International Food Research Journal 25(5): 2173-2176 (October 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Isolation of glucosamine HCl from *Penaeus monodon*

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Article history

Received: 20 June 2017
Received in revised form: 27 September 2017
Accepted: 14 October 2017

Keywords

Glucosamine HCl
Tiger prawn
Chitin
Chitosan
FT-IR spectrophotometry

Abstract

Glucosamine is a compound of amino sugar that is found widely in the cartilage and has a very important role for health and joint flexibility. Glucosamine is generally produced by the hydrolysis of the exoskeletons of marine animals, one of the types of tiger prawns. In the tiger prawn shells contain chitin and chitosan compounds that can be derivatives further into glucosamine HCl. This study aimed to isolate and characterize the content of glucosamine HCl by hydrolysis of chitin and chitosan from the tiger prawns shells. Glucosamine HCl was hydrolyzed chemically using concentrated hydrochloric acid. Characterization glucosamine HCl has done by Fourier Transform Infra Red (FT-IR) spectrophotometry. Glucosamine HCl standard was used as a comparison to calculated match factor (MF). The determination of glucosamine level was performed by UV-Vis spectrophotometry with using glucosamine HCl standard as comparison. The results of the qualitative analysis showed a content of glucosamine HCl in tiger prawn shells either hydrolyzed from chitin and chitosan. It was characterized by the spectrum appears at wave number 1032cm^{-1}, 1537cm^{-1}, 3292cm^{-1} for chitosan and 1032cm^{-1}, 1539cm^{-1}, 3294cm^{-1} for chitin that each showed their vibration ether, CH_2 and amine group (NH). MF price of chitin and chitosan is 916.66 and 976.74, which shows that the resulting spectra are identical. The results of the quantitative analysis showed that the levels of glucosamine in chitin and chitosan 698.41 and 1,119.31 mg/g, respectively. Thus the level of glucosamine HCl in chitosan is higher than in chitin.

Introduction

Shrimp shell wastes of seafood are a potential source of manufacture of chitin and chitosan, a biopolymer that is commercially potential in various industrial fields (Puspawati *et al*., 2010). The use of chitin is limited by the properties of insoluble and difficult to separate with other materials, mainly bound to proteins, so the need for utilization of chitin is first converted into chitosan (Restuati, 2011). The process of changing chitin into chitosan can be done with a chemical method (Saleh *et al.*, 2015).

Forms deacetylation of chitin known as chitosan, which is composed of 2-amino2-deoxy-D-glucose (glucosamine). One important derivative of chitin and chitosan are Glucosamine HCl (Dutta *et al.*, 2004). Glucosamine is an amino monosaccharide that acts as a substrate is referred to the constitution of glycosaminoglycan chains that are the basic ingredients of the articular cartilage (Fattahi *et al.,* 2008). Glucosamine is also a substrate to produce aggrecan and proteoglycan that gives hydrophilizing against cartilage that is then used for the treatment of osteoarthritis (Sibi *et al.*, 2013).

Glucosamine is an amino sugar (hexamine) with a molecular weight of 179.17, which is naturally present in the human body and hard-shelled animals such as crabs. Glucosamine can be obtained by acid hydrolysis using a strong acid or enzymatic hydrolysis using bacterial (Pichyangkura *et al*., 2002). Techniques used to hydrolyze chitin reflux with 37% hydrochloric acid (1:5 w/v) at a temperature of 100°C and under different reaction time (Leite *et al*., 2002), or by using 36.5% HCl at a temperature of 50 and 70°C (Novikov, 2004). Thus, Li *et al*. (2007) reported an increase in temperature of 60 till 90°C to optimize the preparation process of glucosamine HCl (Li *et al.*, 2007). Based on the explanation above, this study aimed to isolate and characterize the content of glucosamine HCl by hydrolysis of chitin and chitosan from shrimp shell.

Materials and Methods

Chemicals and standard solution

Chitosan and Glucosamine G8475-25G was obtained from Sigma Chemie GmbH Aldrich 5 g with a purity of ≥99% over the analysis using UV-Visible spectrophotometry. Pro analysis grade of acetone, concentrated hydrochloric acid (HCl), potassium

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thiocyanate (KSCN), iron (II) sulphate ($FeSO_4$), sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), potasium hydroxide (KOH) and calcium dihidroxide ($Ca(OH)_2$) was purchased from Merck (Darmstadt, Germany). Distilled water was obtained through a Millipore-Q50 Ultrapure water system (Sartorius). The stock solution (c = 1000 µg/mL) was prepared by dissolving 100 mg of glucosamine standard with 100 mL of distilled water.

Preparation of chitin

Tiger prawn shells *(Panaeus monodon)* was washed and dried then pulverized and sieved then performed demineralization, deproteination, depigmentation and deacetylation stage (Rekso, 2004). Mineral disappearances carried out at a temperature of 25–30°C using 1 M HCl solution with a comparison 1:10 (g/ml), and then stirring for 120 minutes, filtered to take sediment. The precipitate was washed with distilled water until neutral pH, then filtered and dried of sediment. The next process is deproteination, the sediment was carried out at a temperature of 60-70°C using 1 M NaOH solution with comparisons was 1:10 (g/ml), and then stirring for 60 minutes. The mixture was separated and filtered to take sediment. The precipitate was washed with distilled water until neutral pH, then filtered and dried sediment. Furthermore, depigmentation by adding acetone then was bleaching with NaOCl 0.315% (w/v) for 5 minutes at room temperature, the comparison is 1:10 (w/v), and then it was washed with distilled water until neutral pH, then was filtered and dried sediment, this sediment known as chitin (Hargono and Sumantri, 2008).

Preparation of chitosan

Chitin was added NaOH solution with a concentration of 60% at a temperature of 90-100°C while stirring at a constant speed for 60 minutes. The result is a slurry was then filtered, washed with distilled water then added HCl 1M to make the pH neutral then washed again with distilled water, filtered and dried (Hargono and Sumantri, 2008). Chitosan is obtained weighed and characterized using FT-IR spectrophotometer (Rekso, 2004). Strong base generating the best degree of deacetylation then optimized to obtain better chitosan. Degree of deacetylation (DD) determined by Domzy and Robbert line method (Hargono and Sumantri, 2008).

Isolation of glucosamine HCl on chitin

Chitin material was dissolved in 12 M HCl (1:20 w/v) which had previously been heated at 60°C, then put into a beaker. Let the mixture at a temperature of 68-85°C with stirring until dissolved solids perfect (Benavente *et al*., 2015). Hydrolysis is added distilled water (1:1 v/v) and then filtered using Whatman filter paper. Distillate 10% activated charcoal is added and the solution is heated at 60°C for 30 minutes. Distillate form of a pale yellow color solution was evaporated over the water bath. Once formed crystals are washed with 96% ethanol. To obtain glucosamine hydrochloride (HCl) is dried at 50°C in an oven (Xavier, 2006).

Isolation of glucosamine HCl on chitosan

Chitosan material is hydrolyzed with concentrated HCl (1:20 w/v) at 90°C for 75 minutes. The resulting brownish black solution dissolved in distilled water (1:1 v/v) (Sibi *et al*., 2013). The mixture was filtered using Whatman filter paper to remove insoluble particles and other impurities. Distillate 10% activated charcoal is added and the solution is heated at 60°C for 30 minutes. Distillate form of a pale yellow color solution was evaporated over the water bath. Once formed crystals are washed with 96% ethanol. To obtain glucosamine hydrochloride (HCl) is dried at 50°C in an oven (Xavier, 2006).

FT-IR spectrophotometry determination

Identification and characterization of glucosamine HCl is done by comparing the spectrum produced between glucosamine HCl standard with glucosamine HCl isolated from chitin and chitosan. Glucosamine HCl is made into pellets with KBr to form a thin layer of transparent. Furthermore, the uptake was measured using FT-IR spectrophotometry at the wave range 4000-800 cm^{-1} (Sibi *et al.*, 2013).

UV-Vis spectrophotometry determination

Glucosamine HCl standard was prepared by series concentration of 50, 100, 200, 300, 400 and 500 µg/ml. For each concentration 3ml of standard and sample 100 µg/ml was then added 0.75 ml of acetyl acetone 4% in 1.25 N sodium carbonate solution, the mixture was heated at 900°C for 1 hour, cooled to room temperature, add 0.75 ml of reagent Ehrlich, allowed to stand for 30 minutes. The absorbance was measured at the maximum wavelength (512.36 nm). The sample was done by three replications (Sitompul, 2011).

Data analysis

A calibration standard curve for glucosamine was obtained by running on UV-Visible spectrophotometer and then plotting absorbance against concentrations. The best fit of the line curve was calculated by equation of line. Linearity was evaluated through

Table 1. Characteristics and levels of glucosamine HCl

GlcN	Wavenumber (cm^{-1})			Match Factor (MF) variable					Concentration (mg/g)			
	-O-	CH_2	NH_2	Intensity	X	Y	X.Y	MF	Abs 1	Abs 2	Abs 3	Conc.
Standard	1032	1539	3293	55.08	0.15	0.25	0.04	-	-	-	-	-
Chitin	1032	1539	3294	41.12	0.24	0.38	0.09	916	0.38	0.37	0.32	698
Chitosan	1032	1537	3292	58.25	0.12	0.14	0.01	976	0.41	0.43	0.38	1119

GlcN : Glucosamine HCl
Abs : Absorbance sample in UV-Visible spectrophotometer

the correlation coefficient (R^2). The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation:

Y= bx + a, where:

Y = Absorbance
b = Slope
x = Concentration
a = Intercept.

Match factor determined to show the identical between standard and sample using the equation below:

$$MF = \frac{103\ \{\sum X.Y - (\sum X.\sum Y)\}^2}{\left\{\sum X^2 - \left(\frac{\sum X.\sum Y}{n}\right)\right\}\left\{\sum Y^2 - \left(\frac{\sum Y.\sum Y}{n}\right)\right\}}$$

MF : 900-1000 showed the value of the two spectra are identic
X : Absorbance spectrum of standard
Y : Absorbance spectrum of sample

Results and Discussion

Isolation mechanism

Generally, glucosamine HCl from chitin and chitosan hydrolyzed by chemical method that the hydrolysis process using a solution of concentrated hydrochloric acid. Chitin is obtained through three stages, namely, deproteination or known by the removal of proteins, demineralization or mineral removal process and depigmentation as color removal. Deacetylation performed to remove the acetyl group of chitin by adding of strong base like NaOH, and the result of this process namely chitosan. To transform chitin and chitosan become glucosamine HCl, both of chitin and chitosan was performed hydrolysis by HCl, respectively.

Analysis of glucosamine HCl

Glucosamine HCl crystals that formed were analyzed by a FT-IR spectrophotometer. Samples were prepared using a KBr pellet, then determined spectrum. Spectrum formed compared to the spectrum of glucosamine HCl produced standard with the same measurement with the sample. From the results of spectra appears (Figure 1, 2 and 3) identical fingerprint area between the sample and standard glucosamine HCl.

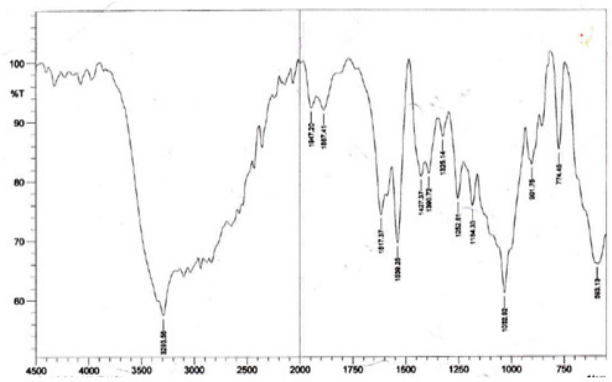

Figure 1. The spectrum of glucosamine HCl standard by FTIR spectrophotometer

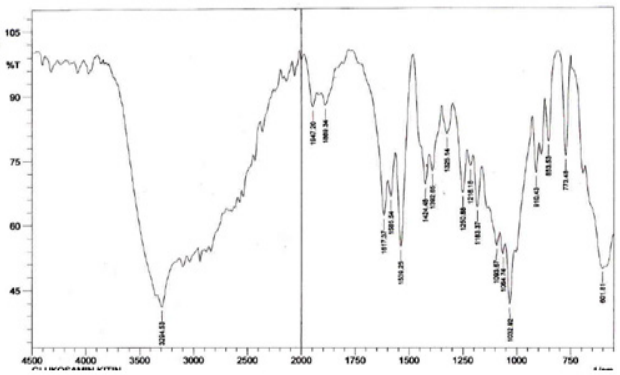

Figure 2. The spectrum of glucosamine HCl of chitin by FTIR spectrophotometer

The Table 1 clearly saw the presence of vibration ether. It can be seen in absorption at wavenumber 1032 cm^{-1}. In addition most of the compounds can also be noted in absorption between 4000 cm^{-1} and 1400 cm^{-1}, the area is known as the principal vibration. In

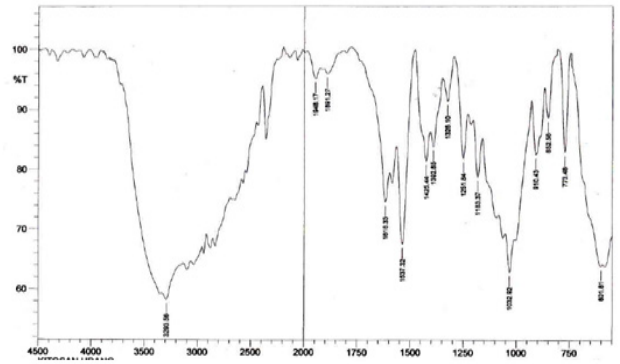

Figure 3. The spectrum of glucosamine HCl of chitosan by FTIR spectrophotometer

the area, there is fundamental vibration-absorption uptake derived from the functional groups, which showed the presence of glucosamine HCl content in the samples of chitin and chitosan. The total content of glucosamine has been determined by UV-Visible spectrophotometer.

To comparing the functional groups of glucosamine HCl with a sample standard, also conducted the comparison similarities based on pricing parameters match factor (MF). The value of MF is 900-1000, If the value of MF ≤900 spectra generated is not identical whereas if MF ≥900 spectra produced identical. Table 1 clearly seen that between glucosamine standard and sample has a similarity.

Conclusion

Based on identification process of glucosamine HCl of chitin and chitosan, standard functional groups of glucosamine HCl and glucosamine HCl from chitin and chitosan have the same wavenumber, and the result of MF calculation show that the spectra were generated between the spectra of glucosamine HCl standard and samples of chitin and chitosan is identical. The total concentration of glucosamine HCl on chitosan higher than on chitin, it was found by spectrophotometry measurement.

Acknowledgements

The authors acknowledge the financial support received from Dean of Faculty of Pharmacy, Universitas Muslim Indonesia (Rachmat Kosman, S.Si., M.Kes., Apt), Head of the Laboratory of Pharmaceutical Chemistry Faculty of Pharmacy, Universitas Muslim Indonesia (Zainal Abidin, M.Pharm., Apt) for their support and encouragement in carrying out his college work.

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