Chapter 16

Utilization of shellfish processing discards

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The commercial aquaculture for crustaceans in India has become a huge success due to the introduction of new species, the improved hatchery production of seeds, scientific management of culture practices and the availability of good quality feed and other input. Introduction of new species like Letopenaeus vennamei has resulted in increased yield and productivity. The farming of this species has already been established in coastal Andhra Pradesh, Karnataka and Tamil Nadu and gaining momentum in Kerala and other states.

Similarly, farmers in both coastal and land locked States have gone for large scale farming of Giant Freshwater Prawn (Macrobrachium rosenbergii) popularly called "Scampi" which is having high demand in both domestic and international markets. In order to meet the raw material requirement of large number of processing units established for export and also to meet the domestic demand. The state of Andhra Pradesh accounts for more than 50 per cent of the cultured Scampi production and also in terms of area under culture. During the year 2013-14 the estimated production of L. vannamei was 406018 tons whereas the black tiger export of cultured prawn from the country was to the tune of 41947 tons and that of scampi, it was 1401 tons (MPEDA 2008).

Industrial processing of prawn results in huge quantities of waste in the form of head and shell. Since the exported shrimp products are mainly of peeled items, the shell waste produced is quite high. The head and shell constitute nearly 60% by weight of the whole prawn depending on the species and size of the prawn. In India its availability is estimated to be 100,000 tonnes annually and it is the single largest fishery waste of the country. Crab shell and squilla are other important raw materials available from marine sector.

Proximate composition

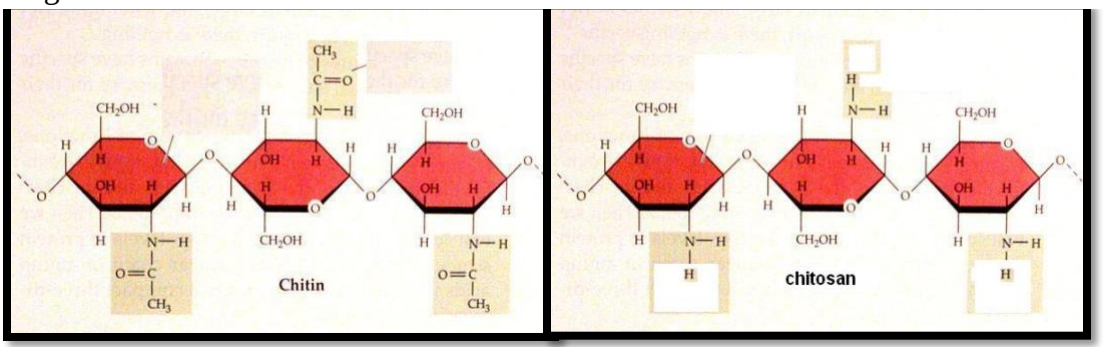
Characteristic	Prawn waste	Squilla	Crab shell
Moisture %	75-80	60-70	60-65
Ash (% dry weight basis)	30-35	33-37	45-50
Protein (% dry weight basis)	35-40	40-45	30-35
Chitin (% dry weight basis)	15-20	12-16	13-15
Fat (% dry weight basis)	3-5	2-3	1-1.5

The investigations carried out at the Central Institute of Fisheries Technology, Cochin, paved the way for production of valuable food and industrial products namely protein extract, chitin and its

derivatives chitosan and glucosamine hydrochloride from the head and shell waste of prawns, crab and squilla.

Chitin (anhydro- N-acetyl- D-glucosamine (N-acetyl 2- amino 2- deoxy D-glucose) is the second most ubiquitous natural polysaccharide after cellulose on earth. It is estimated that chitin is produced annually almost as much as cellulose. Chitin is a white hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates. The monomer units are linked by β (1-4) glycosidic bonds as in cellulose. It is insoluble in water and most organic solvents. It is the most important organic constituent of the exoskeleton of arthropods. The tough and resilient property of chitin is utilized by the living organism as skeletal support and body armor against attack by other marine animals.

The waste of these natural polymers is a major source of surface pollution in coastal areas. Chitin is considered as under utlised resource which has got high potential in new functional biomaterial in various fields. The most important economical source of this material is the shrimp processing industry. Apart from shrimp, the shells of lobster, crab, squilla and squid pens also provide chitin in large quantities. Deacetylation of chitin gives chitosan, a high molecular weight linear polymer of amino-D-glucose.



Production process for chitin and chitosan

Chitin is present in the shells of shrimp or crab or squilla as chitin protein complex along with minerals mainly calcium carbonate. The process for chitin production comprises demineralisation and deproteinisation to isolate chitin. In commercial production demineralisation is done by dilute hydrochloric acid and deproteinisation by dilute aqueous sodium hydroxide. The chitin thus isolated is deacetylated using con.aqueous caustic soda for production of chitosan.

Raw materials

The wet fresh head and body peelings of prawn collected from the peeling centers, crab shell from the processing plants and squilla caught along with prawns can be used either directly immediately on arrival at the plant or can be dried and stored and can be used when required as per the production programme. Shell can be collected from distant centers in the dry form in which case transportation is comparatively easy and economical. Care should be taken to see that the shell should not contain sand and extraneous matter to any significant level.

Selection of raw material

If production of shrimp protein extract is envisaged during the production of chitosan more care has to be taken in the selection of raw material. Only fresh prawn waste can be used for the extraction of protein. It should be iced and hygienically stored and transported. Commercial dry shell gives only very dark coloured protein paste. Moreover, cleaning of the shell is not practical as it normally contains objectionable foreign matters. But, if chitin/chitosan alone is the desired end product dry commercial shell can be used as the starting material.

Production process

The process involves two important stages. (1) Isolation of chitin from shell (2) Conversion of chitin to chitosan.

Isolation of chitin

Chitin is isolated from the shell by demineralisation followed by deproteinisation. If extraction of protein is envisaged for production of shrimp extract only hygienically collected fresh prawn shell has to be taken for processing. The fresh shell has to be treated first with 0.5% dilute aqueous caustic soda and the alkaline protein solution is drained out and kept separately for neutralization, concentration and drying. The residual shell is then deproteinised followed by demineralisation.

Demineralisation

Demineralisation is the process by which the minerals are removed from the shell. If recovery of the protein is not envisaged the wet or dried shells can be directly treated with dilute commercial hydrochloric acid at concentration around 1.25 N at room temperature. The demineraliser is an open cylindrical tank of size 2 m x 1.5 m made of S.S. or M.S. or brick masonry lined inside and outside with fiber glass having perforated false bottom made of S.S wire mesh with 3 mm mesh size and with sufficient reinforcement at the lower end of the cylindrical portion. The vessel is fitted with a propeller agitator of 60 rpm and 80 cm sweep driven by a 5 HP electric motor from the top for gentle agitation of the mass to facilitate the reaction and to avoid floating of the shell. The vessel is so installed that the acidic effluent can be drained by gravity to the fiber glass lined collection tank constructed in brick masonry by the side of the demineraliser. The demineraliser is to be housed in a well-ventilated place with suitable exhaust facility to remove the acid fumes as well as the carbon dioxide coming out during demineralisation of the shell. Demineralisation is an important step in the production of chitin and chitosan. The degree of demineralisation determines to a great extent the characteristics of chitosan.

Deproteinisation

The deproteinisation is the process by which the protein is removed from the chitin protein complex. The shell after demineralisation and washing free of acid is shifted to the deproteiniser where it is treated with 5% aqueous caustic soda at 70-80^{0}C with continuous stirring at an rpm of around 100 using propeller type stirrer for about 30 minutes in a false bottomed steam jacketed open cylindrical

M.S. vessel having arrangements for heating either by steam or by thermic fluid heat exchanger. By 30 minutes the protein from the shell will dissolve in the alkali which can be drained off. The residue is washed well with water to make it free from alkali. This requires at least three washings in potable water with agitation. The product is wet chitin.

Deacetylation of chitin to chitosan

The wet chitin from the deproteiniser is transferred to the cemented collection tank and there to the centrifuge/hydraulic press/screw press for removal of water to the extent possible. The dewatered chitin cake is charged to the deacetylator where it is treated with 50% (w/w) aqueous caustic soda solution at 90-95^{0}C for 1.5 to 2 hours or longer till the deacetylation reaches the required level. After deacetylation, which is ascertained by checking the solubility in 1% acetic acid, the alkali is recovered for reuse. The residue washed twice with minimum quantity of water and collected for reuse making up the concentration. The alkaline chitosan mass is washed well either in the same vessel or after transferring to a storage tank and taking in small quantities to a S.S. washing vessel to remove residual alkali.

Dehydration

The alkali free chitin or chitosan from the washing vessel is collected in canvas bags and pressed under a screw press or hydraulic press or centrifuged to remove the adhering water as far as possible. The residue is wet chitin/chitosan with moisture content around 70%.

The wet alkali free chitin/chitosan cake is taken out, fluffed and spread in clean aluminium trays and dried in hot air drier at temperature 65-70^0 C. Alternatively it can also be sun dried by spreading in open cemented floor protected from dust and other contaminants to moisture content below 7%.

Pulverization

The dried chitin/chitosan is sorted manually to remove any foreign material before pulverizing. The pulverizing can be done in a swinging hammer type or a pin type pulveriser fitted with a balloon or cyclone collector to the desired particle size by suitably changing the screen. Sorting is an important step for getting high quality chitin. The foreign matter like match stick, feather, nylon pieces etc. which are normally present in the shell will be carried to the product even after demineralisation and deproteinisation. No mechanical separation is as effective as manual separation although it involves considerable labour.

Bagging and storage

The powdered chitin/chitosan can be bagged in polythene lined HDPE (high density polythene) woven sacks. Usually a bag of size 100 cm x 65 cm is used for this purpose which can hold 25 kg chitin of 1 mm size or 40 kg chitosan of 0.25 mm size produced from prawn waste or 30 kg chitin or 50 kg chitosan from crab shell. Such bags can withstand all transporting hazards.

Product quality

In commerce chitin and chitosan with the following characteristics are acceptable to the end users.

Characteristics	CHITIN	CHITOSAN
Moisture %	<10	<7
Ash %	<2	<1
Protein %	<2	nil
Colour	off white	off white
Particle size	10-20 mesh	60-80 mesh
Solubility in 1% acetic acid	nil	soluble
Insolubles in 1% acetic acid	N.A	<0.5
pH	7.0-7.5	8-9
Nitrogen %	6.5-6.8	7-7.5
Deacetylation %	N.A	>80
Viscosity (m pa s) in 1% acetic acid at 1% level at 28°C	N.A	<100

The process described above will give chitosan of medium viscosity from commercial dry prawn shell. For low, high and special grade chitosan for specified end use parameters like time, temperature, concentration of acid and alkali during demineralisation and deproteinisation and deacetylation are to be suitably modified in addition to raw material selection. Strict quality control measures are to be adopted for minimising batch to batch variation.

Glucosamine:

Hydrolysis of chitin with concentrated hydrochloric acid causes deacetylation and breakdown of the polymer releasing the monomer as glucosamine hydrochloride. Dry Chitin powder was hydrolyzed with concentrated Hydrochloric acid in a glass lined reactor equipped with reflux condenser in a thermostatically controlled digital water bath with occasional stirring. The temperature of the reaction mixture was slowly raised to the optimum level and maintained at that level for the completion of reaction until the solution no longer gives opalescence in dilution with water. During the process the liberated HCl gas was absorbed in water. The excess acid can be distilled off under vacuum after completion. The undissolved residue, was filtered after adding equal quantity of water. To this mixture 10% activated charcoal was added and the solution was warmed to 60o C for 30 minutes and filtered. If the filtrate still coloured repeat the treatment with little quantity of charcoal. This pale yellow solution was evaporated to dryness in a reduced pressure and mixture was washed with alcohol and dried under vacuum. Glucosamine hydrochloride is an approved nutraceutical product. It is prescribed as a remedy for osteo arthritis and approved by USFDA. It is found to have antiflamatory and antiulcerogenic properties.

Chitooligosaccharides (COS) applications:

Production of COS is of immense interest since these oligosaccharides are thought to have several interesting bioactivities. COS produced using endochitinase showed antibacterial activity

against bacteria, that cause diarrhoeal and emetic syndromes in humans. Potential effects of COS reported were: as drugs against asthma, antibacterial agents, anti-fungal agents, ingredients in wound-dressings, reduce metastasis of tumors, increase bone-strength in osteoporosis, inhibit chitinases in *Plasmodium* parasites and thereby prevent malaria, immune modulators, and a lowering effect on serum glucose levels in diabetics.

Applications of Chitin, Chitosan and Glucosamine

Chitin and its derivatives, particularly chitosan (deacetylated chitin) find industrial application in various fields namely flocculation, paper making, textile printing and sizing, ion exchange chromatography, removal of metal ions from industrial effluents, manufacture of pharmaceuticals and cosmetics and as an additive in food industry. Several versatile applications of chitosan have been developed during the last three decades. There are about 200 current and potential applications of chitin and its derivatives in industry, biotechnology, food processing, pharmacy and medicine.

The application of chitosan for improvement of quality and shelf life of food products have been well documented. It can be directly incorporated into the food or can be used as coatings for food products or can be made as an integral part of the packaging materials. All these techniques are found to have beneficial effects on the food during processing and storage. Edible coatings can be used as a vehicle for incorporating functional ingredients such anit-oxidants, flavours and colors antimicrobial agents and nutraceutical into the food products whereby adding more value to the product. The applications of chitosan in the field of nanotechnology is being studied widely. It was observed that the antimicrobial properties of nano chitosan is far better when compared to natural chitosan. Nanochitosan in conjunction with metal ions have also been found to have applications in different fields.

Glucosamine hydrochloride and sulphate are marketed as food supplements for the treatment of osteoarthritis. Anti-ulcerative effect of glucosamine was recently reported. In the US glucosamine is one of the most common non-vitamin, non-mineral, dietary supplement used by adults. Since glucosamine is a precursor for glycosaminoglycans and glycosaminoglycans are a major component of joint cartilage, supplemental glucosamine may help to prevent cartilage degeneration and treat arthritis. Glucosamine and N-acetyl glucosamine help in building up connective tissue in joints (e.g. gycosamionoglycons (GAG), chondroitin and hyauronic acid). Glucosamine acts not only as a substrate for the synthesis of GAGs but also stimulates their synthesis and prevents degradation. Different combinations of glucosamine are now in use for treatment of arthritis and the annual global consumption of glucosamine exceeds 6000 tons.

Shrimp shell waste can be efficiently utilized by transforming it to value added by-products like chitin, chitosan, glucosamine and chitooligosaccharides that have wide and varied industrial applications.

Further Reading:

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