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Preparation and Comparative Study of Chitosan from Shells of Different Marine and Freshwater Prawns

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Chitin is the main constituent of the exoskeleton or shells of the crustaceans which is treated as a waste but if it is purified we get chitosan which is useful for various industrial and agricultural purposes and used as biomedical agent. Among the various crustaceans, prawns are known to have a higher amount of chitosan. Various species of prawns contains different amounts of chitosan in their shells. The shells of prawns are treated by Deproteinization, demineralization, decolouration and deacetylation methods. Present experiment deals with purification of chitin into chitosan and determining their chitosan content in prawn species *Penaeus indicus, Penaeus monodon, Peneaus aztecus, Macrobranchium rosenbergii* and *Macrobranchium semisulcatus* which are locally available in Mumbai region. This gives us a detail comparison of the amount of extractable chitosan available in each of these prawn species to know the high content of chitosan present among these species. Hence it can be used commercially for mass production for various applications.

Key words: Chitin, *Penaeus*, exoskeleton, treatment, extraction, Chitosan.

Prawns are consumed largely as a protein rich food. Its outer covering exoskeleton or shells constitute almost 50% of its body mass. Exoskeleton generally considered as waste cannot be used as food or as a raw material in any product directly due to its insolubility in water. These shells contain Chitin, which is widely distributed in nature. It is a white, amorphous, semitransparent mass that is insoluble in common solvents like water and alcohol. Since chitin is the second most abundant natural polymer, academic as well as industrial scientists are faced with a great challenge to find new and practical applications for this material (Qin *et al,* 2005). The shells of prawns pose an environmental problem, mainly due to their insolubility in water and very slow biodegradation. (Gamage and Shahidi, 1991*).* The shells are stripped off their lipid, protein and mineral content to form the chitin, which on further treatment gives chitosan. The prawn species *Penaeus indicus, Penaeus monodon, Peneaus aztecus, Macrobranchium rosenbergii* and *Macrobranchium semisulcatus* are collected from Mumbai and Thane region. By using treatment techniques such as, Deproteinization, demineralization, decolouration and deacetylation; the chitin of these 5 species of prawns was converted into chitosan. The chitosan is useful

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for various industrial and agricultural purposes. It is also useful as a biomedical agent in engineering scaffolds, in Drug Delivery System, Surgical sutures, etc. Keeping in view the importance of chitosan the experiment was also carried out to study the chitosan content of each of these 5 varieties of prawn species. The results obtained were further evaluated for their comparative study to know the higher content of chitosan present in these prawn species.

MATERIAL AND METHODS

The variety of the prawn plays an important role in the amount of chitosan produced. The prawns were cleaned, the flesh separated from the shells. The shells were washed under running water to remove soluble organics, adherent proteins and other impurities. The shells were then dried in the oven until completely dried shells were obtained. The dried shell was then ground to obtain a coarse powder of uniform size and used for the treatment.

Deproteinization (DP)

The prawn shell waste powder was subjected to a chemical treatment by sodium hydroxide at an ambient temperature for a desired time period to remove the present proteins thus forming a deproteinized product which is followed by demineralization.

Demineralization (DM)

The deproteinized product was then subjected to a further treatment with concentrated HCl at room temperature to yield a demineralised product which is subjected to decolouration.

Decoloration (DC)

The removal of any dyes or colour present was done by treating it with a mixture of ethanol and acetone. This deproteinized, demineraized and decolorized product is called as chitin.

Deacetylation (DA)

The chitin was purified by removal of the acetyl groups by treatment with a high concentration of NaOH at ambient temperature for a certain period of time. The product thus resulting is called chitosan.

The above steps were carried out for all the 5 varieties of prawn species in triplicates to avoid error in results. During process at every stage weight has been calculated. The chitosan content in each of the varieties of prawns was compared to the other to determine higher chitosan content.

RESULTS

Chitosan is a product which is obtained after the chemical treatment of the prawn shells. In each stage of treatment of shell there is a reduction in the weight. A certain temperature range was set to determine the ideal temperature for each of the respective steps.

Weight after deproteinization: The shell powder was subjected to chemical treatment with sodium hydroxide and was then heated at temperatures of 80°C, 95°C and 105°C. The test was done in triplicates for each variety (Table 1).

Table 1. Weight after deproteinization

Sample name	Weight at 80°C	Weight at 90°C	Weight at 105°C
P.indicus	4.216	3.60	3.503
P.monodon	3.601	3.024	3.018
P.aztecus	4.279	3.708	3.587
M.semisulcatus	3.840	3.145	3.121
M. rosenbergi	4.281	3.871	3.840

The Demineralization of the deproteinized sample was done using conc. HCl. After the demineralization, the sample was then decolourised using a solution of ethanol: acetone. The test was done in triplicates. The averages of the triplicates are given below (Table 2).

Table 2. Weight after demineralization and decolouration at room temp

Sample name	Weight after demineralization	Weight after decolouration
P.indicus	1.889	1.848
P.monodon	1.754	1.736
P.aztecus	1.773	1.772
M.semisulcatus	1.644	1.594
M. rosenbergi	1.426	1.416

The product thus obtained is the chitin which is further deacetylated by treating it with conc alkali such as 50% NaOH at 3 different temperatures (80°C, 90°C and 100°C) to yield chitosan (Table 3).

Table 3. Weight of sample after demineralization

Sample name	80°C	90°C	100°C
P.indicus	1.108	1.107	1.107
P.monodon	1.287	1.287	1.287
P.aztecus	0.928	0.928	0.927
M.semisulcatus	0.977	0.977	0.975
M. rosenbergi	0.980	0.980	0.979

The chitosan of the 5 prawn varieties were weighed. The weight of the chitosan after successive steps of treatment determines the product yield (Table 4).

Among these 5 different prawn species the highest content of chitosan in *P. Monodon*

Table 4. The chitosan weight of 5 different prawn species

Sample name	Chitosan content (gm/ 5 g of powder)	Percent chitosan
P.indicus	1.108	22
P.monodon	1.287	25.7
P.aztecus	0.928	18.56
M.semisulcatus	0.977	19.55
M. rosenbergi	0.980	19.6

DISCUSSION

Chitosan can also be produced from the microorganisms but waste from the microorganisms is not as large as the crustaceans, the maximum being prawns considering the body mass of the organism to its chitinious exoskeleton waste. The present study uses prawns as a source for the production of chitosan as prawns produces a larger amount chitin from its shells. In the deproteinization stage, according to *Varum (2004)* the time taken was very low, at 30°C and 10M of NaOH concentration. In the present study the decrease in concentration of 2M of NaOH and increase in time of 2 hr at 95°C showed very similar results as that of *Varum (2004)* at the cost of less molar of NaOH. The demineralization acid used in the present study is 1N HCl where as the organic acids used in the method of *Bassi, R, Prasher, S.O (2000).* In 1N HCL it was kept for a shorter time period so as not to break the polymer chain of chitin and also does not affect the quality of the final product *Olsen et al, (1996)* stated that the degree of deacetylation of chitosan produced at 110°C ranged from 78 to 84 percent when not stirred (chitosan A) in NaOH (47 percent w/v) for 4 to 24 hours, and from 75 to 93 percent when it was stirred (chitosan B) in NaOH (47 percent w/v) for 1 to 20 hours. However in the present study the deacetylation is carried out at a lower temp of 80°C and with a higher sodium hydroxide concentration. Hence the highest degree of deacetylation was seen at temperature 80°C beyond this temperature the degree of deacetylation remained constant. Concentration of sodium hydroxide does not play a major role on the degree of deacetylation and was maintained as 50% concentration for all 3 temperature ranges.

Decoloration can be done with sodium hypochloride (*Mima, 1983*), which acts as bleaching agent. Sodium hypochloride is expensive and is a harsh chemical. Similar decoloration result was observered in the present study on using ethanol: acetone. The product gets

a uniform off-white colour. There are many methodologies available for the production of chitosan from the crustacean shells. However all these methods involve use of expensive chemicals and instruments.

As seen in the results the maximum yield of chitosan is observed in *P. monodon* (tiger prawns) and the minimum is seen in *P. Aztecus.* Hence habitat of the particular variety does not play a role in determining the amount of chitosan yield.

CONCLUSION

Two factors—economics and versatility-have stimulated interest in the production and utilization of chitosan in various fields. Chitosan derivatives have applications in fields that range from fertilizers to pharmaceuticals. Applications of Chitin and Chitosan is of great interest in bio processing as well as bioengineering, specialists in the biomedical and biopharmaceutical industry, biochemists, food engineers, environmentalists, and microbiologists and biologists who specialize in chitosan technology .

Producing chitosan through the present study is inexpensive since this technique does not require costly instrumentation such as, ultra filtration and molecular sieves. The availability of less expensive chitosan is now possible for its use in various applications.

Present purification method is used as a modification of the production method to produce the chitosan. Although it is not a completely pure medical grade product it requires further purification. Chitin and chitosan have a very promising future in the sector of research and development and in aquaculture technology.

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