Development of A Pressurized Hydrolysis Method for Producing Glucosamine

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ABSTRACT — Osteoarthritis (OA) is one of the main causes of disability in the elderly. The consumption of glucosamine is one way to reduce the effects of osteoarthritis, and the global demand for glucosamine is constantly increasing. Thus there is a need to develop production methods which can produce high quality glucosamine in large quantities. The goal of this research was to develop a safe, practical, and efficient glucosamine hydrochloride production method through the use of low-pressure hydrolysis and to analyse the characteristics of the glucosamine produced. The pressurised hydrolysis method used was applied with various combinations of heating time and acid concentration. The extraction used 30 g of chitosan in hydrochloric acid (HCl), with a ratio of 1:9. HCl concentrations were 5%, 8% and 10% with heating times of 30, 60, 90, 120 and 150 minutes at a pressure of 0,5 ATM. The treatment using 5% HCl concentration for 60 minutes produced the best glucosamine hydrochloride. The glucosamine rendement was 65,33%, with 96.33% solubility, a pH of 5,66, LoD of 0,60%, and LoI of 0,23%. The FTIR spectrum absorption pattern showed 99,44% compliance with the standard, proving that the glucosamine hydrolysis was successful.

Keywords— glucosamine, low-pressure extraction, quality, osteoarthritis

1. INTRODUCTION

Glucosamine is one substance which can be produced from chitosan, and is itself an amino sugar which plays an important role as a precursor in the biochemical synthesis of glycosylated proteins and lipids (Kelly 1998). Glucosamine hydrochloride is also known as 2-amino-2-deoxy-D-glucopyranose, chitosamine hydrochloride, and D-(+)-glucosamine hydrochloride. Structurally, glucosamine is an amino sugar molecule with a chemical formula of $C_6H_{13}NO_5$ HCl and a molecular mass of 215,63 Da. Pure glucosamine is in the form of fine white crystals with a melting point of 190-194 ºC. Glucosamine is highly soluble in water, with a solubility of 100 mg/ml at 20 ºC [10].

Glucosamine can be extracted in many different ways, including chemical hydrolysis, enzymatic processes, fermentation, and various combinations of these methods. [13] extracted glucosamine using the chemical hydrolysis method and investigated the effect of three factors (acid concentration, acid of chitin ratio, and reaction time) on yield obtained. In their results, the highest yield was 87,3%, obtained with 37% hydrochloric acid, 9:1 (volume/weight) acid solution to solid ratio, and 4 h of reaction time. [16] extracted glucosamine in HCl at 90 °C for 75 minutes, obtaining a rendement of 3.32 mg/g from shrimp shells, which had a 30% yield of chitosan. [16] reported that extracting with 0,5M HCl for 8 hours produced a high rendement. [8] used an enzymatic process with an 0,1 M phosphate buffer at pH 8 and 37 ºC. The results showed that glucosamine production peaked at around 24 h with a yield of around 42%. Glucosamine was produced through fermentation using Aspergillus sp by [6], and after 168 hours the rendement was 5,48%. [17] mention that glucosamine can be produced using fermentation with Escherichia coli at pH 5. Hydrolysis in an acid environment such as acetic or hydrochloric acid is then used for the deacetylation of the chitosan monomer to produce glucosamine.

Glucosamine production using enzymatic processes or fermentation has generally been at the laboratory scale. The most commonly used industrial glucosamine extraction process is chemical hydrolysis using a combination of the acid HCl and the base NaOH at specified concentrations.

In this context, there is still a need for research into appropriate concentrations and processing times for producing glucosamine when using the pressure hydrolysis chemical extraction method. The specific goal of this research was to determine the optimum treatment for producing glucosamine with the pressure hydrolysis method. In addition, this research aims to improve knowledge regarding safe glucosamine production methods.

2. MATERIALS AND METHODS

2.1 Material and Equipments

The materials used in this research were chitosan from tiger prawn (Penaeus monodon) waste, distilled water, HCl 5%, 8% and 10%, NaOH 50%, isopropyl alcohol (IPA). Production equipment included a stainless steel pressure cooker, gas stove, digital balance, and glassware. Equipment and materials used to analyse glucosamine specification included an electric oven and furnace, dessicator, petri dishes, porcelain basins, 200 ml glass piala, thermometer, filter paper, pipettes, reaction tubes, Erlenmeyer flasks, a pH meter, and Fourier Transform Infrared Spectroscopy (FTIR) apparatus.

2.2 Experimental Procedure

The glucosamine extraction process pressure method was carried out with HCl concentrations of 5%, 8%, and 10%, heating times of 30, 60, 90, 120, and 150 minutes and a solid/acid weight/volume ratio of 1:9. The samples were pressure-cooked with a pressure of ±0,5 ATM Alcohol was then added to separate the sample form any impurities and neutralized with Isopropyl Alcohol (IPA) until the pH was between 3 and 5. The glucosamine was then dried in an oven at 40 ºC for 48 hours to produce glucosamine hydrochloride ready for use.

2.3 Scope of Research

This research was conducted in two stages, i.e. producing of chitosan as raw material. Characterization chitosan was done by analyzing deacetylation spectrum using the Fourier Transform Infrared Spectroscopy (FTIR) and specification glucosamine.

2.4 Analysis of Glucosamine

Some analysis conducted in this study were yield glucosamine, loss on drying [7], pH values [18], Solubility, Loss on ignition [15], and deacetylation spectrum of the glucosamine using Fourier Transform Infrared Spectroscopy (FTIR) .

2.5 Data Analysis

Result of refining process was statistically processed using SPSS software version 16.0 to see the regression parameter coefficients, percent significance (confidence interval), and the pattern of interaction of factors that significantly influence the response. Duncan's test was used to see significant effects of factors.

3. RESULTS AND DISCUSSION

3.1 Yield glucosamine

Yield is the ratio of the amount of desired substance obtained to that of the original raw material. Glucosamine yield yield is shown in Figure 1.

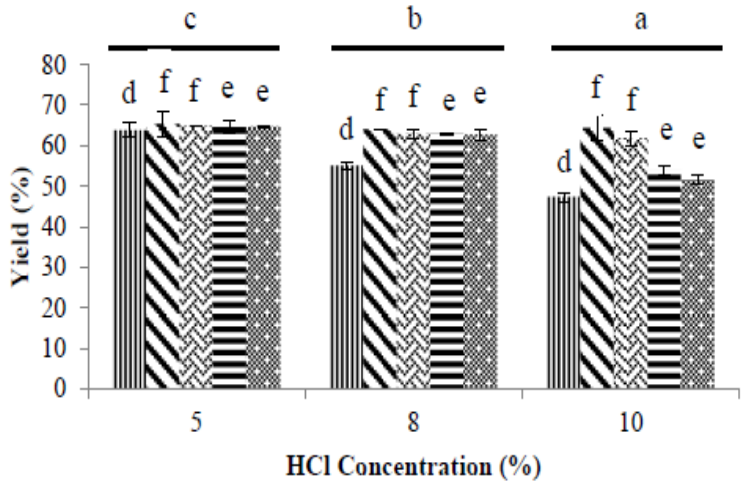

Figure 1. Glucosamine yield. Heating (pressure-cooking) time: ▥ 30 minutes, ◪ 60 minutes, ▨ 90 minutes, ▬ 120 minutes, and ▩150 minutes. Treatments with the same superscript were not significantly different from each other (p>0.05).

The analysis of variance showed that in the pressure hydrolysis method, both acid concentration and heating (pressure-cooking) time had a significant effect on rendement (p<0,05). In addiiton the interaction between HCl concentration and heating time also had a significant effect. A negative association was observed between yield and increase in the acid concentration and heating time. The highest glucosamine yield was 65,33%, obtained with a hydrochloric acid highest on treatment concentration of 5% followed by 63,98% with 8% HCl and 64,34% with 10% HCl, all at a pressurised heating time of 60 minutes.

The pressurised hydrolysis method with HCl consentration 5% and 60 minutes pressurised heating produced a high yield, because of the principles on which this method is based, i.e the joint effects of pressure and acid concentration. The hydrochloric acid (HCl) works to split the amin group from the acetyl group, and the pressure is more effective in accelerating the process of dividing the chitosan polymer to produce glucosamine at lower acid concentrations. The combination of pressure and temperature can also directly speed up the depolymerisation of chitosan to produce glucosamine, so that the cooking time can be reduced compared to conventional (non-pressurised) methods. In their research on glucosamine extraction using a chemical hydrolysis method, [13] obtained their best results (87,3% yield) with an HCl concentration of 37% and a 4 hour extraction time, using a solid/acid ratio of 1:6. While [16] required 8 hours to obtain the highest yield, using an HCl concentration of 0,5 M.

3.2 Loss on drying

The loss on drying (LoD) analysis measures the amount of water and other volatile substances present in the sample, and lost during drying under specified conditions and/or at a set temperature. The loss on drying results are shown in Figure 2.

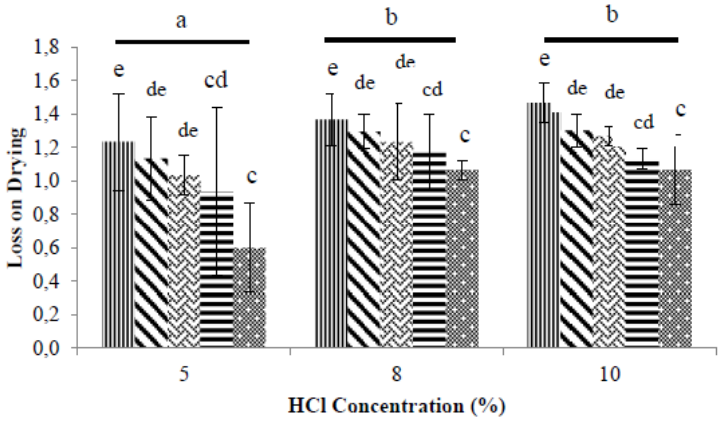

Figure 2. Glucosamine LoD ratios. Heating (pressure-cooking) time: ▥ 30 minutes, ◪ 60 minutes, ▨ 90 minutes, ▬ 120 minutes, and ▩150 minutes. Treatments with the same superscript were not significantly different from each other (p>0.05).

The analysis of variance showed a significant effect (p<0,05) on the LoD of both the HCl concentration and the heating time using this pressure hydrolysis method. The effect of the interaction between HCl concentration and heating time was also significant. The LoD increased with HCl concentration and reduced with pressurised heating time. The best (lowest) glucosamine LoD corresponded to the longest cooking times of 150 minutes, and were 0,60% for the HCL concentration of 5%, 1,07% for 8% HCl and 1,07% for 10% HCl. Based on Duncan's multiple range test, heating time had a significant effect, reducing LoD values. The application of this pressure method was shown to reduce LoD by 0,08%.

The average values for LoD showed that the weight of glucosamine fell by 0,60% to 1,47% after 2 hours at 105 °C. The volatile components consist of covalent molecules with low boiling points, so that some substances can evaporate at low temperatures. We suspect that high LoD values are due to the presence of volatile substances in the product, resulting from imperfect extraction at the chitosan production stage. [11] opined that these volatile substances are composed of small carbon-containing organic molecules which can readily be distilled at atmospheric pressure. It is hoped that using the pressure hydrolysis production method will help to refine the glucosamine from volatile substances, due to the combined action of heat and pressure. However the LoD values in this research do not yet meet [1] [2] standards of 1%.

3.3 pH Values

The pH scale is used to indicate the acidity or alkalinity of an element, a liquid or a solid. The pH values of the glucosamine produced can be seen in Figure 3.

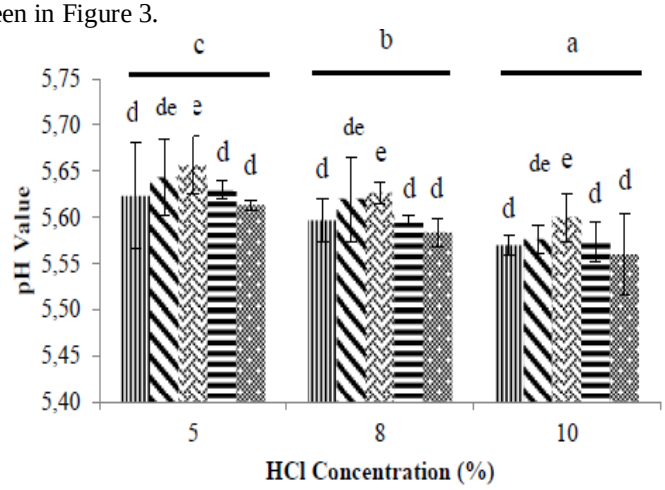

Figure 3. Glucosamine pH. Heating (pressure-cooking) time: ▥ 30 minutes, ◥ 60 minutes, ▨ 90 minutes, ▬ 120 minutes, and ▩150 minutes. Treatments with the same superscript were not significantly different from each other ($p>0.05$).

The analysis of variance showed that with the pressure hydrolysis method, the HCl concentration and heating time both had a significant effect on the pH ($p<0,05$), as does the interaction between them. The extracted glucosamine pH values were lower at higher acid concentrations. The best or highest glucosamine pH values for each HCl concentration were respectively 5,66 at 5% HCL, 5,63 at 8% HCl, and 5,6 at 10% HCl. Duncan's multiple range test also showed that varying the HCl concentration had a significant effect on product pH.

The pH of the glucosamine produced using this pressurised hydrolysis method was between 5,60 and 5,66. Although these pH values are somewhat acidic, they are within the guidelines for human consumption based on [1] [2] which state that the pH of glucosamine may vary between 3,0 and 5,0.

The chemical glucosamine extraction process using hydrochloric acide produces glucosamine hydrochloride, and the neutralisation process can be quite time-consuming. Neutralisation is done using a solution of isopropyl alcohol with a pH between 5,0 and 6,0. [6] state that glucosamine extraction using fermentation produces a product with a neutral pH. This is because the optimum fermentation pH in their experiments was pH 7, over a period of 7 days. Fermentation in a media with neutral pH will produce glucosamine which also has a neutral pH.

3.4 Solubility

Solubility is the maximum amount of a dissolved chemical substance (solute) which can form a homogenous solution in a given solvent. The solubility of the glucosamine produced in water at room temperature can be seen in Figure 4.

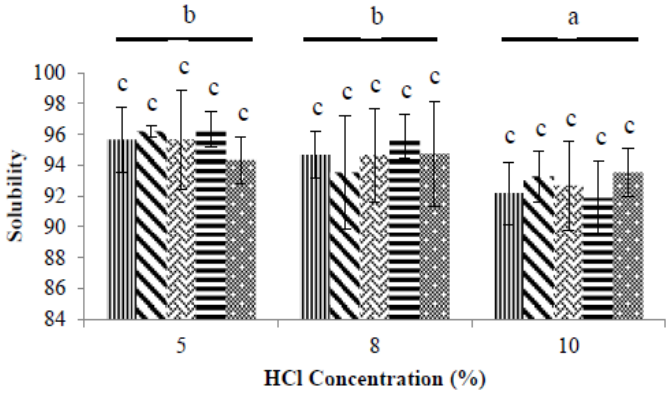

Figure 4. Glucosamine solubility. Heating (pressure-cooking) time: ▥ 30 minutes, ◥ 60 minutes, ▨ 90 minutes, ▬ 120 minutes, and ▩150 minutes. Treatments with the same superscript were not significantly different from each other ($p>0.05$).

The analysis of variance showed that the HCl concentration had a significant effect on the solubility of the glucosamine produced ($p<0,05$). However the effect of heating (pressure-ccoking) on solubility was not statistically significant. The interaction between HCl concentration and heating time also had no statistically significant effect on the solubility of the glucosamine produced with this pressure hydrolysis method. The highest glucosamine solubility was 96,33%, obtained with the 5% HCl concentration, followed by 95,87% at HCl 8% and 93,53% with 10% HCl, all for the 60 minute heating treatment. Duncan's multiple range test showed that HCl concentrations of 5%, 8% and 10% produced significantly different levels of glucosamine solubility. We consider that this is because using a lower HCl concentration produces glucosamine with a higher level of purity and which is therefore more soluble.

The glucosamine hydrochloride solubility tests were carried out using water at 24 ºC and produced solubility rates of 91,87% to 96,33%. Solubility increases or decreases with the temperature of the solvent. [10] stated that glucosamine can easily be dissolved in water at a temperature of 20 ºC. If a substance can be readily dissolved at low temperatures, this indicates that it is highly soluble. Our results show that the glucosamine produced with this pressurised hydrolysis method compled with the relevant standard, which is a minimum solubility of 90,00% [1] [2]

We suspect that the high solubility of the glucosamine obtained from the pressurised hydrolysis method is related to the way in which pressure, temperature and acid concentration work together to split the acetyl group from the chitosan molecule. This process not only removes the acetyl group but also splits up the chitosan polymer into shorter units so that it is easier for the chloride ions (Cl^-) from the hydrochloric acid HCl to bind with the chitosan amine groups to form NH3Cl. The hydroxyl bond between O-H and NH3Cl makes glucosamine hydrochloride readily soluble in water. The glucosamine standards in [1] [2] state that the appearance of glucosamine should be white. When glucosamine is dissolved in water, the solution is generally clear and colourless. However the research results showed that the solution was clear but in some cases had a somewhat yellowish colour. We suspect this was caused by the presence of some pigments and other protein impurities in the chitosan due to imperfections in the extraction process.

3.5 Loss on Ignition

Loss on ignition (LoI) is determined based on the inorganic residue from the process of combustion or oxidation of organic components in food. Total loss on ignition of the glucosamine produced is shown in Figure 5.

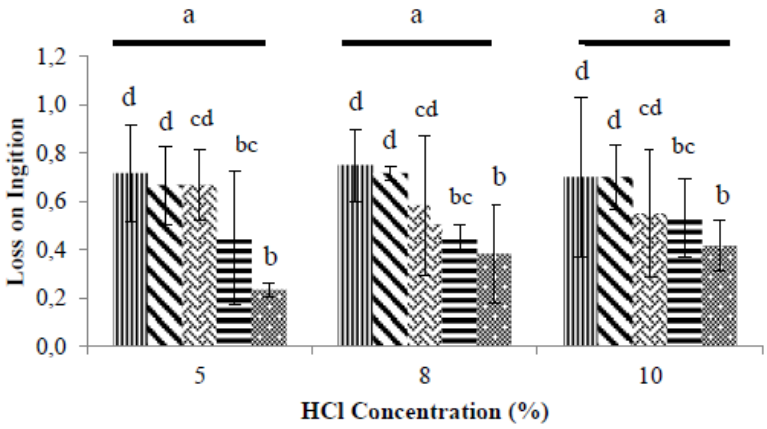

Figure 5. Glucosamine LoI. Heating (pressure-cooking) time: ▥ 30 minutes, ◥ 60 minutes, ▨ 90 minutes, ▬ 120 minutes, and ▩150 minutes. Treatments with the same superscript were not significantly different from each other ($p>0.05$).

The analysis of variance showed that heating time did have a significant effect on the glucosamine LoI however neither the HCl concentration nor the interaction between the two factors had a statistically significant effect ($p>0,05$) on the LoI of glucosamine produced using the pressurised hydrolysis method. The best glucosamine LoI values were produced by the 150 minutes heating treatment, with 0,233% at 5% HCl, 0,383% at 8% HCl and 0,417% at 10% HCl. Duncan's multiple range test showed that different heating times, i.e. 90, 120 and 150 minutes, had a significant effect on glucosamine LoI.

The average values indicate that the LoI from the combustion of glucosamine ranged from 0,233% to 0,750%. Longer heating times during glucosamine extraction produced glucosamine with higher LoI values. This is related to the concentration of the acid used, as higher acid concentrations will result in higher residues. This is intimately linked to the power of the acid to fully break up the chitosan to produce glucosamine with a high purity. In this research, the higher concentrations of hydrochloric acid were less effective in producing glucosamine so that there was were more (large) organic molecules remaining in the glucosamine, thus increasing the LoI. The LoI values in this research are above the [1] [2] standard of 0,1%.

3.6 Level of glucosamine deacetylation

The deacetylation level is determined by counting the amide bonds and the presence of amino groups in the Fourier Transform Infrared Spectroscopy (FTIR) output. The deacetylation levels of the glucosamine can vary depending on the relative volume of acid, reaction time and reaction temperature. High quality glucosamine has a high purity, which is often evaluated based on the level of deacetylation [14].

The deacetylation level of the glucosamine produced from this research was 99,44% (Figure. 7), higher than the standard for glucosamine which is 97,99%. This result shows that the purity of the glucosamine produced using this pressurised hydrolysis method complies with established standards for the commercial production of glucosamine set at 75,0-95,00% by the [2] and 98,00% by the [1]. The FTIR spectrum of the glucosamine produced can be seen in Figure 6.

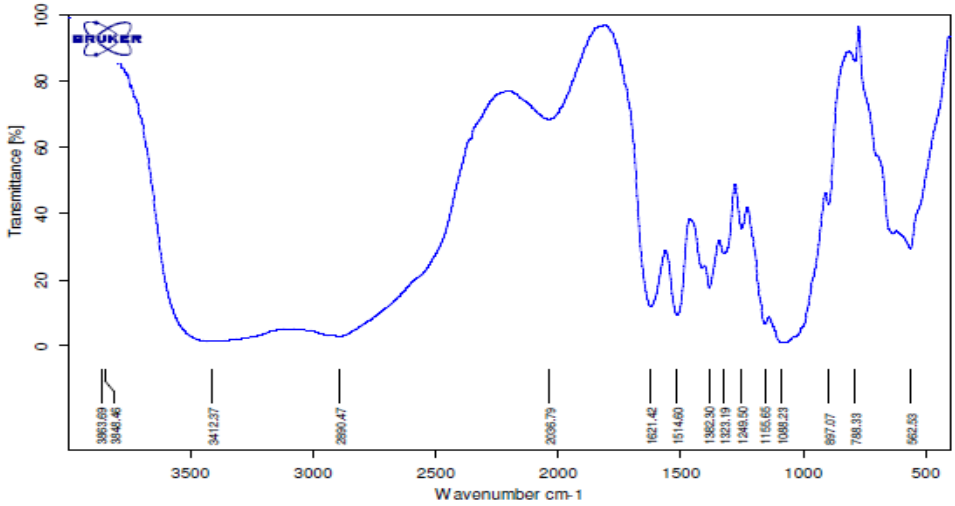

Figure 6. Deacetylation spectrum of the glucosamine research

The FTIR spectrum (Figure 7) shows the standard glucosamine spectrum, with a dominant OH^- group indicated by a wide and strongly marked line at a wavelength of 3676,50 cm^{-1}. The spectrum of the glucosamine produced by the pressurised hydrolysis method (Fig. 6) showed a dominant OH^- group at a wavelength of 3863,69 cm^{-1}. [4] state that the glucosamine monomer will have an OH^- group trace at a wavelength of 3350 cm^{-1} while if it is in polymer form the OH^- group trace will be closer to 3450 cm^{-1}. The dominant N-H trace in glucosamine produced through hydrolysis by [13] was at 3333 to 3380 cm^{-1}.

The primary amide N-H absorption trace can be seen at 3450 cm^{-1} while the secondary amide trace is at 1566 cm^{-1}. For solid samples, the primary amide trace is in the 1640-1620 cm^{-1} range. According to [3] the secondary amide trace was around 1550 cm^{-1}, while [3] give values in the range 1535-1583 cm^{-1}.

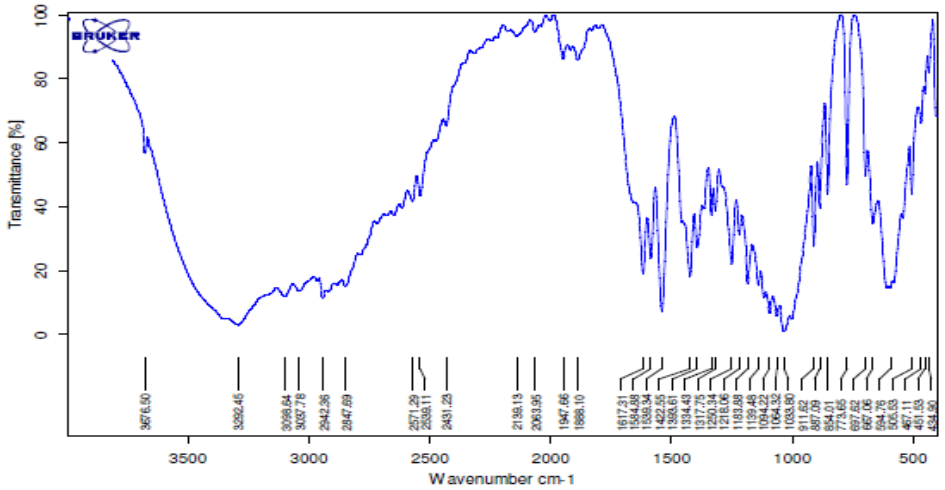

Figure 7. Standard glucosamine deacetylation spectrum

Overall the absorption traces for the GlCN group for glucosamine produced by the pressurised hydrolysis method are similar to and tend to be a little higher than the glucosamine standard, albeit with some variation in wavelength. This can happen due to the range in possible absorption wavelengths for each functional group. Differences in absorption

wavelengths between the standard sample and production samples are considered normal and acceptable as long as the values are still within the wavelength ranges for each functional group.

4. CONCLUSIONS

The pressurised hydrolysis method applied to glucosamine production using an HCl concentration of 5% with 60 minutes pressurised heating time produced a rendement of 65,33%, with 96,33% solubility and purity of 99,44%. The pH was 5,66 with a heating time of 90 minutes, while for a heating time of 150 minutes the LoD was 0,60% and the LoI was 0,233%.

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