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Study of the Effect of Hydrochloric Acid Concentration on the Physicochemical Properties and Glucosamine Content of Vaname Shrimp Shells

Aef Permadi[1], Raudhatul Jannah[2*], Simson Masengi[1], Niken Dharmayanti[2], Resmi Rumenta Siregar[1], Jaulim Sirait[1]

[1]Fisheries Processing Technology Study Program, Jakarta Technical University of Fisheries, AUP Street, Pasar Minggu, South Jakarta, Indonesia

[2]Fisheries Resources Utilization Study Program, Jakarta Technical University of Fisheries, Ragunan Street, Pasar Minggu, South Jakarta, Indonesia

*Corresponding author: raudhatuljannah.aup@gmail.com

Abstract - Glucosamine is a monosaccharide amino compound commonly found in shrimp shell chitin and has the potential to be used as an active ingredient in cosmetic and health products. This study aims to analyze the effect of HCl concentration on the physicochemical properties and glucosamine content extracted from vaname shrimp shells. The HCl concentrations used were 5.5%, 6.5%, and 7.5%. The characteristics observed included glucosamine content, appearance and color, solubility, *loss on drying*, *loss on ignition*, pH, and yield. The results showed that the higher the HCl concentration, the higher the glucosamine content produced. The glucosamine content produced ranged from 420-937.95 mg/kg, and the HCl concentration affected the glucosamine content; the higher the HCl concentration, the higher the glucosamine content. The yield obtained ranged from 67.9-78.3%, and the HCl concentration affected the yield. The pH value of glucosamine ranged from 3.32-3.44, and the HCl concentration affected the pH value. The HCl concentration did not affect the *loss on drying*, *loss on ignation*, and solubility values.

Keywords: Extraction, Glucosamine, HCl, Shell, Vaname shrimp.

I. INTRODUCTION

Shrimp is one of Indonesia's leading fishery commodities due to its high nutritional value and strong global market demand. According to the Ministry of Marine Affairs and Fisheries [1] Indonesia's fishery trade balance recorded a surplus of USD 3.87 billion during January–September, reflecting a 7.2% increase compared with the same period in the previous year. Among the major commodities, shrimp contributed the highest export value, reaching USD 1.18 billion or 28.1% of the country's total fishery exports.

The increasing scale of shrimp production and processing generates substantial amounts of solid waste, including the head, shell, tail, and legs, which account for 30–70% of the total shrimp weight [2]. Despite its abundance, shrimp shell waste remains underutilized, even though it contains valuable components such as minerals (27.6%), proteins (34.9%), chitin (18.1%), and other minor constituents [3]. Inadequate waste management may lead to environmental problems, including foul odors and the accumulation of organic waste [4]. However, shrimp shells possess significant potential as raw material for high-value biopolymers such as chitin and chitosan. [5] stated that shrimp shell waste contains 20-50% chitin.

Chitosan is a deacetylated derivative of chitin and is the second most abundant natural polysaccharide after cellulose [6]. Research by [7] explains that chitosan is a compound derived from the deacetylation process of chitin from the shells of arthropods such as shrimp, crabs, and shellfish. It is widely used in biomedical applications due to its biodegradability, biocompatibility, and non-toxic properties [8]. In addition, chitosan forms ionic gel networks in the presence of anionic crosslinking agents such as sodium tripolyphosphate (TPP) through ionotropic gelation [9]. Chitosan derived from *Litopenaeus vannamei* shells can be further converted into glucosamine through deacetylation and hydrolysis.

Glucosamine is a biologically active compound widely recognized in pharmaceutical and cosmetic industries for its role in the biosynthesis of glycoproteins and lipids, as well as its presence in human connective tissues and cartilage [10]. Typically, glucosamine extraction involves chemical hydrolysis using strong acids such as HCl or H_2SO_4. However, previous studies have reported suboptimal yield and purity, indicating the need for improved extraction approaches. [11] demonstrated that the application of physical treatment using autoclave pressure significantly enhanced glucosamine quality, achieving a yield of 96.32%. Pressure-assisted extraction integrates the effects of acid concentration, temperature, heating duration, and applied pressure to improve hydrolysis efficiency.

Therefore, the present study aims to evaluate the quality and characteristics of glucosamine extracted from chitosan derived from *Litopenaeus vannamei* shells using HCl extraction under a pressure of 1 atm. The application of controlled pressure during extraction is expected to enhance glucosamine quality and provide a promising alternative method for effective and scalable production.

II. MATERIALS AND METHODS

2.1 Materials

The materials used in the process of producing glucosamine from vaname shrimp shells through chemical hydrolysis are shrimp shells, NaOH (99% caustic soda flakes), HCl (37% fuming E-Merck for analysis EMSURE®), distilled water, isopropyl alcohol (*geochem*), and aluminum foil.

2.2 Methods

The production of glucosamine refers to the research by [12] with modifications to the concentration of HCl solvent. The raw material for glucosamine comes from shrimp shells (*Litopenaeus vannamei*) obtained from PT. Red Ribbon, North Jakarta. This study began with the production of chitosan, referring to [13]. Glucosamine extraction was carried out by hydrolyzing chitosan in HCl with concentrations of 5.5%, 6.5%, and 7.5% with a chitosan to HCl solution ratio of 1:10 (w/v). The hydrolysis process used an autoclave at a pressure of 1 atm and a temperature of 115°C for 1 hour. The precipitated extract was then washed to remove residual water and impurities using 90% isopropyl alcohol (IPA) until it reached a pH of 3-5. The resulting glucosamine was dried in an oven at 50°C for 24 hours. The characteristic and quality test parameters of glucosamine powder include glucosamine content [14], solubility test [15], appearance and color analysis [15], Loss on Drying test [16], LoI test [17], pH test [18], and yield [19]. This study used a one-factor Randomized Block Design (RBD) with three replicates. The data obtained were analyzed using analysis of variance (ANOVA) with Tukey's post hoc test (HSD) to determine the differences between treatments. All statistical analyses were performed using SPSS 22.0 software.

III. RESULTS AND DISCUSSION

3.1 Characteristics of Glucosamine Powder

Glucosamine is a monomer derived from chitosan and possesses distinct physicochemical characteristics. In its pure form, glucosamine appears as a white powder containing hydroxyl (–OH) and ammonium chloride (NH_3Cl) functional groups on its smallest carbon unit, which contribute to its solubility in water. Appearance and color serve as observable physical parameters

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commonly used to characterize the quality of a product. The detailed characteristics of the glucosamine produced in this study are presented in Table 1.

Table 1. Characteristics of Glucosamine

Parameter	HCl concentrations		
	5.5%	6.5%	7.5%
Glucosamine content (mg/kg)	420± 6.05^{a}	628.09± 0.35^{b}	937.95± 1.39^{c}
Appearance and color	Brownish white powder	Brownish white powder	Brownish white powder
Solubility (%)	96.80±0.1^{a}	96.88±0.1^{a}	97.04±0.2^{a}
Loss on Drying (%)	0.97 ± 0.2^{a}	0.94 ± 0.3^{a}	0.89 ± 0.4^{a}
Loss on Ignation (%)	0.23±0.4^{a}	0.30±0.2^{a}	0.31±0.4^{a}
pH value	3.32±0.03^{a}	3.34±0.01^{a}	3.44±0.01^{b}
Yield (%)	78.3 ± 1.4^{a}	74.7 ± 1.9^{b}	67.9 ± 2.3^{b}

Note: Different superscript letters (a, b, c) in the column indicate significant differences ($p < 0.05$).

3.2 Glucosamine Content

The highest glucosamine concentration was obtained using 7.5% HCl, yielding 937.95 ± 1.39 mg/kg. In contrast, the lowest concentration, 420 ± 6.05 mg/kg, was produced using 5.5% HCl, while the 6.5% treatment resulted in 628.09 ± 0.35 mg/kg. ANOVA analysis showed that HCl concentration had a significant effect on glucosamine levels ($p < 0.05$). These results indicate that glucosamine yield increased with increasing HCl concentration. According to [20], higher concentrations of HCl enhance the cleavage of glycosidic bonds in chitin, thereby producing greater amounts of glucosamine monomers.

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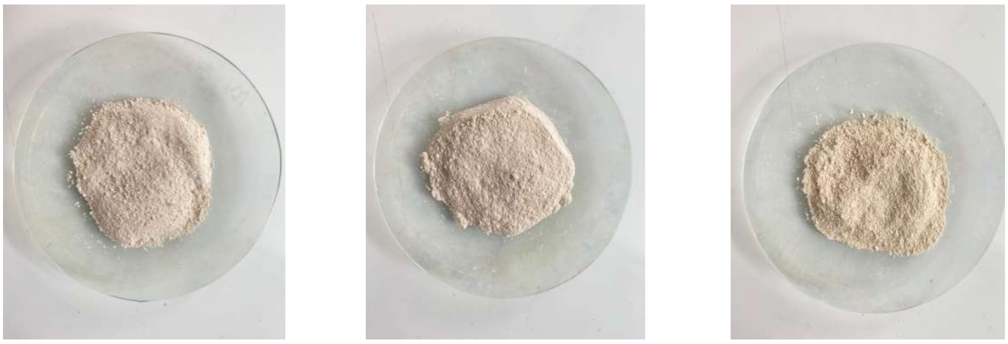
3.3 Appearance and Color



(A) (B) (C)

Figure 1 Hydrochloride glucosamine powder

Notes:

(A) Glucosamine extraction with 5.5% HCl concentration;

(B) Glucosamine extraction with 6.5% HCl concentration;

(C) Glucosamine extraction with 7.5% HCl concentration.

In this study, the extracted glucosamine appeared as a slightly brownish-white powder (Figure 1). The quality of glucosamine can be visually assessed from its appearance after the grinding and refining stages. According to [15] good-quality glucosamine is characterized by a fine, well-refined powder. The glucosamine obtained in this research met this criterion, with an approximate particle size of 80 mesh. The visual characteristics, including appearance and color, were generally consistent with standard hydrochloric glucosamine. However, the slight brown coloration likely resulted from the Maillard reaction, a non-enzymatic browning process that occurs between reducing sugars and amino acids under heat [21].

Glucosamine derived from chitosan is easily pulverized because the monomeric bonds in chitosan are fully broken during hydrolysis. Variations in HCl concentration had a clear effect on the final color of the product. Samples extracted using 5.5% HCl showed a lighter color and were closer to the standard appearance of high-quality glucosamine. In contrast, samples treated with 6.5% and 7.5% HCl exhibited progressively darker shades. This darkening is likely associated with increased degradation of organic components and intensified browning under stronger hydrolysis conditions.

3.4 Solubility

The main indicator used in determining the success of chitosan/chitin in producing glucosamine hydrochloride is its high solubility in water at a temperature of 27°C. Treatment with HCl concentrations of 5.5%, 6.5%, and 7.5% in this study generally produced glucosamine that was soluble in distilled water. Analysis of variance showed that the production of glucosamine with the addition of different HCl concentrations did not have a significant effect on the solubility value of the glucosamine produced ($p>0.05$). The highest solubility test result was obtained using a 7.5% HCl concentration, which was 97.04±0.2%, while the lowest solubility was obtained using a 5.5% HCl concentration, which was 96.80±0.1%, and a 6.5% HCl concentration, which was 96.88±0.1%.

The solubility of glucosamine in this study was higher than that reported by [22], who found glucosamine solubility to be 96.33%. [15] stated that glucosamine from chitosan has good solubility. The higher the temperature of the solvent used, the faster the solubility of the substance will occur. When chitosan is hydrolyzed with HCl, the process no longer breaks the acetyl group but only cuts the chitosan polymer into smaller units so that the Cl- ions from HCl can more easily bond with the amino group of

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chitosan to form NH_3Cl. The presence of a hydroxyl bond between O-H and NH_3Cl causes glucosamine hydrochloride to be soluble in water.

According to [23] glucosamine appears white when viewed visually. When glucosamine is dissolved in water, the solution tends to be clear and colorless. This differs from the color of hydrolyzed glucosamine in this study, which is clear but tends to be yellowish. This is thought to occur because the original color of the sample (chitosan) still contains a small amount of pigment or impurity protein. The results of [20] study show that glucosamine is insoluble in water at HCl concentrations of 20, 25, and 10%. The insolubility of glucosamine indicates that the chitosan has not been completely hydrolyzed. This is due to several factors, one of which is the concentration of acid used. Using HCl concentrations that are too high or too low, as well as inappropriate temperature and pressure in the autoclave, can cause the chitosan to not be hydrolyzed properly.

3.5 Loss on Drying (LoD)

Loss on Drying (LoD) analysis is performed to determine the amount of water and volatile components present in a sample by subjecting it to drying under controlled conditions. As shown in Table 1, the highest LoD value was obtained at an HCl concentration of 5.5% (0.97%), while the lowest value was observed at 7.5% HCl (0.89%). Lower LoD values indicate higher glucosamine purity, as they reflect reduced moisture and fewer volatile impurities.

The application of pressure-assisted hydrolysis was found to reduce the LoD value by 0.09%. This result aligns with the findings of [22], who reported that pressure hydrolysis decreased the LoD value by 0.08%. The ANOVA results indicated that neither HCl concentration nor pressure had a significant effect ($p > 0.05$) on the LoD values. Overall, the LoD values obtained in this study complied with the United States Pharmacopeia [23] specification, which requires an LoD of ≤1%.

3.6 Loss on Ignation (LoI)

Loss on Ignition (LoI) represents the inorganic residue remaining after the combustion or oxidation of organic components in a sample. LoI is part of proximate analysis and is used to assess the nutritional composition of a product, particularly its total mineral content. As presented in Table 1, the highest LoI value was obtained at an HCl concentration of 7.5% (0.31 ± 0.04%), while the lowest value was recorded at 5.5% HCl (0.23 ± 0.04%). The analysis of variance indicated that HCl concentration had no significant effect on LoI values ($p > 0.05$).

The LoI values of the glucosamine hydrochloride produced in this study did not meet the [23] specification, which requires an LoI of ≤0.1%. The elevated residue levels are likely attributable to the high calcium content in shrimp shells. Thus, further optimization of the demineralization process is necessary, particularly due to the substantial $CaCO_3$ content in shrimp shells [24].

3.7 pH Value

The pH value, or degree of acidity, is used to indicate the acidity or alkalinity of a substance or solution, and is an important parameter in the production of glucosamine hydrochloride. The pH measurements obtained in this study are presented in Table 1. The lowest pH value was observed at an HCl concentration of 5.5% (3.32 ± 0.03), while the highest pH value occurred at 7.5% HCl (3.44 ± 0.01), confirming the acidic nature of the product. ANOVA results showed that variations in HCl concentration had a significant effect on the pH of the resulting glucosamine hydrochloride ($p < 0.05$). A decrease in pH value was associated with increasing acid concentration during the extraction process.

The pH values of glucosamine produced using the pressure-assisted hydrolysis method remained acidic but were within the acceptable consumption range recommended by [23], the [25], and the [26], namely between pH 3 and 5. The relatively low acidity is attributed to suboptimal neutralization using isopropyl alcohol (IPA) [11]. The acidic pH of glucosamine results from the release of excess H^+ ions during chemical hydrolysis with concentrated HCl. An increase in H^+ ion concentration may occur due to heightened acidity caused by H^+ ion extraction through the membrane. [27] reported that glucosamine in acidic form exhibits good stability.

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3.8 Yield

Yield is an important parameter that reflects the final mass of a material obtained after processing, and higher yields indicate more efficient production. In the present study, the yield of glucosamine hydrochloride ranged from 67.9% to 78.3%. The highest yield was obtained at an HCl concentration of 5.5% (78.3 ± 1.4%), whereas the lowest yield was recorded at 7.5% HCl (69.7 ± 2.3%). ANOVA results showed that HCl concentration significantly affected the glucosamine yield ($p < 0.05$). The yields obtained were higher than those reported by [12], who achieved a yield of 78.22% using 5% HCl at 0.45 atm. Meanwhile, [15] reported a yield of 69.80% using 8% HCl with an autoclave at 1 atm/15 psi for 60 minutes.

The reduction in yield at higher HCl concentrations is attributed to excessive hydrolysis, which can lead to degradation and the formation of impurities, thereby lowering glucosamine recovery [28]. Increasing the acid concentration intensifies chitin breakdown, potentially causing over-degradation that reduces the final glucosamine yield.

The principle of pressure-assisted hydrolysis involves the combined effects of acid concentration and pressure to accelerate the conversion of chitosan into glucosamine. The mechanism is closely related to reaction kinetics, which are influenced by surface area, concentration, temperature, pressure, and reaction time. Reaction rate describes the change in reactant concentration over time, with reactants decreasing and products increasing as the reaction progresses. The use of a pressure autoclave offers advantages, such as shorter processing time and reduced production costs [29].

Extraction under pressure accelerates hydrolysis due to a puffing mechanism. Puffing refers to the expansion of materials caused by heat, during which gas is introduced into the sample, expands, and is released, resulting in disruption of the cellular or polymer structure [30]. This structural breakdown facilitates the conversion of chitosan into glucosamine. Hydrochloric acid (HCl) contributes to this process by cleaving the β-1,4-2-acetamido-2-deoxy-D-glucose linkages in chitosan. Chitosan contains hydroxyl (–OH) and amine ($–NH_2$) groups, and the chloride ion (Cl^-) from HCl interacts with the protonated amine group (NH_3^+) to form glucosamine hydrochloride ($NH_3^+Cl^-$).

IV. CONCLUSION

This study demonstrates that variations in HCl concentration during the hydrolysis process significantly influence the physicochemical characteristics of the resulting glucosamine hydrochloride. The glucosamine produced from shrimp shells met the required standards for yield, appearance and color, loss on drying, and pH. However, the loss on ignition values did not comply with the United States Pharmacopeia (USP) specifications, indicating the need for further optimization of the demineralization process. ANOVA results confirmed that HCl concentration had a significant effect on glucosamine content, yield, and pH, with higher acid concentrations contributing to increased glucosamine content.

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