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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.

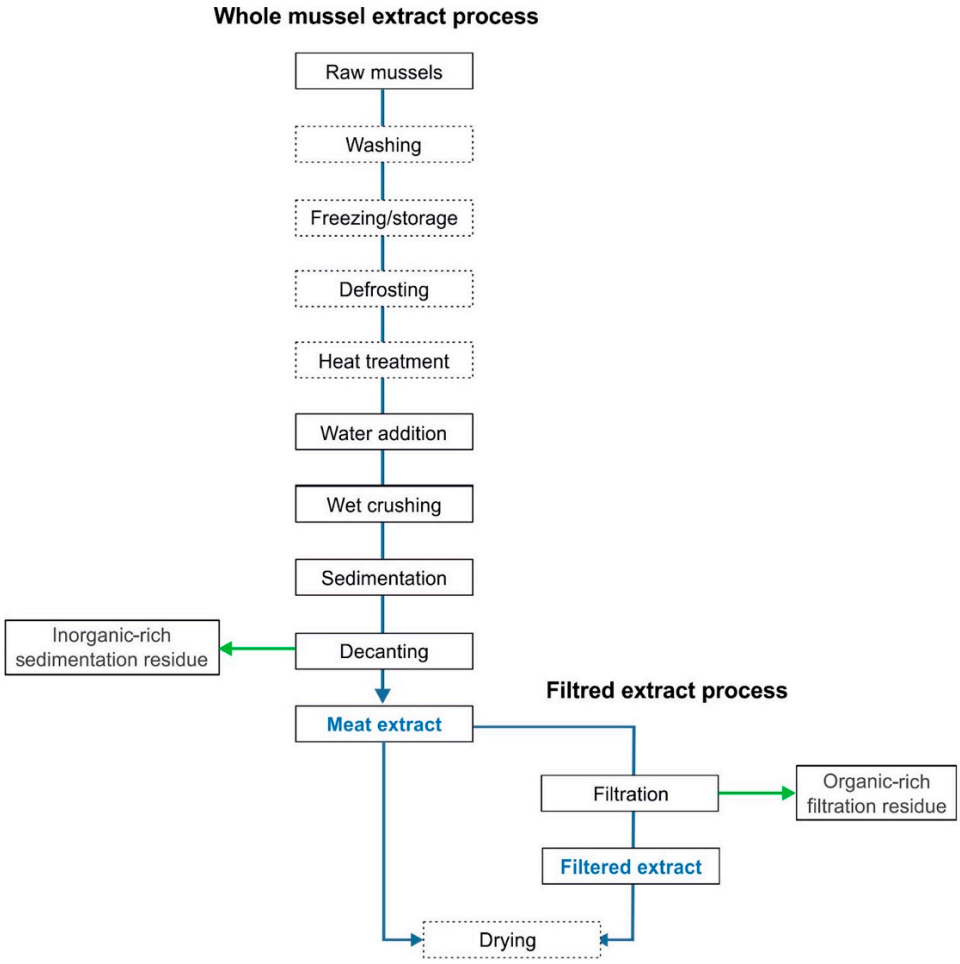

Figure 1. Schematic diagram for meat and protein separation. Dashed boxes represent optional processing steps.

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).

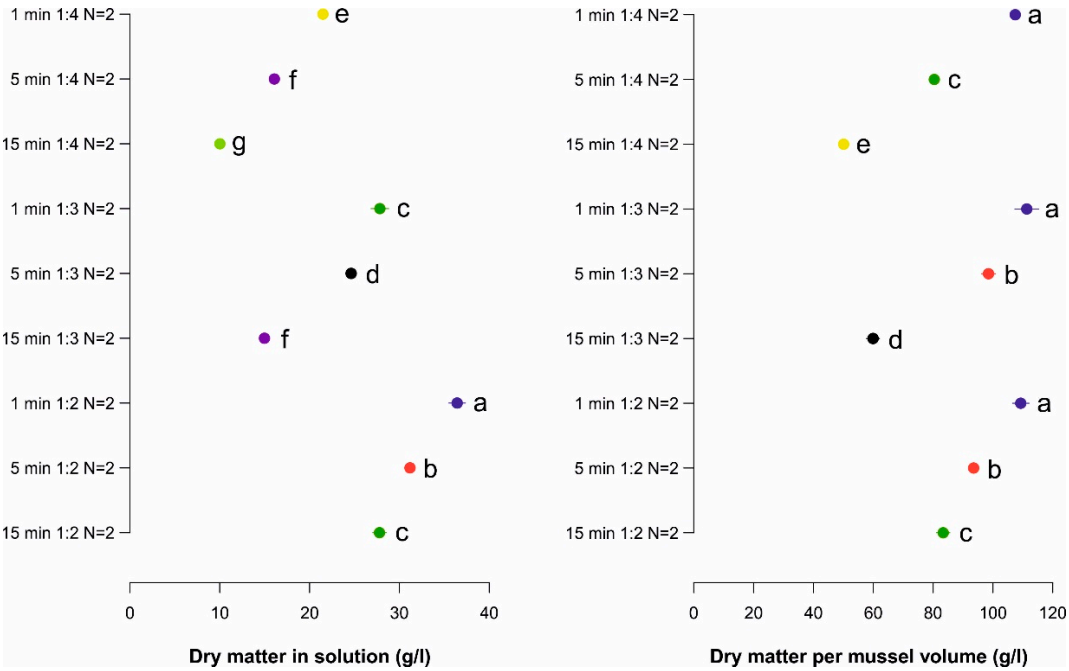

Figure 2. Average dry-matter content with 95% CI (g per liter suspension or g per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

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).

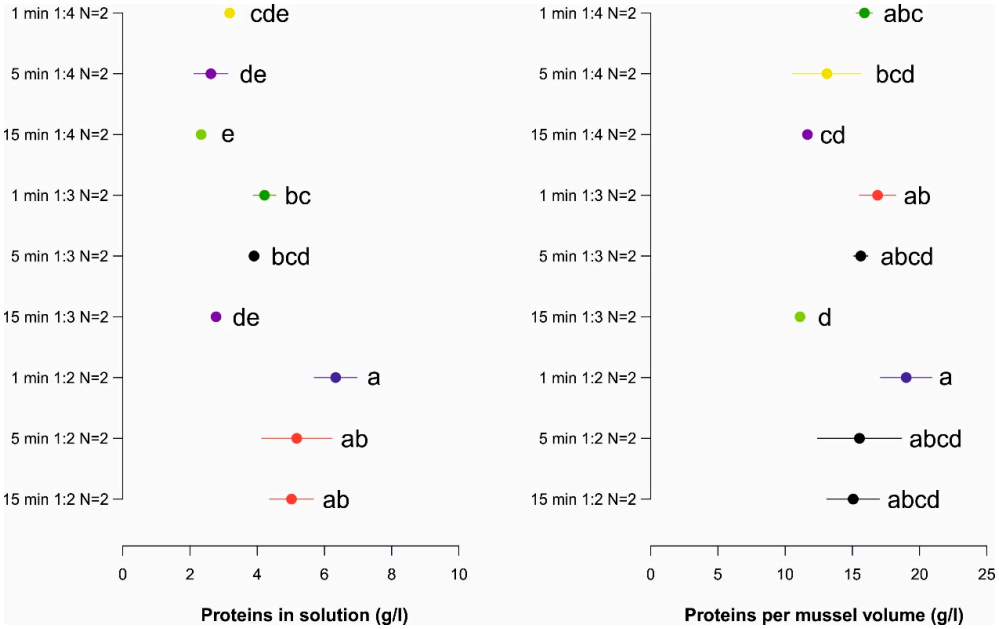

Figure 3. Average protein content with 95% CI (g per liter suspension or g per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

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).

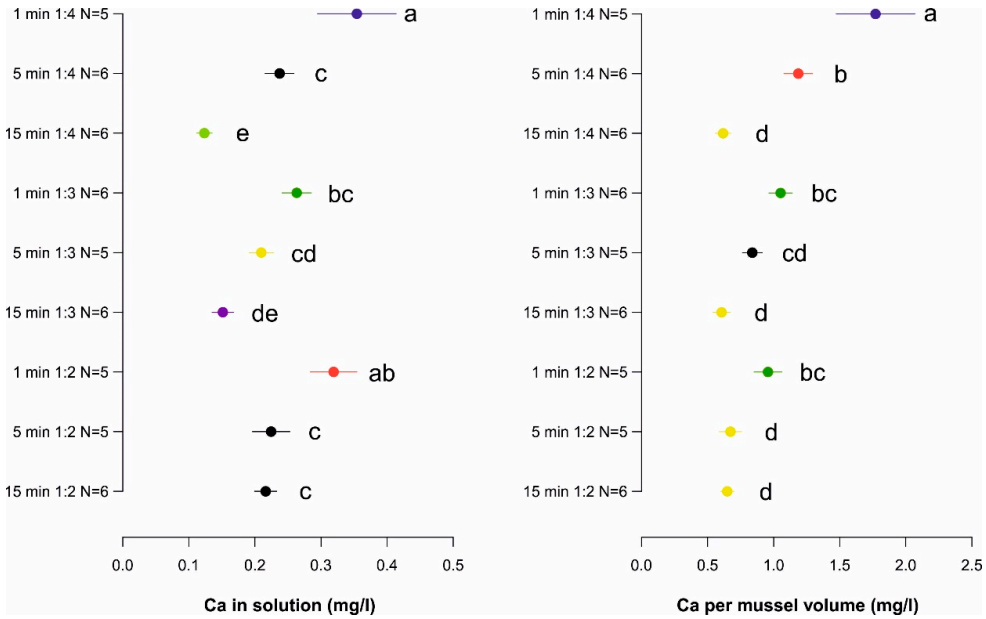

Figure 4. Average calcium ion content with 95% CI (mg per liter suspension or mg per liter mussel volume) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

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).

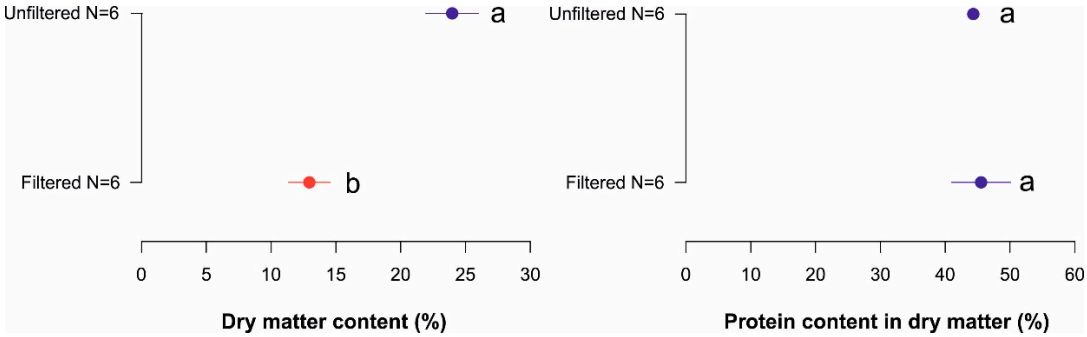

Figure 5. Average content of dry matter and the content of proteins in dry mass with 95% CI (%) among different treatments. The mean values with different letters are significantly different from each other at $p < 0.05$. N indicates the number of replicates.

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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