Preparation of Glucosamine Hydrochloride from Crustacean Shell Waste and Its Quantitation by RP-HPLC


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Abstract: Various products derived from crustaceans have food and medicinal values. Glucosamine is an amino monosaccharide acting as substrate for the production of aggrecan and proteoglycans and thus have therapeutic activity in osteoarthritis. The present study has been aimed to prepare glucosamine hydrochloride (Glu-HCl) from various crustacean shells namely Penaeus monodon (Indian shrimp), Portunus pelagicus (blue crab) and Portunus sanguinolentus (three spot crab) by acid hydrolysis and its quantitation by reversed phase high performance liquid chromatography (RP-HPLC). The yield of chitin after demineralization with 0.5M HCl was 87.83%, 89.18% and 51.11% and deacetylation of chitin with 2N NaOH resulted in the yield of 68.91%, 75.67% and 30% for P.sanguinolentus, P.pelagicus and P.monodon respectively. HPLC analysis of obtained glucosamine hydrochloride revealed species of Portunus (21.64 mg g⁻¹ and 21.83 mg g⁻¹) were better source of Glu-HCl than P.monodon (3.32 mg g⁻¹). Further, this study describes the recycling of crustacean wastes to a value added product which is having potential applications in the field of food and medicine.

Key words: Glucosamine HCl • HPLC • Crustaceans • Chitosan • Chitin

INTRODUCTION

The major economically important group of crustaceans includes lobsters, shrimps and crabs. About 40-50% of total weight of crustaceans goes as waste while processing for human food and the slower degradation of crustacean shell waste has become the major concern in sea food processing industries [1, 2]. Further, it has resulted in waste collection, disposal and pollution problems [3]. Proper use of crustacean wastes allows recovery of value added by products which are having potential applications in the field of food and medicine [4-8]. Glucosamine has been prepared from various crustaceans [9-10]. Glucosamine produced by hydrolysis of chitosan has therapeutic activity in osteoarthritis [11]. Methods have been described for the quantitation of glucosamine in chitin by HPLC [12-15]. Chitin is mainly produced from cuticles of various crustaceans mainly by crabs and shrimps. Chitin is the major structural component of exoskeleton of invertebrates and cell wall of fungi and is composed of 2-acetamido-2-deoxy-β-D-glucose (N-acetyl glucosamine). Deacetylated form of chitin is known as chitosan which is composed primarily of 2-amino-2-deoxy-β-D-glucose (glucosamine). Glucosamine is an amino monosaccharide acting as a preferred substrate for the constitution of glycosaminoglycan chains. It is also a substrate for the production of aggrecan and proteoglycans which gives hydrophilicity to the cartilage thus beneficial in treatment of osteoarthritis [16]. Glucosamine has anti-cancer [17, 18], anti-inflammatory [19] and antibacterial [20] effects.

The present study was aimed to prepare glucosamine hydrochloride using crustacean (Penaeus monodon, Portunus pelagicus and Portunus sanguinolentus) shell waste involving demineralization, deproteination and deacetylation processes. Both quality and quantity of obtained Glu-HCl were determined by Fourier Transform Infrared Spectroscopy (FT-IR) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC).
MATERIAL AND METHODS

Demineralization: Crustacean (shrimp and crab) shells were collected from local market and washed in cold water tap water by removing dirt and loose tissues. Legs and heads were separated and the shells were dried in hot sun for 24 hours. After drying, the shells were treated with 0.5M HCl (1:14 w/v) for 8 hours at room temperature and the squasy shells were rinsed in water to remove calcium chloride.

Deproteination: The demineralized shells were treated with 1N NaOH (1:12 w/v) at 90°C for 2 hours and the residue was neutralized in running tap water. The deproteinized shells were dried in sun to obtain the chitin material which was further used to produce chitosan by deacetylation process.

Deacetylation: Chitin was treated with 2N NaOH (1:14 w/v) solution to remove the acetyl groups at room temperature for 8 hours in a shaker. The resulting chitosan was washed in running tap water followed by distilled water and dried in sun.

Preparation of Glucosamine HCl: The chitosan material was coarsely grinded and hydrolyzed with conc. HCl at 90°C for 75 minutes. The resultant brownish black material was dissolved in distilled water and decolourized with activated charcoal. The solution was filtered and the filtrate was evaporated at 45°C to recover glucosamine hydrochloride (Glu-HCl). The crystals were washed in ethanol and dried at 50°C in hot air oven and analyzed by high performance liquid chromatography.

FT-IR Spectrum: Standard glucosamine hydrochloride and obtained Glu-HCl were compared using FT-IR spectra for identification and comparing purity. The FT-IR spectrum was recorded on a Cary 640 FT-IR (Agilent Technologies) in the form of Kbr discs. The resolution was in 2 cm⁻¹ and the scanning range was 4000-800 cm⁻¹.

HPLC Method: HPLC analysis was performed using 1260 infinity series LC system installed with a G1311C pump, a G1329B autosampler and a G13166 column compartment and G4212B DAD Detector. Chromatographic separation was carried out on a Zorbax Eclipse XDB-C8 Analytical column (4.6 × 250mm, 5 µ; Agilent Technologies). The mobile phase used was orthophosphoric acid (pH 2.5): acetonitrile (70:30) with the flow rate of 0.6 ml min⁻¹ in column at ambient temperature with infusion volume of 20 µl in each experiment. Injection volume was 10 µl and detection was by UV absorbance at 195 nm.

RESULTS AND DISCUSSION

Glucosamine is part of the structure of chitosan and chitin which compose the exoskeletons of crustaceans, arthropods and fungi. The hydrolysis of chitosan results in monomers of β-(1-4)-linked-D-glucosamine (GlcN), an amino monosaccharide with physiological importance to the human body.

In the present study, crustacean wastes were used to produce chitin and chitosan by acid hydrolysis method. The yield of chitin after demineralization was 87.83%, 89.18% and 51.11% and deacetylation of chitin with 2N NaOH resulted in the yield of 68.91%, 75.67% and 30% for P.sanguinolentus, P.pelagicus and P.monodon respectively (Table 1).

The finger prints of the FT-IR spectra of standard and prepared Glu-HCl does not show any excess peaks (Fig 1). The absorption bands at particular wavelength are due to the formation of NH₃⁺ in Glu-HCl which was in accordance with previous data [21].

Peak identification of glucosamine was done by comparing with the retention time of pure standard. Purity of the peaks was confirmed with the characteristic spectra obtained from the detector. Fig 2 represents the chromatograms glucosamine hydrochloride prepared from crustacean shells. In the figures, y axis is milli absorbance unit (mA) and x axis is retention time (RT) in minutes. There were two peaks occurred close which are related to adsorption of glucosamine in the standard chromatogram (Fig 2). From the HPLC analysis, the yield of Glu-HCl prepared from crustacean wastes was 21.64 mg g⁻¹, 21.83 mg g⁻¹ and 3.32 mg g⁻¹.

Previous studies have revealed that acid hydrolysis is the preferred method to release glucosamine from chitin material [22]. Several factors such as time, temperature,
Fig. 1: FT-IR spectra of Glu-HCl

a. *Portunus sanguinolentus*

b. *Portunus pelagicus*

c. *Portunus montodon*

Fig. 2: HPLC chromatograms of glucosamine hydrochloride from crustacean waste
Table 1: Yield of chitin, chitosan and Glu-HCl from crustacean waste

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chitin</th>
<th>Chitosan</th>
<th>Glu-HCl (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portunus sanguinolentus</td>
<td>87.83%</td>
<td>68.91%</td>
<td>21.64</td>
</tr>
<tr>
<td>Portunus pelagicus</td>
<td>89.18%</td>
<td>75.67%</td>
<td>21.83</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>51.11%</td>
<td>30%</td>
<td>3.32</td>
</tr>
</tbody>
</table>

pH and acid concentration are attributed to the release of glucosamine from chitin or chitosan. Acid hydrolysis of chitin with concentrated HCl for longer time leads to breakdown of glucosamine and decreased recovery [23]. Low concentrations of hydrochloric acid slow down the chitin hydrolysis. Mojarrad et al. [15] described the optimized conditions for the preparation of glucosamine HCl as 30% and 37% HCl (9:1 v/w) for 4 hours from Metapenaeus monoceros. In the present study, HCl (0.5M) treatment for 8 hours produced a better yield of chitin (89.83 %) from P. pelagicus. Purchase and Braun [24] isolated glucosamine from crab shells. Stacey and Webber [25] described the simple production of glucosamine from crab shells of low protein content. The present findings revealed that exoskeleton of P. sanguinolentus and P. pelagicus were better sources of Glu-HCl than P. monodon under the described conditions. Ferrer et al. [26] has obtained 80% yield of glucosamine during the acid hydrolysis of shrimp shell wastes. The yield of chitin, chitosan and Glu-HCl from P. monodon was comparatively lower than that of crab species used in this study.

**CONCLUSION**

The present study was found as simple, efficient and suitable for the preparation of glucosamine from crustacean shells thereby recycling crustacean wastes. Further, HPLC method allows the detection and quantitation of glucosamine prepared by acid hydrolysis of chitosan from exoskeleton of crustaceans and could be used as routine method for analysis of Glu-HCl in raw materials and pharmaceutical formulations.

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**REFERENCES**


