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(RESEARCH ARTICLE)

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Quality estimation of crustaceans and mussels based on the recovery of high value-added compounds and properties of their exoskeletons

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Abstract

Large amounts of marine by-products generated during the processing of crustaceans, such as exoskeletons of shrimp, crabs, lobsters and mussels, are often discarded as waste despite their potential to serve as valuable raw materials. Exoskeletons of these species are rich in high-value compounds such as chitin, chitosan, bioactive peptides, lipids, and minerals, all of which have vast industrial, biomedical, and agricultural applications. Therefore, efficient quality estimation of these exoskeletons is crucial to optimize the recovery of these compounds and ensure sustainable practices in the seafood industry.

Keywords: Chitin; Chitosan; Glucosamine; By-products; Crustaceans; Exoskeleton

1. Introduction

The seafood industry generates a significant amount of by-products during processing. For instance, in the case of crustaceans, the exoskeletons, which account for a large proportion of their total biomass, are typically discarded or used for low-value applications such as animal feed or fertilizer together with agricultural by - products [1-3]. The shellfish industry, which processes mussels, faces similar challenges, as the shells are typically discarded after consumption. Despite these shells being composed of valuable materials, such as chitin, chitosan, glucosamine, they remain largely untapped, representing a significant loss of potential resources.

The exoskeletons of crustaceans and mussels serve as protective armor for these organisms, providing both structural support and defense against environmental threats. These exoskeletons are complex and composed of various organic and inorganic materials, each contributing to the physical properties of the shell.

Crustacean exoskeletons are predominantly composed of chitin, a long-chain polysaccharide, which is the second most abundant biopolymer after cellulose. Chitin forms the structural backbone of the exoskeleton, providing strength and flexibility. Additionally, crustacean shells contain proteins, lipids, and minerals (primarily calcium carbonate), which contribute to the hardness and durability of the shell. Mussels have shells composed primarily of calcium carbonate, which makes up the majority of their exoskeleton. However, mussel shells also contain proteins, chitin, and polysaccharides [4,5].

Chitin, when extracted from exoskeletons, can be transformed into chitosan, a biopolymer used in diverse fields such as biomedicine, cosmetics, agriculture, and food processing. Additionally, bioactive peptides derived from exoskeleton proteins possess antioxidant, antimicrobial, and anti-inflammatory properties, making them highly valuable for nutraceuticals. Furthermore, calcium carbonate found in mussel shells has applications in biomaterials and

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environmental technology. As a result, accurately estimating the quality of these exoskeletons is essential for maximizing the recovery of these compounds and promoting sustainable practices within the seafood industry [6,7].

This article examines the relationship between the quality of exoskeletons from crustaceans and mussels and the effectiveness of extracting high-value compounds. It also examines the methods used to assess quality, the extraction techniques involved and the potential applications of these compounds. The aim is to demonstrate how a more organized approach to managing exoskeleton waste can lead the seafood industry towards enhanced sustainability and better resource efficiency.

2. Material and methods

2.1. Reagents

All the chemicals and solvents used were purchased from Sigma-Aldrich, were of analytical grade and used without purification. All solutions were freshly prepared in distilled water.

A total of 64 specimens were collected. Initially, the shells were removed from the animals. The specimens were then packed in polyethylene bags, placed on ice, transported to the laboratory, and stored in a freezer at -20°C until needed. Prior to grinding, the larger shell fragments were broken into smaller pieces. The samples were dried in a drying oven (model R. Espinar, S.L.) at 95°C until a constant weight was achieved. The dried samples were subsequently ground into smaller particles using a POLYMIX® PX-MFC 90 D mill.

pH measurements were made using a digital laboratory pH meter (model WTW pH 525) which was calibrated using certified pH= 4.0 and pH= 7.0 buffer solutions, according to the official method.

The ether extract (EE) was determined using method of Soxhlet. Approximately 2 g of solid sample were mixed with anhydrous sodium sulfate, placed in an extraction thimble and were extracted using an appropriate solvent in the Soxhlet extractor.

Ash contents were determined using dry ashing method. The samples (2 g) were ashed for about 8h until a grey ash residue had been obtained using a furnace (model P. Selecta, 3000 W) where temperature had been gradually increased from room temperature to 450°C in 1 hour. [8]. (AOAC, 2002).

The solubility of chitosan was carried in dilute solution of acetic acid. 1000 mg of chitosan obtained from the deacetylation process was dissolved in 100 mL of 1% acetic acid solution and stirred by magnetic stirrer until a homogeneous solution was obtained. The chitosan acidic solution was then filtered and the insoluble content was calculated from the weight of insoluble particles obtained on the filter and the weight of chitosan dissolved.

The water binding capacity (wbc) was calculated as follows; 10 ml of distilled water with 1000 mg of chitosan was mixed on a vortex for 15 min and centrifuged at 3500 rpm for 30 min. After centrifugation, supernatant water was poured off and the sample was weighed. WBC (%) = [Bound water (g)/Initial chitosan weight (g)] * 100. The oil binding capacity (obc) was calculated as follows; 10 ml of sunflower with 1000 mg of chitosan was mixed on a vortex for 15 min and centrifuged at 3500 rpm for 30 min. After centrifugation, supernatant oil was poured off and the sample was weighed. OBC (%) = [Bound oil (g) / Initial chitosan weight (g)] * 100.

2.2. Extraction of chitin

Deproteination (Dp): Dry samples of raw shrimp and mussel shell waste were treated with diluted solution of NaOH at different solid to solvent ratio (w/v), with constant stirring for twelve hours at different temperatures, with pH ranged from 11-13. After that, the solution was filtered and the samples were washed with distilled water to neutrality. Demineralization (Dm): Samples from deproteination process were treated with diluted solution of HCl at different solid to solvent ratio (w/v), with constant stirring for twelve hours with pH value ranged pH 1.0-2.5 at room temperature. After that, the solution was filtered and the samples were washed with distilled water to remove acid and calcium chloride. The samples were dried until constant weight was obtained. The dried sample is now known as chitin.

2.3. Chitosan production

Deacetylation (Da): The deacetylation process was conducted by soaking dried chitin prepared from demineralization in a concentrated solution of NaOH or (w/v) KOH with constant stirring for one day at room temperature. After that,

the product is known as chitosan. Chitosan was washed with tap water until neutral (pH 6.5-8.0) and dried as described in deproteination and demineralization.

Extraction of glucosamine: Chitin was grinded to fine particles and hydrolyzed with concentrated solution of HCl at different temperatures, then was filtrated to remove the solids and finally addition of absolute ethanol at cooling temperature was performed to recover glucosamine. The mixture was cooled for three weeks to crystallize and finally the solid crystals were washed with ethanol and dried in an oven for eight hours [9-12].

3. Results

Flow diagram of chitin, chitosan and glucosamine extraction protocol presented in Figure 1.

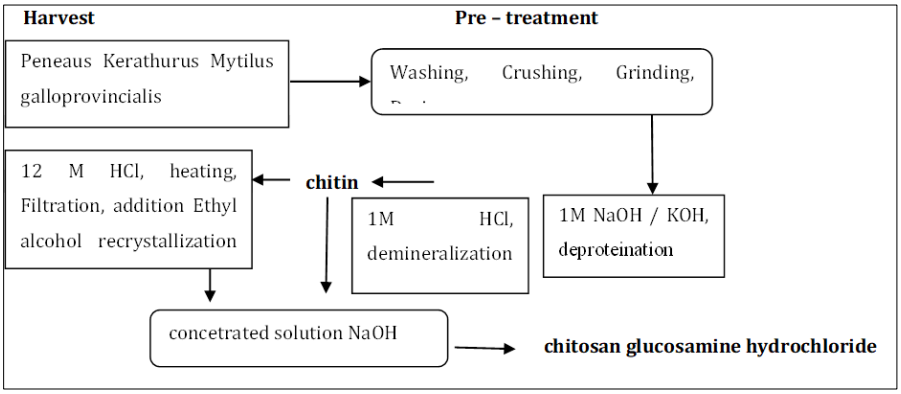

Figure 1 Process flow diagram for the extraction of chitin, chitosan, and glucosamine from the shells of Mytilus galloprovincialis and the exoskeleton of Penaeus kerathurus.

3.1. Extraction from shrimps

Recovery (%) of glucosamine and experimental details and from shrimps were presented in Table 1 and Table 2.

Table 1 Experimental details and recovery (%) of glucosamine from shrimps

exp	Chitin (g)	HCl (ml)	ratio	Molarity (M)	Time(h) / temperature (°C)	Addition water/ ethanol (ml)	Glucosamine recovery (%)	rpm
1	2	30	1/15	12 M	1.30/55	15/15	47	400
2	1	10	1/10	12 M	1.3/50	15/30	45	400
3	1	15	1/15	12 M	3/62	15/20	53	400

Table 2 Experimental details

	Experiment		
Parameters	I	II	III
Normality of solution for deproteination	1.0 N	1.0 N	1.0 N
Solid to solvent ratio	1:15	1:20	1:25
Normality of solution for demineralization	1.0 N	1.0 N	1.0 N
Solid to solvent ratio	1:10	1:10	1:10
Normality of solution for deacetylation	12.5 N NaOH	10 N KOH	12.5 N NaOH
Solid to solvent ratio	1:10	1:10	1:10
Stirring period for Dp, Dm, Da/Rpm	24 h/250	24 h/ 250	24 h/ 250
Centrifugation (wbc, obc)/Tim	3500 rpm 30 min		

Table 3 Yield % of chitin and chitosan

Experiment	Shrimp shells (g)	Chitin (g)	Chitosan (g)	Chitin yield (%)	Chitosan yield (%) (as a ratio to initial dry shell)
I	5.00	0.87	0.55	17.41	11
II	7.00	1.37	1.08	19.56	15.5
III	20.00	3.96	1.94	19.82	9.7

Table 4 Physicochemical and functional properties of chitosan (values are expressed as Mean± S.D (n=5)

Parameters	Value
pH	8.3±0.1
ether extract	0.41±0.2
Ash	0.21±0.08
solubility in 1 % acetic acid	93±2.13
Wbc	565±50.43
Obc	384±26.92
Color	Whitish slightly brow

3.2. Extraction from mussels

Table 5 Experimental details

	Experiment			
Parameters	**I**	**II**	**III**	**IV**
Molarity of solution for deproteination (Dp)	2 N KOH	1.0 N NaOH	1.0 N KOH	1.0 N NaOH
Solid to solvent ratio	1:7	1:5	1:5	1:7
Solution Molarity for demineralization (Dm)	1.0 N HCl	1.0 N HCl	1.0 N HCl	1.0 N HCl
Solid to solvent ratio	1:8	1:8	1:10	1:10
rpm	without	without	400	400
Time (d) / temperature	2 d/ room temp			

Table 6 Yield % of chitin

Experiment	Mussel shell (g)	Chitin (g)	Chitin yield (%)
I	10.00	5.77	57.70
II	13.04	7.73	59.31
III	18.60	11.67	62.72
IV	15.79	9.54	60.40

Table 7 Experimental details and recovery % of glucosamine from mussels

Experiment	Chitin (g)	HCl (ml)	ratio	Molarity of HCl (M)	rpm	Time (h) / Temperature (°C)	Glucosamine recovery (%)
1	1	15	1/15	12 M	400	1/60	11
2	2	20	1/10	12 M	400	2/50	9.5
3	1	20	1/20	12M	400	24/room temperature	9.85

Table 8 Physicochemical and functional properties of chitin

Parameters	Value
pH	8.6
ether extract	<1 %
Ash	< 1%
solubility in water	Insoluble
solubility in acetic acid	Almost completely dissolved
Color	Whitish slightly gray

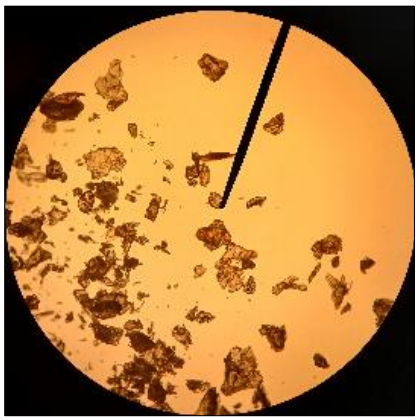

Figure 2 Crystals of glucosamine

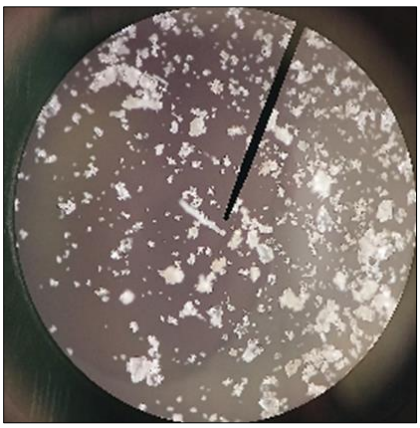

Figure 3 Chitosan

The effects of solution molarity, time, heating temperature, solid-to-liquid ratio, and agitation on the production of chitin and chitosan, as well as the acid hydrolysis of chitin, were investigated to optimize glucosamine yield and crystal quality in mussels and shrimps. The glucosamine yield after acid hydrolysis ranged from 45% to 53% in shrimps and from 9.5% to 11% in mussels. The highest recovery in shrimps was observed in experiment 3, while in mussels, it was in experiment 1. To enhance the crystallization rate, ethanol was added, as the recrystallization process was slow at room temperature. Low temperatures (5°C) combined with ethanol facilitated the formation of glucosamine crystals. The optimal glucosamine yield in shrimps was achieved with a solid-to-liquid ratio of 1:15, using a 12M HCl solution for acid hydrolysis at 62°C with agitation (400 rpm) for 3 hours (figure 2). For mussels, the best yield was obtained with the same solid-to-liquid ratio, using a 12M HCl solution for one day without heating, also with agitation at 400 rpm. The experimental parameters and details are outlined in Tables 1, 2, 5, and 7. Variations in the results can be attributed to differences in the normality of solutions (NaOH, KOH, HCl) used for deproteination, demineralization, and deacetylation, as well as variations in the solid-to-solvent ratio. Consequently, the yield percentages differ across experiments. The yields of chitin and chitosan are presented in Tables 3 and 6, highlighting differences across experiments. In shrimp-based experiments, the yield of chitin ranged from 17.4% to 19.8%, with the highest value observed in experiment III. The yield of chitosan ranged from 9.7% to 15.5%, with the highest value in experiment II. In mussel-based experiments, the yield of chitin ranged from 57.7% to 60.4%, with the highest value in experiment IV. The functional and physicochemical properties of chitin and chitosan studied in this work are summarized in Tables 4 and 8, showing a range of characteristics. In shrimp exoskeletons, chitosan (figure 3) appears whitish to slightly brown, with water-binding and oil-binding capacities of 565% and 384%, respectively. Chitosan demonstrates high solubility in 1% acetic acid (93%), with ether extract and ash content below 0.5%. In mussels, chitin is whitish to slightly gray, with ether extract and ash content below 1%.

4. Discussion

Exoskeletons of crustaceans and mussels are rich in high-value-added compounds like chitin, chitosan, glucosamine and calcium carbonate. These compounds have diverse industrial applications, ranging from pharmaceuticals to environmental applications. Chitin, is a long-chain polysaccharide (consists of N-acetylglucosamine) which is the second most abundant biopolymer after cellulose and provides the exoskeleton with its structural foundation, offering both strength and flexibility. Chitin can be chemically modified into chitosan. Chitosan has gained attention for its versatility in biomedical applications, including wound healing, drug delivery, and tissue engineering [10, 13]. Additionally, chitosan is used in water treatment due to its ability to adsorb heavy metals and dyes. Calcium carbonate exists in high concentrations in crabs and lobsters shrimps and mussels, provides hardness to the exoskeleton and has industrial applications in construction, bioplastics, and cosmetics. Glucosamine is a natural monosaccharide with the chemical formula $C_6H_{13}NO_5$. It is commonly found as glucosamine sulfate ($C_6H_{14}NO_6S$) or glucosamine hydrochloride ($C_6H_{14}NO_5Cl$) [14,15]. Glucosamine is derived from the chitin in the shells of crustaceans (shrimp, crab, and lobster) and certain marine mollusks like mussels. Glucosamine is a key building block of cartilage and contributes to the synthesis and maintenance of joint tissues. Supports the production of glycosaminoglycans and proteoglycans, which are essential for cartilage elasticity and shock absorption. Provides anti-inflammatory properties reducing inflammation in joints by modulating cytokines and enzymes involved in inflammation. Also plays a role in the repair and regeneration of connective tissues [16-18]. The quality of crustacean and mussel exoskeletons plays a critical role in determining how effectively compounds can be recovered. Several factors, such as storage conditions, treatment process and chemical composition, affect the overall quality of the exoskeletons and, by extension, the yield and purity of the extracted compounds. The physical condition of exoskeletons is another important factor in compound recovery. Exoskeletons with cracks or other damage are less suitable for extraction due to the loss of structural integrity. Chemical makers such as the degree of chitin deacetylation and mineral content are critical quality indicators. Exoskeletons with higher chitin content and a greater degree of deacetylation are preferable for chitosan extraction, as they offer better reactivity and solubility [19]. However, maximizing their potential requires efficient recovery techniques and accurate quality estimation. The results of this study underscore the variability in the yield percentages of glucosamine, chitin, and chitosan. These variations emphasize the significance of the deproteination, demineralization, hydrolysis, and deacetylation processes, which are affected by factors such as the molarity of the solutions (NaOH, KOH, HCl) and the solid-to-solvent ratio used during treatment [7, 12, 20]. The functional and physicochemical properties of the chitosan produced indicate a high-quality product. Solubility is a key property that determines chitosan's quality, with greater solubility signifying superior quality. Chitosan is soluble in dilute organic acids, such as acetic or formic acid, but is insoluble in water and basic pH solutions. Its solubility is influenced by the distribution of N-acetyl and free amino groups. In an acidic aqueous solution, the amino groups in chitosan become protonated, which enhances its solubility. Additionally, higher solubility values are associated with an increased degree of deacetylation due to the removal of acetyl groups from chitin. In this study, it was observed that the chitosan demonstrated an elevated degree of solubility when subjected to acetic acid, exhibiting a coloration that ranged from whitish to a slightly brown hue, which is analogous to the findings reported in various other investigations on the subject [5, 7, 11]. The capacities for binding

both water and oil displayed by the chitosan were found to be significantly higher when compared to the values that have been documented in prior research; however, it is noteworthy that these capacities remained well within the anticipated range established by existing literature. Furthermore, the remarkably low content of ash and ether extract served to further corroborate the purity of the chitosan, thereby indicating the successful implementation of the demineralization and deproteination processes, which collectively validate the overall quality of the final product obtained. Taking into account the various experimental parameters and the percentages of yield observed, it is proposed that the extraction process holds potential for optimization, which could ultimately lead to the attainment of enhanced yields of chitin, glucosamine, and chitosan.

5. Conclusion

The seafood and shellfish industry generates a large volume of by-products during processing. In crustaceans, the exoskeletons, which make up a considerable portion of their total biomass, are often discarded or used in low-value products like animal feed or fertilizer.The recovery and quality estimation of high-value-added compounds from crustacean and mussel exoskeletons hold immense promise for achieving sustainability and economic benefits. By adopting innovative technologies and leveraging the unique properties of these biocompounds, industries can transition to a circular bioeconomy, reducing waste and enhancing resource efficiency.

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