

Optimum Conditions for Preparation of Glucosamine Hydrochloride and Glucosamine Sulfate from Shrimp-Shell Chitin

Piyabutr Wanichpongpan

Department of Chemical Engineering
King Mongkut's University of Technology Thonburi
Thailand

Shewin Attasat

Pilot Plant Development and Training Institute
King Mongkut's University of Technology Thonburi
Thailand

Abstract

The shrimp processing industries produce a large quantity of wastes, such shrimp shell, and head, varying from 45 - 55% of the weight of raw shrimp. Therefore, it has been very useful to utilize these wastes into the value-added byproducts. In this study, the optimum conditions for preparation of glucosamine by the hydrolysis of chitosan extracted from shrimp shell were determined. The conditions involve the ratio of mixture to hydrolysate for preparation of crude glucosamine hydrochloride, the ratio of ethanol to hydrolysate for preparation of crystalline glucosamine hydrochloride, and the optimum temperature and reaction time for preparation of glucosamine sulfate. Finally, the purity of the glucosamine sulfate was determined by Gas Chromatography-Mass Spectrometry analysis.

Keywords: Shrimp shell; Chitin; Chitosan; Hydrolysis; Glucosamine hydrochloride; Glucosamine sulfate

1. Introduction

Glucosamine is an amino monosaccharide synthesized in the body from glucose through hexosamine pathway and it naturally occurs in the connective tissues contributing to maintenance of strength, flexibility, and elasticity of these tissues. Glucosamine is a precursor in the production of cartilage, mucous membranes, and synovial fluid. Hence it has been used to treat or relieve the symptoms of osteoarthritis (Haupt et al., 1999; Setnika, et al., 2013). In addition, glucosamine was also reported having anti-inflammatory, antibacterial, and anti-cancer effects (Nagaoka, et al., 2011; Sibi, et al., 2013).

Besides being synthesized in the body, glucosamine can be derived from chitin, the second most abundant natural polysaccharide after cellulose, which most found in the exoskeletons of crustaceans such as shrimp, crab, and squid. The structure and function of chitin is analogous to those of cellulose, but the hydroxyl group (-OH) at the C-2 in cellulose is replaced by an acetamide group (-NHCOCH₃). Chitosan is a derivative of chitin. It can be produced from the deacetylation of chitin by conversion of acetamide groups into amino groups (-NH₂). The structure of cellulose, chitin, and chitosan are shown in Figure 1. For this reason, chitosan has three types of reactive functional groups, i.e. an amino group, and both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Shahidi et al., 1999). Chemical modifications of these groups have provided numerous useful materials in different fields of application (Kurita, 1986).

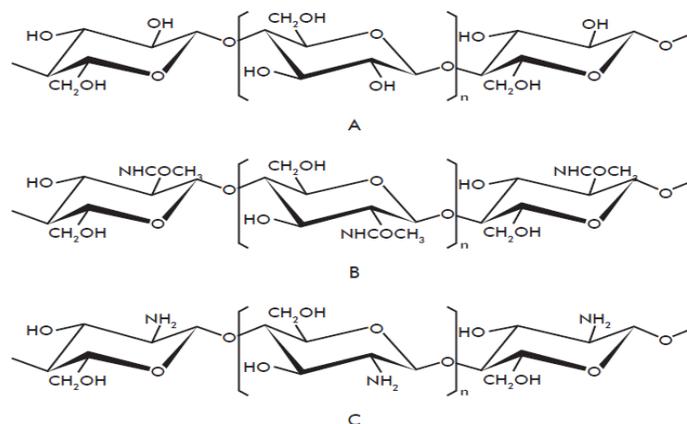


Figure 1: The structural representation of A) cellulose, B) chitin, and C) chitosan (Sharp, 2013)

Thailand is a major exporting country of shrimp to the world market. The average annual shrimp export from 2009 to 2013 is over 184 thousand metric tons, worth approximately US\$ 1.4 billion per year (OAE, 2014). During the processes to obtain shrimp meat, the approximately 45 - 55% of the weight of raw shrimp are shells and heads considered as waste (Lertsutthiwong et al., 2002), which are sold into the animal feed industry at a very low price. Therefore, it would be very useful to utilize these waste into value-added applications, such as production of chitin, chitosan, and as well as glucosamine.

Glucosamine can be produced from shellfish waste by the various processes involved the deproteinization, demineralization, decoloration, and then hydrolysis using hydrogen ions as a catalyst or using enzymes to digest molecular chain of chitin into N-acetyl glucosamine monomers. While, D-glucosamine can be produced by hydrolysis of chitosan into D-glucosamine monomers as showed in Figure 2.

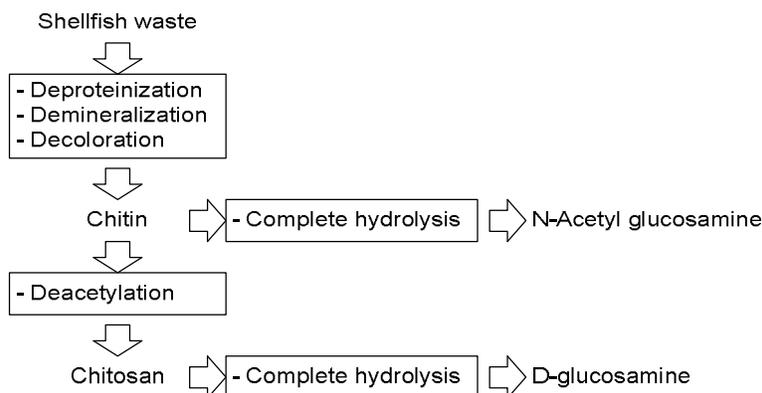


Figure 2: Production of glucosamine (adapted from Shahidi et al., 1999)

Most of glucosamine used for treatment of osteoarthritis is available in three salt forms, i.e. N-acetyl glucosamine, glucosamine hydrochloride, and glucosamine sulfate. The structure of glucosamine hydrochloride and glucosamine sulfate is shown in Figure 3. Among these forms, glucosamine sulfate is found to be the majority of medical studies in Thailand (Wangroongsub et al. 2010).

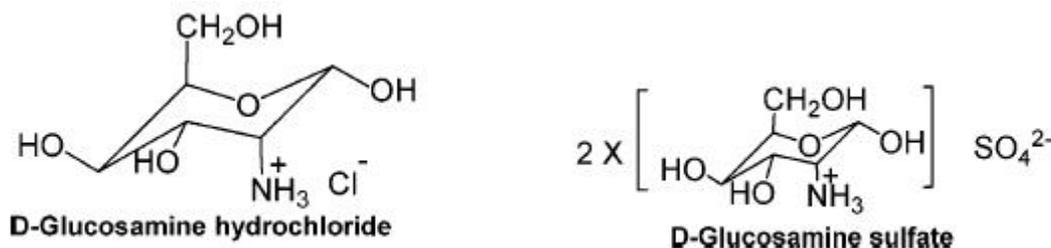


Figure 3: Structure of glucosamine hydrochloride and glucosamine sulfate (Mojarrad, et al., 2007)

The objective of this study is 1) to find the optimal conditions for hydrolysis of chitosan extracted from shrimp shells to glucosamine hydrochloride in hydrochloric solution, and 2) to find the optimum temperature and reaction time for preparation of glucosamine sulfate from the reaction of glucosamine hydrochloride with sodium sulfate.

Materials and Methods

Chitosan, purchased from Marine Bio Resources Co., Ltd., Thailand, was prepared from shrimp (*Litopenaeus vannamei*) shell, and had the degree of deacetylation and molecular weight of 90% and 50,000 g mole⁻¹ respectively.

Study of the optimum ratio of initial mixture volume to hydrolysate volume for crystallization of crude glucosamine hydrochloride

A 10-g chitosan extracted from shrimp shells was hydrolyzed by adding 300-ml of 14.4% w/v hydrochloric acid and heated under temperature of 80°C for 6 hours. The mixture was filtered with filter paper to remove solid residue and the filtrate was evaporated to the final volume of 100-ml. This product, called hydrolysate, was left for crystallization of crude glucosamine hydrochloride for 24 hours. The crude crystalline glucosamine hydrochloride was filtered out with filter paper, dried in a vacuum oven, and then weighted. The procedure was repeated by changing the final volumes of hydrolysate to 200 and 300-ml, which were designed as the volume ratios of the initial mixture to the final hydrolysate of 3:1, 1.5:1, and 1:1 respectively.

Study of the optimum ratio of ethanol volume to hydrolysate volume for crystallization of glucosamine hydrochloride

The crude crystalline glucosamine hydrochloride in 100-ml hydrolysate, prepared using the method described above at the volume ratios of the initial mixture to the final hydrolysate of 3:1, was further purified by re-crystallization in ethanol. A 50-ml of 95% ethanol was slowly added to the hydrolysate while continuous stirring, being careful not to turbid solution. The solution was left in the refrigerator at 4°C for 24 hour. The crystalline glucosamine hydrochloride was filtered out with filter paper and dried in an oven and then weighted. The procedure was repeated by changing the amount of ethanol added to the hydrolysate to 100 and 150-ml, which were designed as the volume ratio of ethanol to hydrolysate of 0.5:1, 1:1, and 1.5:1 respectively.

Study of optimum temperature and reaction time for preparation of glucosamine sulfate from glucosamine hydrochloride

The crystalline glucosamine hydrochloride was weighed about 5.0-g and put into a two-necked round-bottomed flask. A 30-ml of distilled water and a 2.0-g of sodium sulfate were added and heated for reaction by controlling the constant temperature at 30°C for 0.5, 1.0, 1.5, and 2.0 hours respectively. The obtained crystalline glucosamine sulfate was dried in a vacuum oven. The procedure was repeated by changing the reaction temperature to 40, 60, and 80°C respectively.

Results and Discussion

The optimum volume ratio of initial mixture to hydrolysate for crystallization of crude glucosamine hydrochloride

The experimental results showed that the amount of crude glucosamine hydrochloride increased as increasing the volume ratio of the initial mixture to the final hydrolysate (Figure 4). At the ratio of 1:1 glucosamine hydrochloride was crystallized in small quantities, mostly was in the form of suspended solids. The amount of crystals increased as increasing the ratio to 1.5:1, and reached the highest amount at the ratio of 3:1. This concentration was probably suitable for crystallization of glucosamine hydrochloride and being optimum for evaporation.

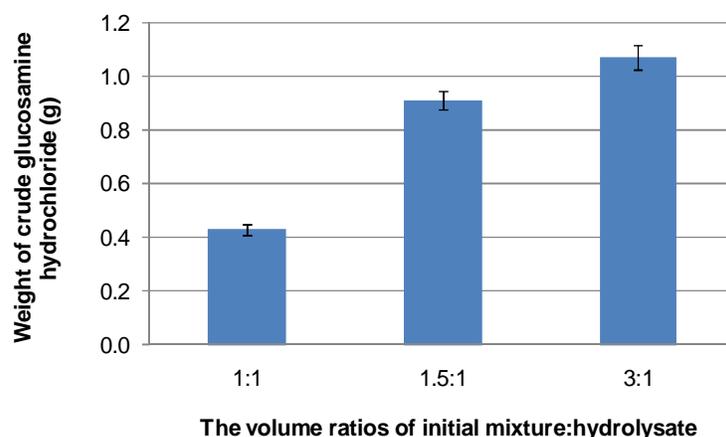


Figure 4: The relationship between weights of crude crystalline glucosamine hydrochloride with the volume ratios of initial mixture to hydrolysate (error bars represent standard deviation, n=3).

The optimum volume ratio of ethanol to hydrolysate for crystallization of glucosamine hydrochloride

Glucosamine hydrochloride was soluble in water, but slightly dissolved in ethanol. The addition of 100-ml and 150-ml of 95% ethanol to a 100-ml hydrolysate, i.e. the volume ratio of ethanol: hydrolysate of 1:1 and 1.5 respectively, produced slightly difference in amounts of glucosamine crystalline, while addition of 50-ml of 95% ethanol could not form the crystalline (Figure 5). These results indicated that the dosage of ethanol affected the yield of glucosamine hydrochloride. In this study, the optimum volume ratio of ethanol: hydrolysate for crystallization of glucosamine hydrochloride was 1:1. Although, the product yield was slightly lower than the ratio of 1.5:1, but ethanol was used in very lower quantities.

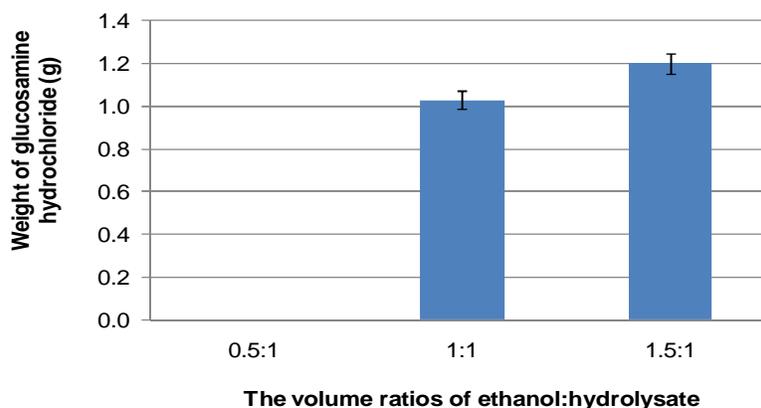


Figure 5: The relationship between weights of crystalline glucosamine hydrochloride with the volume ratios of ethanol to hydrolysate (error bars represent standard deviation, n=3).

The optimum temperature and reaction time for preparation of glucosamine sulfate from glucosamine hydrochloride

The results in Figure 6 showed that the optimum temperature for preparation of glucosamine sulfate was 40°C, since it gave the maximum amount of crystalline glucosamine sulfate and the crystals still appeared to be whitish. At higher temperature, i.e. 60 and 80°C, the crystals appeared to be brownish and blackish respectively, which was not preferable to be used in industries. The experimental results also showed that the reaction time slightly affected the amount of glucosamine sulfate formed and the period of 0.5 hours was sufficient for the reaction. The higher reaction period could produce a little more product and considered to be waste.

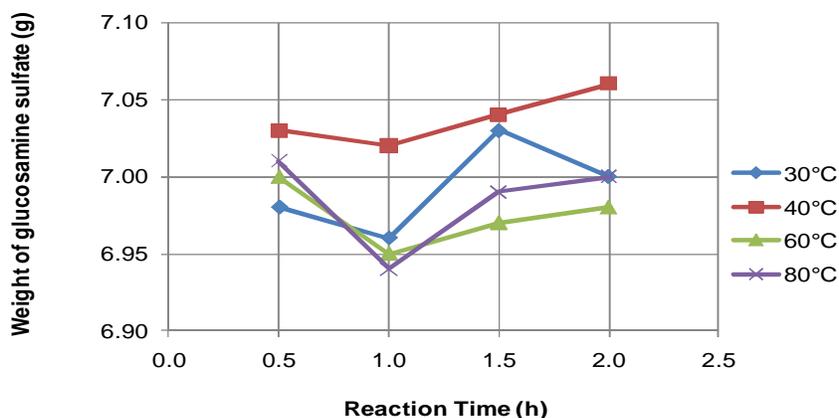


Figure 6: The relationship between the weight of a crystalline glucosamine sulfate and reaction times at various temperatures (each value was a mean of two replicates).

Considering to the yields, a 10-g of chitosan could produce about 1.20-g of glucosamine hydrochloride, representing a yield of 12%. While the reaction between 5-g of glucosamine hydrochloride and 2-g of sodium sulfate could produce 7.03-g of glucosamine sulfate.

To determine the purity of glucosamine sulfate, the gas chromatography - mass spectrometry (GC-MS) spectra of glucosamine sulfate obtained from this experiment was compared to those spectra of the standard glucosamine sulfate. The GC-MS analysis was performed with the HP 5890 series II GC connected to the model 5971A mass-selective detector (Hewlett Packard). The injector temperature was set to 120°C. The oven temperature was initially set to 60°C and increased to 150°C at a rate of 5°C min⁻¹. The MS detector temperature was set to 180°C in order to prevent the condensation of water in the system.

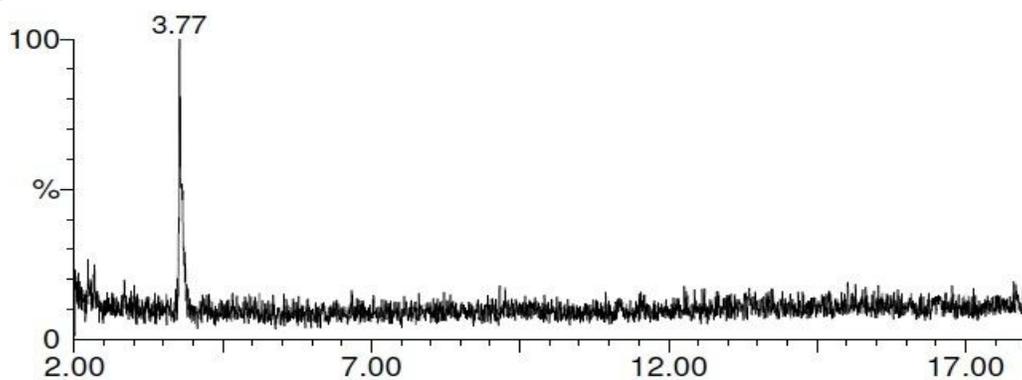


Figure 7: The GC-MS spectra of standard glucosamine sulfate

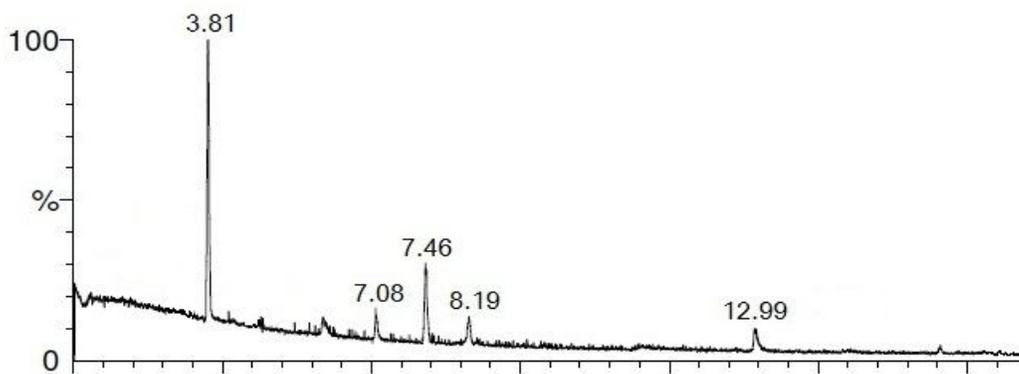


Figure 8: The GC-MS spectra of glucosamine sulfate obtained from this study

The results of the analysis showed that the peak of glucosamine sulfates was apparently observed at 3.77 min (Figure 7), which was comparable to those of the standard glucosamine sulfates observed at 3.81 min (Figure 8). However, the spectra of glucosamine sulfates obtained from this study still contained small peaks corresponding to trace impurities or moisture. The comparison between peaks of glucosamine sulfate and other impurities indicated that the purity of product was 63%.

Conclusion

In this study, the ratio of initial mixture volume to hydrolysate volume of 3:1 was suitable for preparation of crude glucosamine hydrochloride. The ratio of ethanol volume to hydrolysate volume of 1:1 could produce the highest amount of crystalline glucosamine hydrochloride. The optimum temperature and reaction time for preparation of glucosamine sulfate were 40°C for 0.5 hours.

References

- Houpt, J.B., McMillan, R., Wein, C., Paget-Dellio, S.D. 1999. Effect of glucosamine hydrochloride in the treatment of pain of osteoarthritis of the knee. *Journal of Rheumatology*, 26: 2423-2430.
- Kurita, K. 1986. Chemical Modifications of Chitin and Chitosan. In: Muzzarelli, R.A.A., Jeuniaux, C., Gooday, G.W. (Eds.). *Chitin in Nature and Technology* Plenum Press, New York. pp. 287- 293.
- Lertsutthiwong, P., How, N.C., Chandkrachang, S., Stevens, W.F. 2002. Effect of Chemical Treatment on the Characteristics of Shrimp Chitosan. *Journal of Metals, Materials and Minerals*, 12: 11-18.
- Mojarrad, J.S, Nemati, M., Valizadeh, H., Ansarin, M., Bourbour, S. 2007. Preparation of glucosamine from exoskeleton of shrimp and predicting production Yield by Response Surface Methodology. *J Agric Food Chem*, 55(6): 2246-50.
- Nagaoka, M. Igarashi, J. Hua, Y. Ju, S. Yomogida and K. Sakamoto. 2011. Recent aspects of the anti-inflammatory actions of glucosamine. *Carbohydrate Polymers*, 84(2): 825- 830.
- Office of Agricultural Economics (OAE). 2014. Import-export statistic [Online], Available at: http://www.oae.go.th/oea_report/export_import/export.php [Accessed on 28 July 2014].
- Setnikar I, Cereda R, Pacini MA, Revel L. 1991. Anti-reactive properties of glucosamine sulfate. *Arzneim Forsch*, 41:157-161.
- Shahidi, F., Arachchi, J.K.V., Jeon, Y.J. 1999. Food applications of chitin and chitosans. *Trends in Food Science & Technology*, 10(2): 37-51.
- Sharp, R.G. 2013. A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields. *Agronomy*, 3(4): 757-793.
- Sibi G., Dhananjaya K., Ravikumar K.R., Mallesha H., Venkatesha R.T., Trivedi D., Bhusal K.P., Neeraj, Gowda K. 2013. Preparation of Glucosamine Hydrochloride from Crustacean Shell Waste and It's Quantitation by RP-HPLC. *American-Eurasian Journal of Scientific Research*, 8(2): 63-67.
- Wangroongsub, Y., Tanavalee, A., Wilairatana, V., Ngarmukos, S. 2010. Comparable Clinical Outcomes between Glucosamine Sulfate-Potassium Chloride and Glucosamine Sulfate Sodium Chloride in Patients with Mild and Moderate Knee Osteoarthritis: A Randomized, Double-Blind Study. *J. Med Assoc Thai*, 93(7): 805-811.