**Evaluation of Glucosamine hydrochloride from biowaste of** ***Macrobrachium rosenbergii* and *Penaeus monodon*, different ponds of Andhra Pradesh**

**ABSTRACT**

 A variety of goods made from the skeletons of crustaceans have both biomedical and pharmacological abilities. As an amino monosaccharide that serves as a substrate for the synthesis of aggrecan and proteoglycans, glucosamine has therapeutic potential for osteoarthritis. The goal of the current work is to produce glucosamine hydrochloride (Glu-HCl) by acid hydrolysis from a variety of crustacean shells, specifically *Macrobrachium rosenbergii* and *Penaeus monodon* (tiger shrimp), and then quantify the result using reversed phase high performance liquid chromatography (RP-HPLC). The fiber found in crab, lobster, shrimp, and prawns is called chitin. Chitosan, a naturally occurring biopolymer, was created by deacetylating chitin, one of the main structural components that make up the exoskeleton of crustaceans. Chitosan is a linear polymer of a (1–4)-linked 2-amino 2-deoxy b-D glucopyranose. Shell trash is produced in large quantities by the aqua industry and is typically dumped, causing significant environmental nuisance. Glucosamine hydrochloride, chitin, and chitosan can all be obtained commercially from such waste. In this study, the yield % of the tiger shrimp (*Penaeus monodon*) and freshwater prawn (*Macrobrachium rosenbergii*) is being assessed. In comparison to *P. monodon*, *M. rosenbergii* was a superior producer. The recycling of crustacean waste into a product with added value is also covered in this study.

**Key Words:** Glucosamine hydrochloride, Chitin, Chitosan, *M. rosenbergii* and *P. monodon*.

**Introduction:**

 The socioeconomic development of our nation is greatly influenced by the shrimp industrial sector, and shells are frequently thrown away as garbage. A cationic amino-polysaccharide, chitin is composed of N-acetyl-D-glucosamine residues that are connected by β (1-4). Crustaceans, mollusks, diatoms, insects, and fungus are among the species whose exoskeletons contain it [1]. Between 60,000 and 80,000 tonnes of chitinous waste are produced each year in India alone; a significant amount of chitin can be extracted from crustacean bio-waste [2, 3]. Numerous industries, including nanotechnology, pharmaceuticals, tissue engineering, genetic engineering, and the food industry, have found use for chitosan [3]. Because of their antibacterial qualities, coagulation, non-toxicity, biodegradability, and biocompatibility in both plant and animal tissue [4, 5].

Gels, beads, colloids, films, and capsules can all be made from chitosan [6]. They can be used for everything from mechanical support to diet. But because they are thought to be non-biodegradable and take years to return to nature, they have also caused issues with disposal. In other words, they take up a lot of room and constitute a significant problem as environmental contamination raises the need for biopolymers, which have the qualities of being non-toxic, biocompatible, and biodegradable [7]. Nearly 40–50% of the weight of raw shrimp is produced as shrimp biowaste during processing by the shrimp industry [8, 9]. Shrimp trash is produced by these activities, and this solid waste is a problem for the environment [10].

A white to light-red solid powder, chitosan is derived from chitin and is insoluble in water and organic acids yet indigestible by human digestive enzymes [11]. Commercial interest in chitin and its derivative chitosan stems from their superior adsorption, chelating, biodegradability, and non-toxicity. Chitosan, in particular, has a lot of appealing uses in biotechnology, the food and pharmaceutical industries, cosmetics, environmental engineering, agriculture, and aquaculture because of these qualities [12]. In light of the importance and uses of chitosan, the current study aims to compare the yield percentage of glucosamine hydrochloride and quality characteristics of freshwater prawns *Macrobrachium rosenbergii* and marine shrimp *Penaeus monodon*.

**Materials and Methods**

Freshwater prawn bio-waste while the bio-waste of marine shrimp, *Penaeus monodon*, was obtained from shrimp culture ponds at Bapatla District, Andhra Pradesh, *Macrobrachium rosenbergii* was taken from freshwater culture ponds (Fig. 1 & 2). The two samples' moist weights were recorded after the bio-waste (carapace, exoskeleton, appendages, etc.) was removed. Before shipping for additional processing, the shell material was homogenized in a lab mixer and allowed to dry in an oven set at 500C for 24 hours. Weighing both dried shell samples after they had been dried allowed us to calculate their yield. Until they were needed, the collected shell samples were kept in the storage facility at roughly 250C. Biowaste is treated with 4% alkali to separate the protein and 4% acid to remove calcium carbonate. Concentrated 50% alkali can further deacetylate the resultant chitin product to prepare chitosan.

**Deproteinization:** 10 g of shell powder was dissolved in 300 ml of 2mol/dm3 H2SO4 solution at a ratio of 1:30 using crushed biowaste samples. Distilled water was used to wash the sample until the pH was neutral. The sample was demineralized and then dissolved in 300 milliliters of a 2 mol/dm3 NaOH solution to deproteinize it. After being stored for four hours once again, the material was cleaned until its pH was neutral [13].

**Deacetylation:** To perform the deacetylation procedure, 55% NaOH is added, and the mixture is then cooked on a hot plate for two hours at 1000C. After that, the samples are put under the hood and allowed to cool to ambient temperature for thirty minutes. In order to preserve the solid material, which is the chitosan, the samples are then continually cleaned with 50% NaOH and filtered. After being left exposed, the samples were oven-dried for six hours at 1100C. Following cooling, the sample was filtered to extract chitosan and rinsed with 55% NaOH [13, 14].

**Percentage of Yield**

Based on the weight of the shell bio-waste, the percentage of chitin and chitosan yield was determined [15].

 $\% Chitin or Chitosan = \frac{Weight of Residue (g)}{Weight of initial dry raw materia (g)} X 100$



 **Fig: 1. *Macrobrachium rosenbergii***



**Fig: 2. *Penaeus monodon***

**Preparation of Glucosamine HCL:**

The chitosan was hydrolyzed with concentrated HCL at 90°C for 75 minutes after being coarsely ground. After dissolving the resulting brownish black substance in distilled water, activated charcoal was used to remove the color. To recover glucosamine hydrochloride (Glu-HCL), the solution was filtered, and the filtrate was then evaporated at 45°C. After being cleaned with ethanol and dried in a hot air oven at 500 degrees Celsius, the crystals were subjected to high performance liquid chromatography analysis.

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**Fig: 3. Glucosamine Hydrochloride**

**Results and Discussion:**

Crustacean and other arthropod shells are made of chitosan and chitin, both of which include glucosamine. A monosaccharide of critical physiological importance in the human body, β-linked D-glucosamine, is produced when chitin/chitosan is hydrolyzed. Table No. 1 shows the bio-waste yield from the freshwater prawn *Macrobrachium rosenbergii* and shrimp *Penaeus monodon*. The yield of glucosamine hydrochloride, which is the result of the current study (Fig.4). 580g of biowaste, 60.9 percent chitin and 40.2 percent chitosan, and 11.2 percent glucosamine hydrochloride were all created from one kilogram of prawn *Macrobrachium rosenbergii* sample used in this test. The *Penaeus monodon* produced 439g of biowaste, 49.6 percent of chitin & 35.5 percent of chitosan and finally 9.9 % of Glucosamine hydrochloride.

**Table - 1: The hydrolysis of chitosan results in monomers of yield of bio-waste from experimental organisms**

|  |  |  |
| --- | --- | --- |
|  **Experimental Species** |  **Bio-waste (g)** | **Total sample weight (g)** |
| *Macrobrachium rosenbergii* | 580 | 1000 |
| *Penaeus monodon* | 439 | 1000 |

**Fig: 4. Yield of Crude bio-waste, Chitin and Chitosan and Glucosamine hydrochloride from the *M. rosenbergii* and *P. monodon***

The shells of the crustaceans are cleaned, dried and ground into powder. Chitosan is obtained from chitin which is the primary structural polymer [16, 17]. The production of chitin from crab shells may range from 60% to 70% [18]. A higher yield, or 78%, from mud crabs was noted by Ali *et al.,* [19]. Chitosan's economic importance has increased recently because to the advantageous qualities of its soluble derivatives, which can be used in food processing facilities, cosmetics, and medicine to suppress tumor growth and cell viability [20, 21]. According to Diya *et al.,* [22], shrimp shells yield 30-36.7% chitosan. The source and isolation process of chitin determine the quality of chitosan, and chitosan with varying degrees of deacetylation is being marketed. Chitosan's uses are determined by its properties and molecular weight. Utilization of such shell wastes production of some useful products such as chitin and chitosan [23] and chemical treatment in anticancer and antitumor activity [24, 25]. Krishna *et al.,* [26] investigated the chitosan yield percentage and quality parameters in *M. rosenbergii*, *P. monodon* and *L. vennamei*.

Fabrics, gauzes, dialysis membranes, antiviral and antifungal medicines, and wound dressings are among the items made with chitin and chitosan [27]. Because it inhibits the growth of bacteria, chitosan aids in the healing process and protects wounds against bacterial invasion. It might work just as well against microbes that cause typhoid [28]. The degree of deacetylation is one quality measure that indicates an acetyl group that can be eliminated from the chitosan yield. A number of other factors impact the quality of chitosan. In the present study the *M. rosenbergii* produced 11.2% and *P. monodon* produced 9.9% of Glucosamine Hydrochloride (Fig.3) from 1kg of shell waste.

Rossi *et al.,* [29] stated that shrimp wastes are promising avenue for sustainable resource utilization and the extraction of valuable bioactive compounds. Jafari *et al.,* [30] reported that the glucosamine hydrochloride could exert beneficial effects to the treatment of diseases related to the inflammation oxidative damage. Hamdan *et al.,* [31] stated that shrimp waste to provide added bio-compounds offers environmental and therapeutic benefits.

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