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Hydrochloric acid producing higher purity of glucosamine than sulfuric acid: a comparison study with different detection approaches

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In this study, HCl and H_2SO_4 were used to compare the best conditions for producing glucosamine from the dry chitin of shrimp shells. The results showed that for HCl hydrolysis, the most favored conditions were 12 M, 80 °C, 2 hours, and a chitin-to-acid volume ratio of 1:20, whereas, for H_2SO_4 hydrolysis, the most favored conditions were 6 M, 90 °C, 5 hours, and a chitin to acid volume ratio of 1:20. HCl produced pured glucosamine with a higher yield than H_2SO_4. Additionally, our findings indicated that glucosamine could be detected using a UV detector with a weak signal, whereas we recommended using an RI detector for a comparably stronger signal. Our production maximum yield of 283.9 ± 13.8 mg Gln g^{-1} chitin from HCl hydrolysis was comparable to that of other studies, with a comparison between using HCl and H_2SO_4 being highlighted.

Keywords: acid hydrolysis, comparison, shrimp chitin, glucosamine hydrochloride, glucosamine sulfate.

INTRODUCTION

Glucosamine (2-amino-2-deoxyglucose, chitosamine, or Gln) is a monosaccharide compound normally produced and found in the human body. It is used as a precursor in the production of macromolecules such as proteoglycans, glycoproteins, glycosaminoglycans, hyaluronic acid, etc.[1]. These substances, which are an essential component in synovial fluid and almost every tissue of the body, especially the cartilage at the end of the joint, make cartilage flexible and can support the movement of bones and joints[2]. As a result, glucosamine plays an important role in joint function. Glucosamine has also been reported to have inhibitory effects on many inflammatory agents, antioxidants, antibacterial, and anticancer[3–6]. As a result, glucosamine is commonly used as a dietary supplement to treat osteoarthritis[7]. Glucosamine products for arthritis are usually formulated as the hydrochloride salt, glucosamine sulfate ($Gln{\cdot}SO_4^{2-}$), glucosamine phosphate, and glucosamine chloralhydrate, or N-acetylglucosamine (NAG), which makes the molecular size and other properties of glucosamine different, such as stability, when made into a product[8]. Glucosamine is available in two forms: glucosamine hydrochloride (Gln·HCl) and $Gln{\cdot}SO_4^{2-}$. Both forms have been studied extensively, but there is still some debate about which form is more effective in terms of absorption and bioavailability for use in the treatment of patients with osteoarthritis, a degenerative joint disease that affects millions of people worldwide[9–10]. Gln·HCl is believed to be more stable than $Gln{\cdot}SO_4^{2-}$, making it a popular choice for dietary supplements. However, studies have shown that Gln·HCl is not as readily absorbed by the body as $Gln{\cdot}SO_4^{2-}$[11]. On the other hand, $Gln{\cdot}SO_4^{2-}$ is considered to be more easily absorbed by the body than Gln·HCl[12]. Some studies have suggested that $Gln{\cdot}SO_4^{2-}$ maybe more effective at reducing pain and improving joint function in people with osteoarthritis[13–15].

Glucosamine is an amino sugar that occurs in deacetylated and depolymerized forms in chitin[16]. Chitin (Fig. 1.)

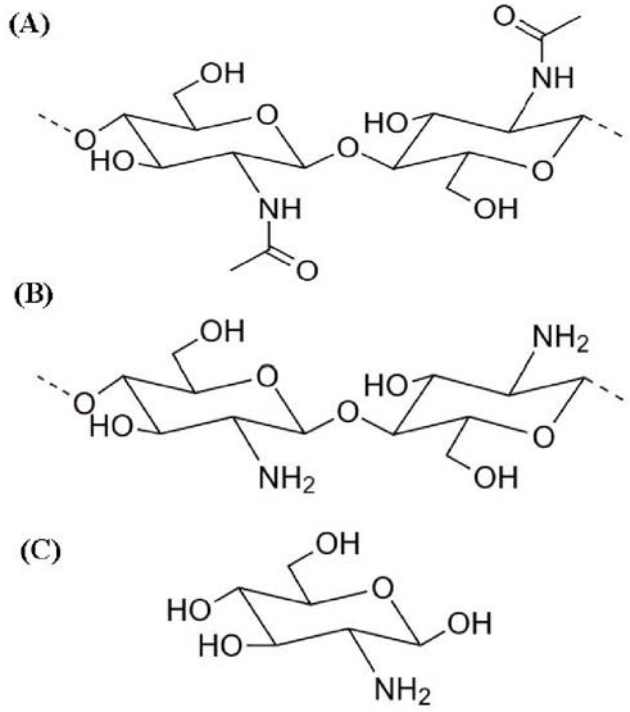


Figure 1. Chemical structure of chitin (a) and its deacetylated form, chitosan (b). Acid hydrolysis hydrolyzes the glycosidic bond in chitosan, releasing glucosamine (c)

is the second most common polysaccharide in nature after cellulose. It is often found in the exoskeleton of various lower organisms, including shrimp, crabs, insects, and the main constituent of almost all fungal cell walls[17]. Glucosamine can be produced from shrimp shells by using concentrated acids as a catalyst or by using enzymatic bacterial chitinase in the hydrolysis process. Acidic hydrolysis of chitin promotes both the cleavage of the glycosidic linkage (depolymerization) and the N-acetyl linkage (deacetylation) reaction to yield glucosamine[18]. High acid concentrations and high temperatures led to chitin depolymerization. Whereas the use of only acid concentrations in the hydrolysis process, chitin was

only moderately influenced by the acid concentration because the acid mainly attacks the β-1-4 linkage from the amorphous regions, and in a lower degree, the acetamide group linked in the C-2 carbon will lead to deacetylation reactions[19]. Based on the benefits of Gln·HCl and $Gln{\cdot}SO_4^{2-}$, hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) were produced from the acid hydrolysis of chitin. HCl is a representative of a monoprotic acid, which means it can donate only one proton (H^+) per molecule when it dissociates in water, while H_2SO_4 is a representative of a diprotic acid, meaning it can donate two protons (H^+) per molecule when it dissociates in water[20]. Therefore, the hydrolysis of monoprotic and diprotic acids can lead to different outcomes due to the number of protons they can donate. Additionally, commercially available $Gln{\cdot}SO_4^{2-}$ is produced by the reaction of Gln·HCl and sodium sulfate (Na_2SO_4), resulting in crystals containing chloride ions (Cl^-) and sulfate ions (SO_4^{2-}) impurities[21]. This is probably not the only type of $Gln{\cdot}SO_4^{2-}$. Therefore, in this study, H_2SO_4 was used in the production of $Gln{\cdot}SO_4^{2-}$ to remove Cl^- from the system and to study the amount of $Gln{\cdot}SO_4^{2-}$ obtained from the production.

There are several methods to determine the glucosamine yield from the hydrolysis process. The most commonly used techniques are Gravimetric Method, DNS Method, and High-Performance Liquid Chromatography (HPLC). The gravimetric method involves the direct measurement of the mass of purified glucosamine. It includes steps such as hydrolysis of chitin, isolation of glucosamine, and subsequent drying to obtain the pure compound. The mass of the obtained glucosamine is then measured using an analytical balance. The yield is calculated by comparing the mass of the obtained glucosamine with the chitin. The DNS (3,5-dinitrosalicylic acid) method is used to determine the concentration of reducing sugars, including glucosamine[22]. When glucosamine interacts with DNS reagents, resulting in a color change. The intensity of the color change is measured using a spectrophotometer, and the concentration of glucosamine is determined based on a calibration curve obtained with known standards. The DNS method is a widely used and relatively simple spectrophotometric method. HPLC can be utilized to separate and quantify glucosamine in a mixture by using detectors such as UV detection, refractive index detection (RI), fluorescence detection, or mass spectrometry[23]. HPLC-UV and HPLC--RI are popular techniques used to measure glucosamine concentrations. HPLC-UV relies on the glucosamine's absorbance properties at a specific wavelength. HPLC--RI measures changes in refractive index caused by the presence of the analyte which is particularly useful for compounds that lack chromophores or have low UV absorbance[24]. Thus, the combined use of these methods allows validation of the results and ensures the validity and reliability of the measured glucosamine output. In addition, using multiple techniques reduces the error or bias that may arise from using a single method.

Thailand has successfully developed the seafood export industry and is now one of the world's largest seafood exporters, especially shrimp exports[25]. According to data from Thailand's chilled & frozen seafood industry in 2018, shrimp is exported at the highest rate than fish (30.5%) and cephalopod (18.8%), resulting in more shrimp waste than other types of seafood[26]. Byproducts of shrimp waste in the form of heads and shells account for approximately 48.5%–56% of the shrimp raw material weight, depending on the species[27]. These shrimp byproducts are usually applied for low-value animal feeds and biological fertilizers. As a result, it is particularly critical to convert by-products into high-value products such as chitin, chitosan, and glucosamine to promote the community economy, increase income for farmers and fishermen, enhance the value of shrimp waste, thereby reducing the impact on the environment, and solve the problem of sustainable shrimp waste management. This study aimed to produce glucosamine from shrimp shell chitin and to find the optimal conditions for acid hydrolysis of HCl and H_2SO_4 to four variables (temperature, reaction time, acid ratio, and acid concentration). Gravimetry, DNS, HPLC-UV, and HPLC-RI are four detection methods used in comparison.

MATERIALS AND METHODS

Materials and Chemicals

Shrimp shell dry chitin was purchased from Sin-Udom (Sinudom Agriculture Products Limited Partnership, Thailand). 37% hydrochloric acid (Qrec, New zealand), 98% sulfuric acid (Ajax Finechem, Australia), 95% alcohol (Lpure, Thailand), 3,5-dinitrosalicylic acid (Himedia, India), sodium hydroxide pellets (Ajax Finechem, Australia), sodium potassium tartrate (Kemaus, Australia), and 65% perchloric acid (CARLO ERBA Reagents, France) were analytical reagent grade. Acetonitrile (RCI Labscan, Ireland) and methanol (RCI Labscan, Ireland) were high-performance liquid chromatography (HPLC) grade. 98% D-glucosamine hydrochloride (Acros Organics, USA) and N-acetylglucosamine (Sigma-Aldrich, USA) were used as standards.

Acid hydrolysis parameters on glucosamine production

Acid hydrolysis was modified from Benavente et al. (2015)[28], as shown in Figure 2. Optimal conditions for HCl and H_2SO_4 hydrolysis were examined by several experimental parameters, including hydrolysis temperature (70, 75, 80, 85, and 90 °C), hydrolysis time (1, 2, 3, 4, 5, 6, 7, and 8 hours), a ratio of raw material to acid volume (w/v, 1:5, 1:10, 1:15, 1:20, 1:25, 1:30, and 1:35), and acid concentration (10, 11, and 12 M HCl and 4, 5, and 6 M H_2SO_4). The mixtures obtained from each experiment were cooled to room temperature and filtered with Whatman No. 1 filter paper to remove the solid particles present in the solution. Added 3V of 95% ethanol and incubated at –10 °C for 24 hours. The solid crystals were washed with 95% ethanol 3 times and dried at 50 °C for 12 h.

Glucosamine detection using gravimetric method

The solid crystals, dried at 50 °C for 12 h, were directly weighed and used in the calculation of the yield of glucosamine in mg Gln g^{-1} chitin.

Glucosamine detection using DNS method

The DNS method was used in a modified method from Miller (1959)[29]. Briefly, glucosamine crystals from

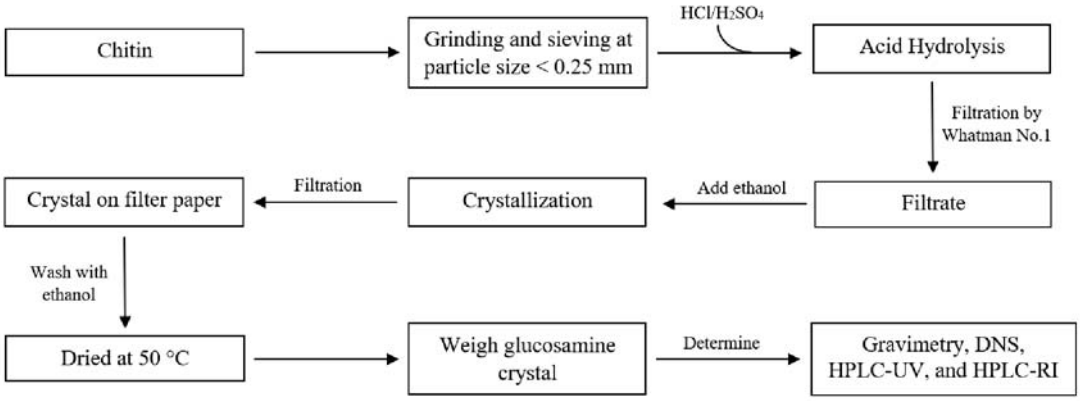


Figure 2. Schematic representation of the main steps in acid hydrolysis of chitin

each experimental parameter were dissolved in water and segmented for DNS analysis. One ml of standard or sample solution and 1 ml of DNS reagent (dissolve 16 g of sodium hydroxide (NaOH) in 200 ml distilled water and then add 10 g of 3,5-dinitrosalicylic acid under continuous stirring until completely dissolved, add 300 g of sodium potassium tartrate, and the remaining volume was filled with distilled water to make 1,000 ml) were added to test tubes. The mixture was vortexed and incubated in a boiling water bath for 5 min. After the solution has cooled to room temperature, adjust the volume with 3 ml distilled water and vortexed solution. The absorbance was then measured with a spectrophotometer at a wavelength of 540 nm.

Glucosamine detection using High-performance liquid chromatography (HPLC) method

Glucosamine was determined by high-performance liquid chromatography (HPLC) according to Magaña et al. (2014)[30] method with minor modifications. The Inertsil ODS-3 C18 (150 × 2.1 mm, 5 μm) column was used for analytical separation. The mobile phase was sodium perchlorate (50 mM perchloric acid, adjusted to pH 6.5 with 1 M sodium hydroxide)–acetonitrile (99:1, v/v) at a flow rate of 0.8 mL min^{-1}. The injection volume was 10 μl. The column temperature was maintained at 35 °C, the run time was set at 15 min, and quantitation was performed using peak area counts. Detection was performed using a UV-visible detector at 193 nm or a refractive index (RI) detector, separately.

Fourier Transform Infrared Spectroscopy (FTIR)

Chitin, standard glucosamine hydrochloride, and glucosamine obtained from HCl and H_2SO_4 hydrolysis experiments under the best conditions of production were determined by FTIR spectra. The scanning range between 400–4000 cm^{-1} was used.

Statistical analysis

All experiments were performed in triplicates. Yield measurements under different conditions were tested for significant differences at the 95% confidence level by one way ANOVA and Duncan tests using SPSS version 23 for Windows (SPSS software, New York, USA).

RESULTS AND DISCUSSION

Optimization of Acid Hydrolysis Parameters on Glucosamine

The hydrolysis of the chitin polymer chain by concentrated HCl and H_2SO_4 is an acid-catalyzed cleavage of the glycosidic linkage that generates the new reducing sugar or glucosamine[31]. The cleavage mechanism of the β-1,4-O-glycosidic bond involves the following steps in Figure 3, including (1) Protonation of glycosidic bonds, protons from acids protonates to oxygen on glycosidic bonds. The positively charged oxygen atom becomes more electronegative; it increases the electrophilicity of the carbonyl carbon. (2) The water in the reaction provides protons to molecules of electrophilic carbon that have been detached from the chitin polymer chain, resulting in the formation of a NAG. (3) The oxygen atom of the carbonyl group is protonated by acids to

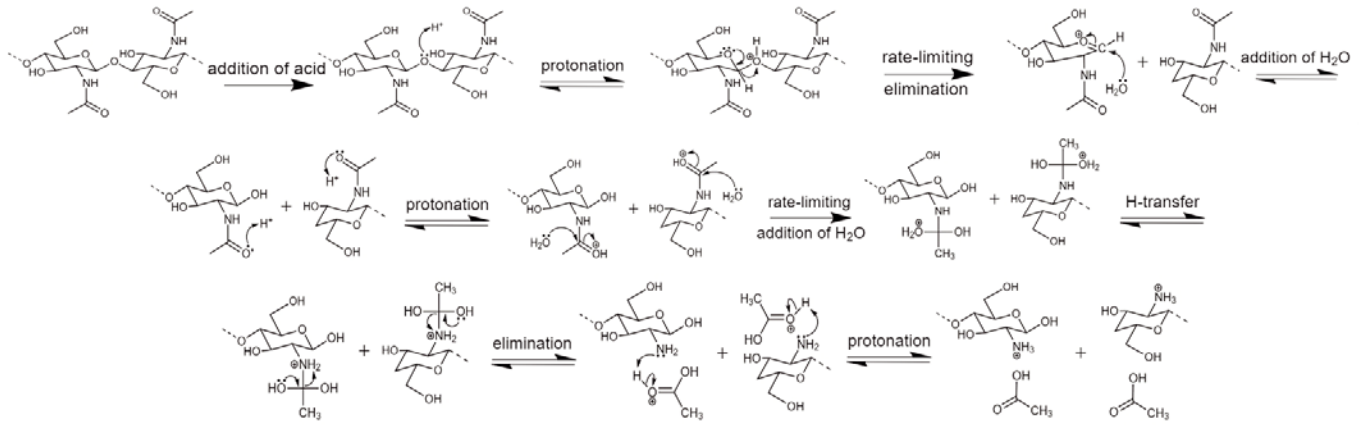


Figure 3. Schematic illustration of the proposed reaction mechanism for the acid-catalysed hydrolysis

form electrophilic carbon. (4) The water molecule attacks the electrophilic carbon, resulting in the formation of a tetrahedral intermediate. (5) Protonation at the -OR group by an acid for the molecule elimination of the -OR group. Carboxylic acids and reducing sugars are formed. Optimization for acid hydrolysis is therefore important in the production of glucosamine. The effect of acid hydrolysis on glucosamine yields depends on four parameters, including temperature, time, ratio of raw material per acid volume, and acid concentration. The glucosamine yields from each parameter were determined by the gravimetric method, the DNS method, and the HPLC method with UV (HPLC-UV) or RI (HPLC-RI) detector.

The hydrolysis temperature is important for the degradation ability of HCl and H_2SO_4. As the hydrolysis temperature increases, the glucosamine yield increases due to the higher activation energy, resulting in the reaction going to a near transition state. The effect of the optimal hydrolysis temperature in HCl ranging from 70 to 90 °C was shown in Figure 4A. The glucosamine yields from all methods tended to increase at temperatures above 70 °C and peaked at 80 °C due to chitin being completely hydrolyzed to form glucosamine. When the hydrolysis temperature rises above 80 °C, the glucosamine yield decreases due to the Maillard reaction of the amino group with the aldehyde group of aldoses which leads to thermal degradation of glucosamine[32]. Likewise, with H_2SO_4 hydrolysis (Fig. 5A), the glucosamine yield

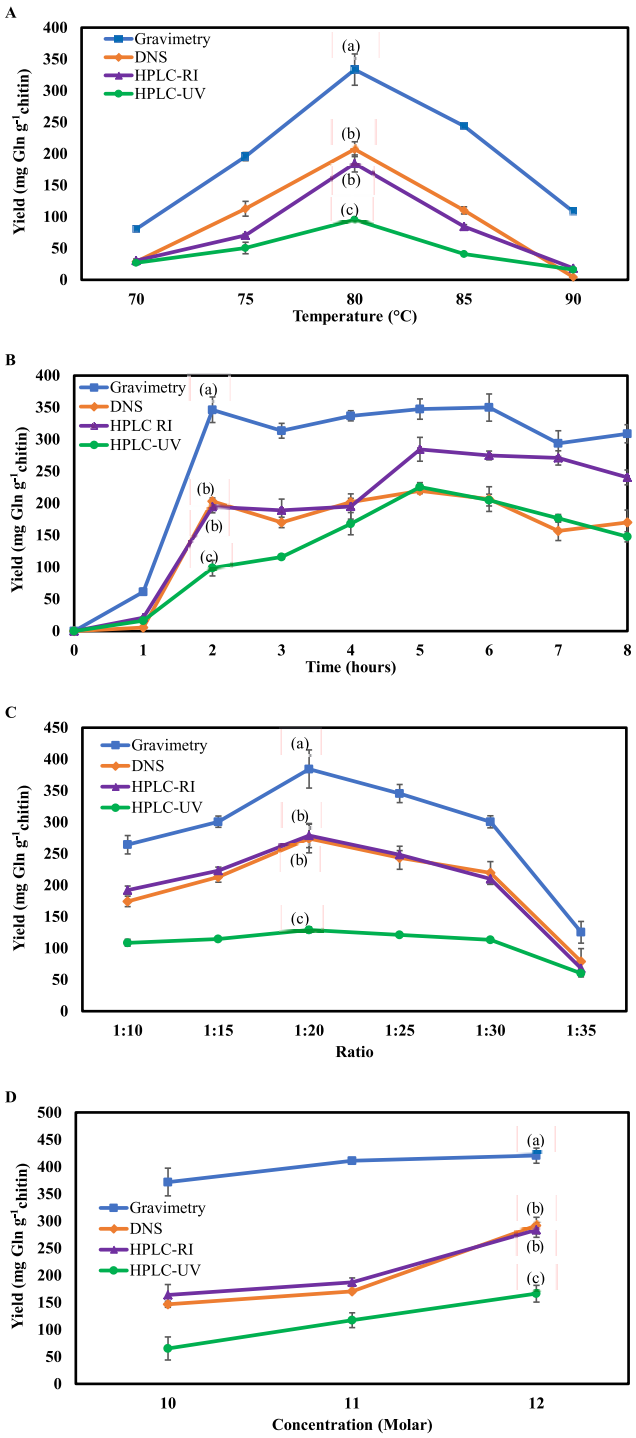


Figure 4. Effect of chitin hydrolysis by hydrochloric acid at various conditions to produce glucosamine. (A) hydrolysis temperature (B) hydrolysis time (C) ratio of raw material to acid volume (D) hydrochloric acid concentration

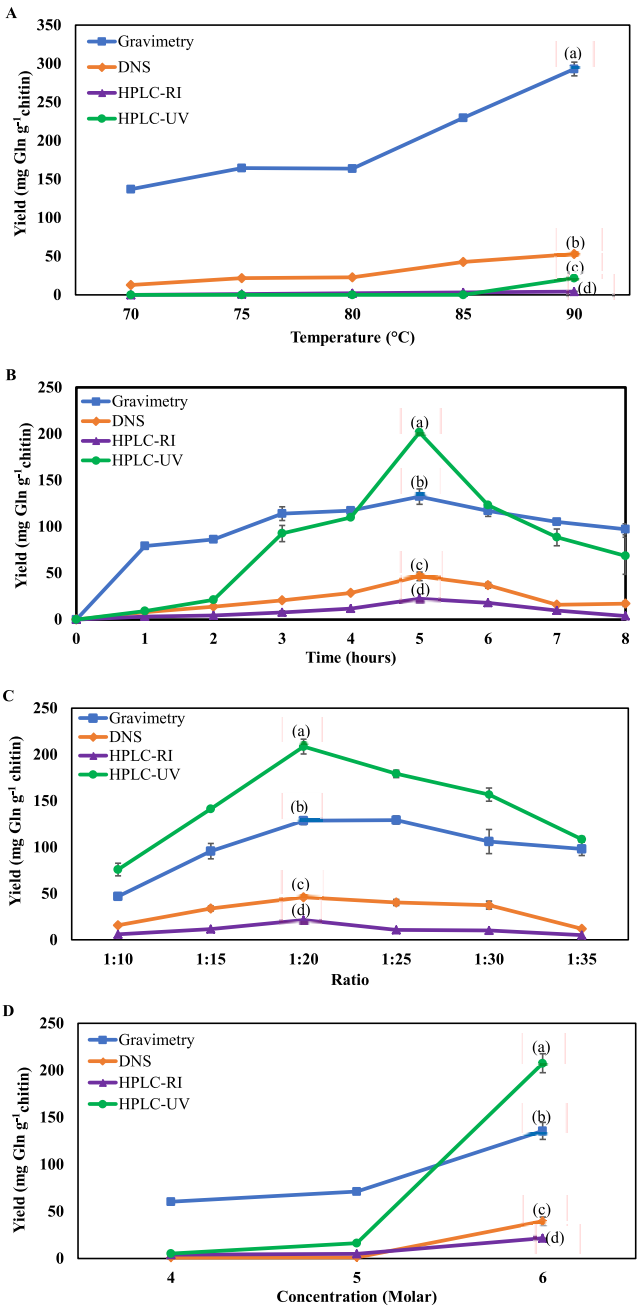


Figure 5. Effect of chitin hydrolysis by sulfuric acid at various conditions to produce glucosamine. (A) hydrolysis temperature (B) hydrolysis time (C) ratio of raw material to acid volume (D) hydrochloric acid concentration

peaked at 90 °C significantly. Our findings are consistent with studies that reported that 80 °C was an optimum temperature for the glucosamine yield by HCl[33–35] and 90–100 °C for the glucosamine yield by H_2SO_4[36–37].

Four detection techniques were used to compare the yield after the chitin was hydrolyzed to form glucosamine. Firstly, the gravimetric method, which measures the dry mass of all substances, including glucosamine and glucosamine oligomers, with the ability to precipitate in 70% ethanol, produced the highest glucosamine yields. At this level of ethanol, no salt co-precipitates. Second, the value of the glucosamine yield obtained using the DNS method was less than the yield obtained using gravimetry. It does so because the reagent's 3,5-dinitrosalicylic acid (DNS) only reacts with substances that contain a free anomeric carbon at C-1 (hemiacetal), aldehyde groups (–CHO), or ketone groups (–CO), or both, in reducing sugars[38]. Glucosamine and its oligomers, which are reducing sugars, are oxidized, resulting in the conversion of DNS (yellow) to 3-amino-5-nitrosalicylic acid (orange-red), and the oxidation of the reducing to an oxidized sugar[39]. So, the color intensity varies with the concentration of glucosamine and glucosamine oligomers that interact with DNS. It is interesting to note that the glucosamine yield obtained from HPLC analysis was lower than that of the gravimetric method and the DNS method because the HPLC method uses specific equipment and operating conditions to measure specific sugars[40]. In addition, HPLC has good absorbency in both the mobile phase and stationary phase and can separate the pure compound from the substance, while the DNS method analyzes the concentration of reducing sugars from the color change[41]. Therefore, HPLC analysis is more reliable than the DNS method. The DNS method was found to be an inaccurate analytical method for estimating reducing sugars when more than one sugar is present in the solution, whereas HPLC is a more accurate analytical method. For this reason, the DNS test for some reducing sugar analysis may not be a true reflection of the sample[42].

HPLC is widely used to determine the concentration of reducing sugars because of its rapidity, high efficiency, sensitivity to compound detection, selectivity of the peak positions on the chromatogram, applicability to very complex mixtures, and the possibility of determining several analytes simultaneously[43]. In addition, the column used was able to separate sugars and oligomers (if any) from other peaks in the chromatogram, and the detector can give a signal in the chromatogram based on the concentration of reducing sugars compared to the standard curve. This provides the most accurate quantitative determination of reducing sugars or glucosamine. Schwald et al. (1988)[44] found that the HPLC method is one of the most promising methods for sugar analysis, due to its universality, time efficiency, accuracy, and selectivity for the quantification of carbohydrates. Similarly, Hasnaoui, et al. (2011)[45] and Sims (1995)[46] reported that the HPLC method is the preferred method for measuring individual sugars in carbohydrate mixtures because of its precision and simplicity. The Ultraviolet detector (HPLC-UV) is the most commonly used in analyzing compounds with specific properties because almost all organic compounds contain a chromophore, resulting in the ability to absorb UV at different wavelengths[47]. The absence of a chromophore in the sugar molecules, on the other hand, allows for UV detection by measuring the absorbance at low wavelengths (< 200 nm)[48]. Although a UV detector can identify all types of sugars present in the mixture, the analytical concentrations for each type of sugar may vary when compared to a refractive index (RI) detector. Because relatively low sugar concentrations can be detected by the RI detector more precisely than by the UV detector, this phenomenon has been observed[49]. Therefore, RI detection may be preferable to UV detection for low concentrations of reducing sugar or glucosamine analysis. However, several studies have reported the use of HPLC-UV[50–52] or HPLC-RI[53–55] to separate and quantitate glucosamine. Since it has been reported that glucosamine monomer can be tracked using UV detection at 195 nm[56], the glucosamine yield from HPLC-UV was examined for comparison with HPLC-RI. The absorption of UV light by glucosamine may be primarily attributed to the presence of bonds and functional groups within the molecule such as hydroxyl (OH), amino (NH_2) groups, and hydroxymethyl group ($HOCH_2$). This makes the absorption of UV light by glucosamine relatively weak compared to some other molecules with extensive conjugation, such as aromatic compounds. The absorption maxima for glucosamine are typically broad and can vary depending on the specific glucosamine derivative, solvent, and pH conditions. The glucosamine yield from hydrolysis by HCl showed a pattern in the following direction: gravimetric method > DNS method > HPLC-RI > HPLC-UV. HPLC-RI provides more glucosamine yield than HPLC-UV, but in hydrolysis by H_2SO_4, HPLC-UV provides more glucosamine yield than HPLC-RI. Therefore, HPLC-RI was primarily focused on the HCl hydrolysis method and HPLC-UV on the H_2SO_4 hydrolysis method.

Glucosamine production over 8 hours was observed as a function of hydrolysis time. The glucosamine yield significantly increased after one hour and rapidly increased to a peak after two hours. Thereafter, the reaction rate stabilized and decreased slowly upon entering 7 hours of HCl hydrolysis (Fig. 4B). While hydrolyzing with H_2SO_4 (Fig. 5B), the glucosamine yield increased within one hour and increased relatively slowly from one to five hours. After that, the reaction rate slowed down, and five hours was chosen as the ideal hydrolysis time. Our findings are consistent with studies that reported that two hours was an optimum hydrolysis time for the glucosamine yield by HCl[57–59], and five hours for the glucosamine yield by H_2SO_4[36].

The influence of different ratios of chitin raw material per volume of acid used in hydrolysis was studied, as shown in Figures 4C and 5C. Different acid volumes were used with a fixed amount of chitin. A higher acid volume indicates a higher concentration of molar equivalent acid, which indicates a higher concentration of hydrolysis catalysis. It was found that the optimal ratio was 1:20 for both HCl[28, 60] and H_2SO_4[36] because the volume of solvent and hydrogen ions provided the best conditions for catalyzing the hydrolysis reaction. Higher acid volume could indicate a slower rate of glucosamine precipitation when ethanol was added later in the precipitation process.

For effective hydrolysis, the acid concentration is just as crucial as the acid volume ratio. A too-low acid concentration will result in a low molar equivalent of an acid catalyst, which will reduce the yield of glucosamine, whereas a too-high acid concentration will cause the Millard reaction, which can also lower the yield. The yield of glucosamine increased when the HCl concentration increased from 10 to 12 M (Fig. 4D). The molar content of hydrogen ions plays an important role in hydrolysis of chitin. The hydrogen ion content increases with the concentration of the acid used. As a result, the yield of glucosamine increased as the acid concentration increased. While the H_2SO_4 concentration was raised from 4 to 6 M (Fig. 5D), H_2SO_4 concentrations above 6M did not increase the yield of glucosamine because the molar content of hydrogen ions in the hydrolysis system reached saturation[36]. Therefore, the optimum concentrations of HCl and H_2SO_4 are 12 M[28, 61–62], and 6 M[36], respectively.

HPLC-UV and RI Detection Comparison

The chromatogram for the isocratic separation of glucosamine obtained from chitin hydrolysis with HCl and H_2SO_4 is shown in Figure 6. The mobile phase was

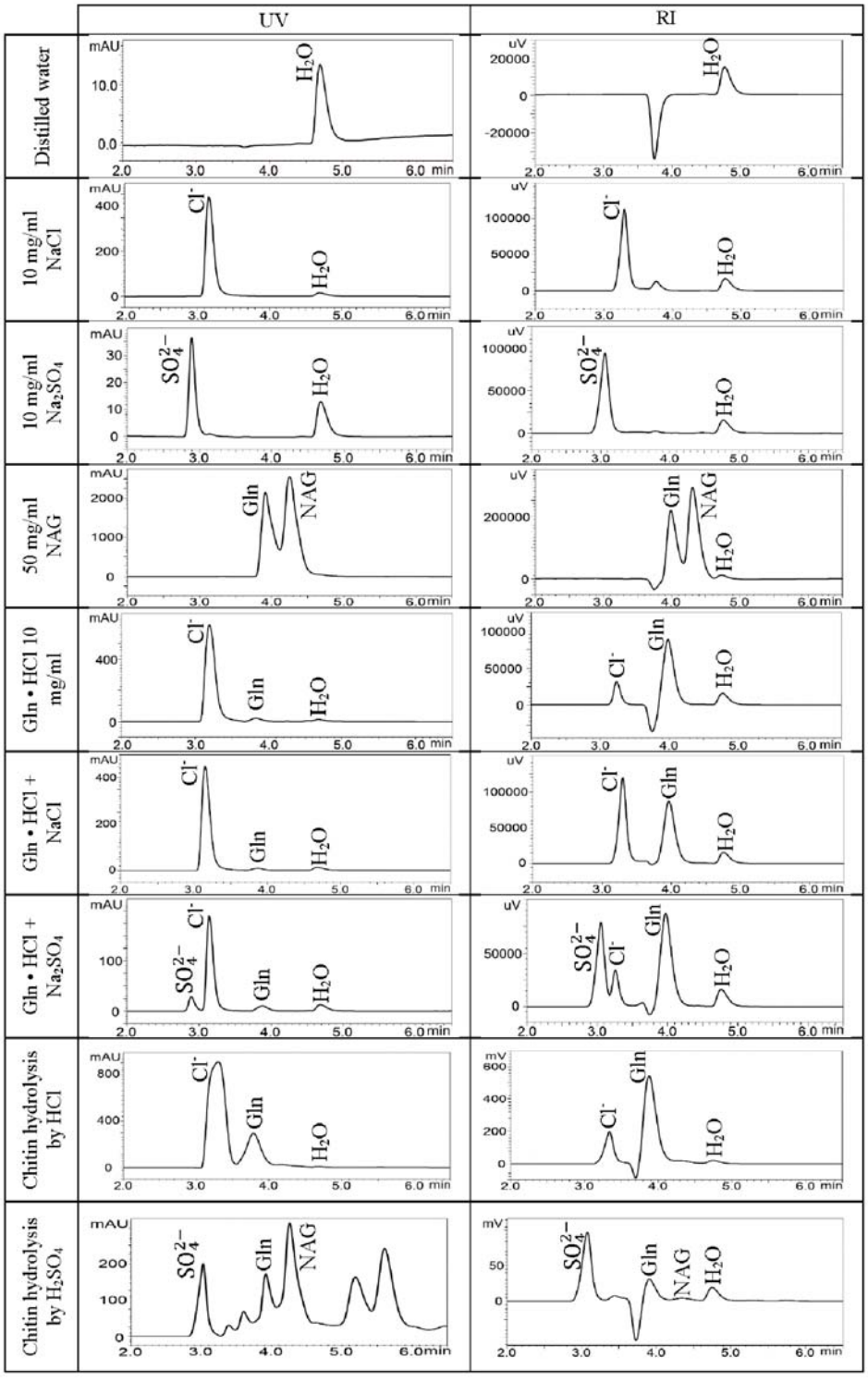


Figure 6. High performance liquid chromatography (HPLC) chromatograms of water, NaCl, Na_2SO_4, standard N-acetylglucosamine, standard glucosamines (in water, NaCl, and Na_2SO_4), as well as chitin-hydrolyzed samples in HCl and H_2SO_4 were obtained using both UV and RI detectors

50 mM sodium perchlorate: acetonitrile (99:1, v/v), and the UV or RI detectors were used for detection. In the UV chromatogram, distilled water (H_2O) showed a low-signal peak with a retention time (RT) of 4.7 min. After injecting 10 mg/ml sodium chloride (NaCl), two major peaks were observed at 3.2 and 4.7 min, with a peak at 4.7 minutes belonging to water. A peak at 3.2 min can be chloride ion (Cl^-) because sodium ion (Na^+) from sodium perchlorate is present at baseline, so the peak of Na^+ from NaCl should not be observed in the chromatogram. The absence of a Na^+ peak was confirmed by injecting 10 mg/ml sodium sulfate (Na_2SO_4). The chromatogram showed peaks at 2.9 and 4.7 min, so 2.9 min peak should be the sulfate ion (). With the standard chemical, 50 mg/ml NAG showed strong peaks at 3.9 and 4.2 min, whereas we expected to see only one peak. This standard chemical was obtained through chitin acid hydrolysis, where the end product, glucosamine, can be formed, so it was possible that these peaks belonged to NAG and glucosamine mixture. When compared to the separation of 10 mg/ml standard Gln·HCl, the chromatogram showed peaks at 3.2 (a Cl^- peak), 3.8, and 4.7 (a water peak) min, indicating that the peak at 3.8 min was glucosamine (Gln). As a result, a peak at 4.2 min in a 50 mg/ml NAG chromatogram must be NAG. In our sample, chitin hydrolysis by HCl produced three peaks with identical retention times to standard Gln·HCl, indicating Cl^-, Gln, and water. In our sample, chitin hydrolysis by HCl resulted in three peaks in the chromatogram with identical retention times to standard Gln·HCl, indicating Cl^- (a 3.2 min peak), Gln (a 3.8 min peak), and water (a 4.2 min peak), whereas chitin hydrolysis by H_2SO_4 resulted in several peaks in separation. Although some oligomers were most likely formed during the reaction, the significant peaks can be found at the correct RT: 2.9 (a peak), 3.8 (a Gln peak), and 4.2 (a NAG peak) min. The results demonstrate a noteworthy finding, as both detection techniques exhibited a linear relationship between the signal peak area and the glucosamine concentration. This observation is evident in Figure S1 (UV detection) and Figure S2 (RI detection), supporting the accuracy and reliability of both methods in quantifying glucosamine levels.

For the RI detection, H_2O, Cl^-, , Gln, and NAG showed peaks at 4.8, 3.3, 3.1, 4.0, and 4.3 min, respectively, with a markedly stronger signal than that from UV detection. Hydrolysis of the chitin sample by HCl showed the same 3 peaks of Cl^-, Gln and H_2O as observed in the UV chromatogram with a slightly different RT, whereas chitin hydrolysis by H_2SO_4 showed the same, Gln, NAG, and other peaks as found in the UV chromatograms but with a slightly different RT. According to this study, when chitin was hydrolyzed with HCl, only the target peak of the Gln was seen, whereas, when it was hydrolyzed with H_2SO_4, peaks for the Gln-product, NAG, and other unidentified peaks were seen. Consequently, we suggest that using HCl rather than H_2SO_4 is preferable for converting chitin to all Gln. The results obtained from this study suggested that RI detection is preferable to UV detection in terms of sensitivity for quantification.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was utilized to investigate the structural changes of chitin hydrolyzed products under optimal conditions using HCl or H_2SO_4. The study compared the products to standard substances, including chitin, chitosan, NAG, and Gln·HCl (Fig. 7). According to the FTIR spectra of chitin hydrolysis by HCl, the overall peak characteristics, such as those at wave number 3,281.68 cm^{-1}, were very similar to those of the standard Gln·HCl. We observed the O-H stretching peak[63] at wave number 3,281.68 cm^{-1}, the C–H stretching peak[64] at wave number 2,937.55 cm^{-1}, the –NH bending peak[65] at wave number 1,535.31 cm^{-1}. The peaks in the region 1200–1000 cm^{-1} were attributed to the stretching of the glycosidic linkage (C–O–C) and C–OH in the sugar ring[66]. In our chitin hydrolyzed by H_2SO_4 sample, we observed a combined spectral pattern between NAG and Gln·HCl standards, which included a broad range of wave numbers 3,392.84 and 2,927.33

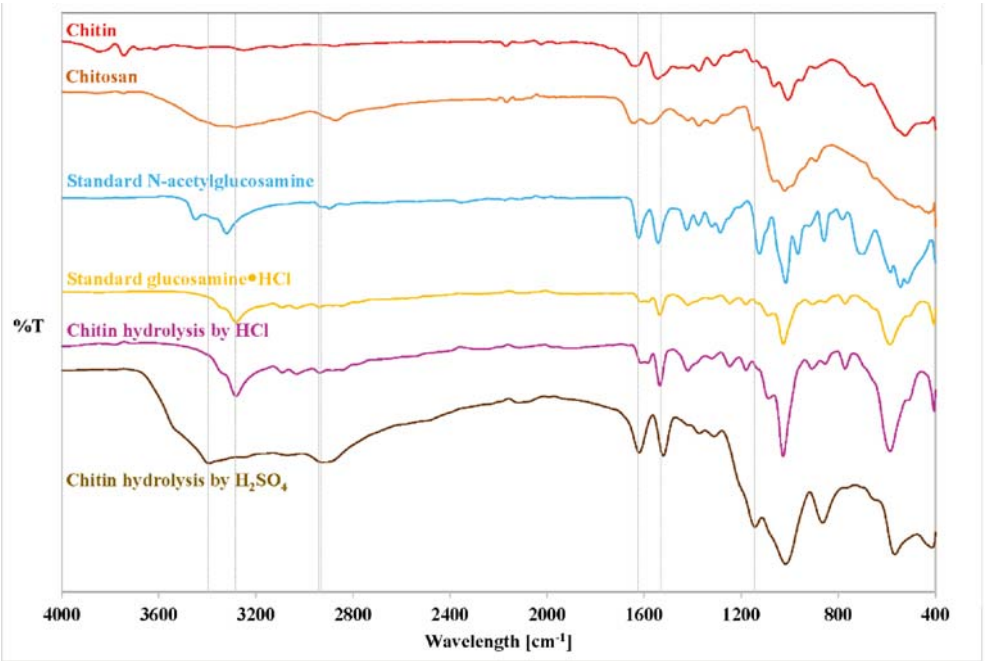


Figure 7. FTIR spectra of chitin, chitosan, standard N-acetylglucosamine, standard glucosamine hydrochloride, and chitin hydrolyzed samples in HCl and H_2SO_4

cm^{-1}, representing N-H and O-H stretching peaks, and the peaks at 1,620.13 and 1,520.58 cm^{-1}, which were amine groups[67]. It was observed that the peak corresponding to the standard Gln·HCl appeared at a wave number of 1,142.91 cm^{-1}. As a result, the product mixture could be found using FTIR analysis, as our HPLC study had previously shown. The FTIR results from this study also provided additional proof that HCl is more effective at chitin hydrolysis than H_2SO_4.

Comparison of Glucosamine Yields

Overall, in terms of acid hydrolysis optimization, the best condition for glucosamine yield from 12 M HCl hydrolysis was at 80 °C for 2 hours, in the ratio of chitin to acid volume of 1:20, the yield detected by HPLC-UV and HPLC-RI were 166.3 ± 15.6 and 283.9 ± 13.8 mg Gln g^{-1} chitin, respectively. On the other hand, the best condition for 6 M H_2SO_4 hydrolysis was at 90 °C for 5 hours with chitin to acid volume ratio of 1:20, yielding 207.5 ± 10.0 and 21.5 ± 0.0 mg Gln g^{-1} chitin as detected by HPLC-UV and HPLC-RI, respectively. Table 1 compares the yields obtained in this study to those obtained in other studies. We found that the glucosamine yield from HCl hydrolysis was 42% lower than that for the yield of Gln·HCl under the condition of 12 M HCl, chitin to acid volume ratio of 1:20, temperature 85 °C for 1 hour[28], but it was found to be higher than those shown in Table 1. While the yield of glucosamine from H_2SO_4 hydrolysis was 135.6 ± 8.9 mg Gln g^{-1} chitin higher than that of the yield of glucosamine under the conditions of 6 M H_2SO_4, chitin to acid volume ratio of 1:10, temperature 100 °C for 6 hours[36]. Our findings show that the optimal conditions for HCl and H_2SO_4 hydrolysis should be important to produce glucosamine from chitin-rich waste streams.

CONCLUSION

The study compared the hydrolysis of chitin using HCl and H_2SO_4 to produce glucosamine. The optimal conditions for HCl hydrolysis were 12 M acid concentration, 80 °C for 2 hours with chitin to acid volume ratio of 1:20. For H_2SO_4 hydrolysis, the optimal conditions were 6 M acid concentration, 90 °C for 5 hours with chitin to acid volume ratio of 1:20. However, the use of H_2SO_4 resulted in a mixture of products, including glucosamine, NAG, and potentially oligomers of these building blocks. The study found that the RI detector was more sensitive than the UV detector for glucosamine detection in the HPLC system. Overall, the results suggest that HCl hydrolysis with a maximum yield of 283.9 ± 13.8 mg Gln g^{-1} chitin is a more suitable method for producing glucosamine from chitin. The ideal conditions obtained from this experiment were able to convert low-cost community chitin into a processed product from shrimp waste. It also adds value to the products that state-owned enterprises in the community can produce to promote a sustainable community economy.

Abbreviations and notation: Gln, glucosamine; HCl, hydrochloric acid; H_2SO_4, sulfuric acid; H^+, proton; Cl^-, chloride ion; SO_4^{2-}, sulfate ions; DNS, 3,5-dinitrosalicylic acid; HPLC, High-Performance Liquid Chromatography; UV, ultraviolet; RI, refractive index; HPLC-UV, HPLC with ultraviolet detector; HPLC-RI, HPLC with refractive index detector; NaOH, sodium hydroxide; FTIR, Fourier Transform Infrared Spectroscopy; H_2O, distilled water; RT, retention time; Na^+, sodium ion; Na_2SO_4, sodium sulfate; NAG, N-acetylglucosamine; Gln·HCl, glucosamine hydrochloride; Gln·SO_4^{2-}, glucosamine sulfate.

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Table 1. Glucosamine production in this study and other studies

Source of chitin	Condition	Yield (mg Gln g^{-1} chitin)		Ref.
		Gln·HCl	Gln·SO_4^{2-}	
Shrimp	80 °C, 2 h, 1:20 ratio, 12 M HCl	420.6 ± 13.7 (GV) 291.9 ± 15.2 (DNS) 166.3 ± 15.6 (HPLC-UV) 283.9 ± 13.8 (HPLC-RI)	n/a	This study
Shrimp	90 °C, 5 h, 1:20 ratio, 6 M H_2SO_4	n/a	135.6 ± 8.9 (GV) 39.5 ± 4.5 (DNS) 207.5 ± 10.0 (HPLC-UV) 21.5 ± 0.0 (HPLC-RI)	This study
Shrimp	85 °C, 1 h, 1:20 ratio, 12 M HCl	580.0 (GV)	n/a	[28]
Shrimp	60 °C, 4 h, 2.2 M HCl	105.0 (GV)	n/a	[19]
Shrimp	90 °C, 2 h, 12 M HCl	137.0 (GV)	n/a	[57]
Shrimp	90 °C, 75 min, 12 M HCl	21.6 (HPLC-UV)	n/a	[68]
Blue crab	90 °C, 75 min, 12 M HCl	21.8 (HPLC-UV)	n/a	[68]
Three spot crab	90 °C, 75 min, 12 M HCl	3.3 (HPLC-UV)	n/a	[68]
Cicada sloughs	90 °C, 2 h, 12 M HCl	205 (GV)	n/a	[57]
Cockroaches	90 °C, 2 h, 12 M HCl	33 (GV)	n/a	[57]
Straw mushroom	100 °C, 6 h, 1:10 ratio, 6 M H_2SO_4	n/a	56.8 ± 3.6 (GV)	[36]

Remark: n/a was not available, GV was gravimetry, and DNS was dinitrosalicylic acid.

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