-derived calcium supplements (e.g., eggshell powder) suggest a potential pathway for regulatory acceptance. This means that an evaluation is required to determine whether mussel powder, including its shell components, can be legally approved for human consumption [55]. Since people traditionally do not consume mussels with their shells, this question needs to be clarified before commercial application.

- Marine biotoxin monitoring under EU food law: In addition to microbiological testing, EU Regulation (EC) No 853/2004 mandates the monitoring of marine biotoxins in bivalve molluscs destined for human consumption. Future studies must address the potential presence of Paralytic Shellfish Poison (PSP), Amnesic Shellfish Poison (ASP), and Diarrhoeic Shellfish Poison (DSP), including toxins such as Okadaic acid, Dinophysistoxins, Pectenotoxins, Yessotoxins, and Azaspiracids, to ensure regulatory compliance and consumer safety. While a prior project under Baltic Blue Growth monitored these toxins across various sites and seasons in the Baltic Sea and did not detect any harmful levels, those findings were not part of this study and cannot substitute for formal analytical confirmation. Therefore, toxicological safety must be established through dedicated biotoxin testing in future investigations.
- Microbiological stability over time: Although the product was free from microbial contamination at the time of analysis, further studies should evaluate microbial stability under different storage conditions to ensure long-term food safety.
- Texture challenges: The grainy mouthfeel and precipitation at higher concentrations suggest that further processing modifications (e.g., colloidal milling or hydrolysis, could improve product dispersibility.
- Functional and physicochemical properties: While the nutritional composition has been analyzed, additional studies on bioavailability, digestibility, and interactions with other food ingredients would provide further insights into how micronized mussel powder performs in different food applications.
- Application trials: Future research should explore how micronized mussel powder performs in real food applications, such as soups, sauces, and protein-enriched snacks, to evaluate its functional properties in formulated products.

By incorporating microbiological safety results into the discussion, this section highlights the product's compliance with food safety standards while reinforcing its potential for food applications. However, before proceeding to commercialization, regulatory clearance under EU food legislation must be thoroughly assessed.

5. Conclusions

This study demonstrates the feasibility of producing microbiologically safe, nutritionally valuable, and sensorially acceptable micronized Baltic mussel powder through optimized drying and milling processes. The drying process effectively reduced moisture to 3%, ensuring prolonged shelf stability and preventing microbial proliferation. The micronization process achieved a high material recovery (98.3%), with finer particle sizes enhancing dispersibility and improving sensory characteristics. Sensory evaluation confirmed that the 4000 rpm micronized powder provided an optimal balance of umami intensity and texture, making it suitable for food applications such as functional foods, soups, and seasoning blends.

Importantly, microbiological analysis confirmed the absence of foodborne pathogens, including *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli*, highlighting the effectiveness of the processing methods in ensuring food safety. The combination of low moisture content and controlled processing conditions played a crucial role in preventing microbial contamination and reinforcing the product's stability during storage.

Despite its promising applications, some limitations were noted, particularly textural challenges at higher concentrations, which require further formulation adjustments. Future research should focus on long-term microbial stability, advanced processing techniques for improved solubility, and application trials in real food systems to fully exploit the potential of micronized mussel powder.

Overall, the results confirm that micronized Baltic mussel powder is a safe and functional ingredient with broad applications in the food industry, provided that proper storage conditions and formulation optimizations are considered.

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Abbreviations

The following abbreviations are used in this manuscript:

CFU/g	Colony-Forming Units Per Gram
EU	European Union
ISO	International Organization for Standardization
O.	Odor
T.	Taste
QDA	Quantitative Descriptive Analysis
rpm	Revolutions Per Minute
w/*v*	Weight/Volume
µm	Micrometer (Micron)
kJ	Kilojoule
kcal	Kilocalorie

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