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Isolation and characterization of chitosan from crab (*Scylla serrata*)) shell waste

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Abstract

Crab shell waste is excellent sources of chitosan (41-53%). Procedures for isolation of chitosan have been developed, along with investigation of its distinctive physicochemical properties. Optimal conditions for deproteinization of crab shell waste were 3.5% NaOH at 65 ^{0}c for 2 hrs with solids to solvent ratio of 1:10(w/v). Optimal demineralization involved treatment with 1 N HCL at ambient temperature for 30 min with a solid solvent ratio of 1:15 (w/v). Removal of the carotenoid astaxanthin from the shell matrix required extraction with acetone before bleaching with 0.315% sodium hypochlorite solution for 5 min with a solid to solvent ratio of 1: 10(w/v).Chitin deacetylation was carried out at 15 psi/121 ^{0}C using 50% sodium hydroxide solution for 15 min. After this step, samples were filtered off, washed with distilled water to neutral pH and dried in an oven at 60 ^{0}C for 24 hrs. These studies demonstrated that process modification of crab production affected different characterization.

Keywords: Crab (*Scylla serrata*) Shells, Chitosan, Isolation and Characterization

Introduction

Chitosan is partially deacetylated polymer of glucosamine (2 acetamido-2-deoxy b-1, 4-D-glucan). It is essentially a natural water soluble derivative of cellulose with unique properties. Chitosan be used as a flocculent, clarifier, thickener, fiber, film, affinity chromatography column matrix, gas-selective membrane, plant disease resistance promoter, anti-cancer agent, wound healing promoting agent and antimicrobial agent. It is used as processing aid and is being trialed for application in fruit preservation, wound dressing, cosmetics, artificial organs and pharmaceuticals (Brine *et al*., 1991).

Chitosan is usually prepared from chitin and chitin has been found in wide range of natural sources (crustaceans, fungi, insects, annelids, mollusks, coelenterate etc.) (Tharanathan and Kittur, 2003). However chitosan is only manufactured from crustaceans (crab, krill, and crayfish) primarily because a large amount of the crustacean's exoskeleton is available as product of food processing. Crab shell is made up of three basic components. These are chitin, protein and a calcium salt of which chitin is most important for scientific studies. Chitin is a fairly completely acetylated polysaccharide in nature, being

only second after cellulose (Adole and Omogbai, 2012).

Shellfish processing waste (i.e. shrimp, crab, lobster, prawn, squid and crawfish) contain 14-35% chitin on a dry weight basis and theses source materials for chitin are becoming a growing waste disposal problem for the fish and shellfish processing industries. Chitosan is a non-toxic, biodegradable and biocompatible polymer.

Over the last several years, chitinous polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification, in chemical plants for waste water treatment and in food industries for food formulations as binding, gelling, thickening and stabilizing agent (Knorr, 1984). The main aim of this study was to extraction of chitosan from crustacean shell waste by using acid and alkaline treatments (demineralization and deproteinization) followed by decolorization by changing process protocol and study their properties.

Materials and methods

Sample collection and extraction of Chitosan

Crab (*Scylla serrata*) shell waste (carapace and other material) were collected from a local sea food processor fisherman and local fish market, Ratnagiri. Upon receipt the shell waste was stored at – 20 ^{0}C until utilized. Crab (*Scylla serrata*) shell waste was dried in hot air oven 60 ^{0}C for 24 hrs. Dried shell waste were packed in polyethylene bag and stored in dry place. Dried shells were pulverized manually.

The abbreviation (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA,) denotes the sequential processes used to prepare crab chitosans:

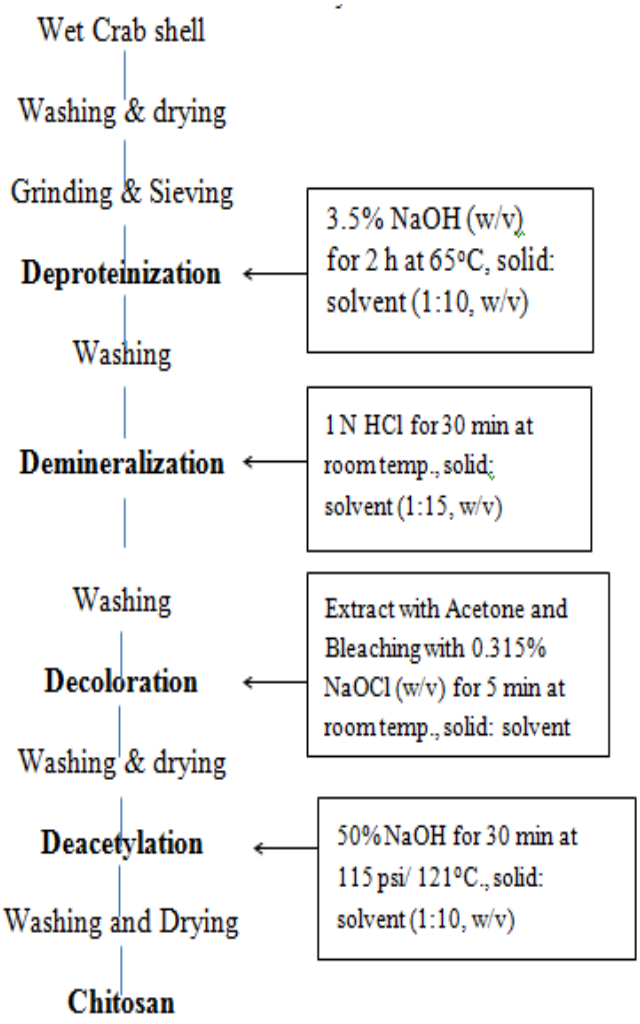
DCMPA = decolorized + demineralized + deproteinized + deacetylated;

DMCPA = demineralized + decolorized + deproteinized + deacetylated;

DMPCA = demineralized + deproteinized + decolorized + deacetylated;

DMPAC = demineralized + deproteinized +deacetylated + decolorized;

DPMCA = deproteinized + demineralized + decolorized + deacetylated.



Production of chitosan involved basic steps of demineralization, deproteinization, decoloration and deacetylation. production order, samples were deproteinized by treating with 3.5% (w/w) NaOH solution for 2 hrs at 65^{0}C with constant stirring at a solid to solvent ratio of 1:10 (w/v) demineralized at room temperature with 1N HCL for 30 min at ambient temperature with a solid to solvent ratio of 1:15 (w/v) for 15 min and

decolorized with acetone for 10 min and dried for 2 hrs under hood, followed by bleaching with 0.32 % (v/v) solution of sodium hypochloride (containing 5.25% available chlorine. After each step, the solid was filtered off, washed with distilled water to neutral pH. Chitin deacetylation was carried out at 15 psi/121 ^{0}C using 50% sodium hydroxide solution for 15 min. After this step, samples were filtered off, washed with distilled water to neutral pH and dried in an oven at 60 ^{0}C for 24 hrs.

Determination of moisture, ash and nitrogen content

Moisture content of the samples was determined according to the standard method (AOAC, 1999) with minor modification. Moisture of samples was determined by drying the samples at 60 ^{0}C for 24 hrs or until the weights were constant. It was then calculated by percentage of weight loss comparing to the initial weight of the samples. Ash and nitrogen contents of chitosan were measured according to a previously described procedure (AOAC, 1999).

Determination of Degree of deacetylation

The degree of deacetylation of chitin and chitosan were measured according to Pradhan and Bedakar, (2002). For actual determination, 1 g dried chitosan sample was taken and refluxed with 20 ml of 12 N Sulphuric Acid for twenty minutes. The clear solution obtained was cooled and 50 ml distilled water was added to it. The resulting acetic acid was distilled out and titrated directly with 0.1 N Sodium Hydroxide until end point was pink using phenolphthalein as an indicator. Taking into consideration the molecular weight of chitin $(C_8H_{13}NO_5)n$ for 100% acetylation and that of chitosan $(C_6H_{11}NO_4)n$ for 100% deacetylation did the calculations for degree of deacetylation.

Statistical analysis

The data were analysed using appropriate statistical method (Snedecor and Cochran, 1967; Zar, 2005).

Results and discussion

Chemical composition of crab shell

Proximate compositions of crab shell waste were analyzed immediately after collection. Proximate composition (wet weight basis) of crab shell waste was observed to moisture - 34.41%, ash-11.93%, protein-5.3%, fat-0.44%. (Table 1 and Fig. 2) Thankappan and Madhavan, (1985) reported proximate of shell in shrimp on dry basis expect moisture (%). They observed that moisture, ash, protein, and fat were in order of 82.25%, 27.92%, 28.38%, and 7. 39% in *P. Stylifera*, 78.82%, 20.00%, 37.00% and 2.56% in *P. indicus*, 74.86%, 18.91%, 39.62%,and 2.95. in *M. dobsoni* and 78.15%, 23.15%, 34.04%, 3.36% inn *M. Monoceros* respectively.

Characterization of crab chitosan

Ash content: Ash content of crab chitosans compared with that of the commercial shrimp chitosan. The crab chitosans contained less than 3% ash with a range of 1.82 % to 3.02 % (Table 2). Commercial shrimp chitosan products contained less than 3 % ash. Ash measurement is an indicator of the effectiveness of the demineralization (DM) step for removal of calcium carbonate. Elimination of the demineralization resulted in products having 31 - 36% ash (Bough *et al*., 1978). The ash content in chitosan is an important parameter.A high qualitygrade of chitosan should have less than 1% of ash content (No *et al*., 1995).

Nitrogen content: No and Meyers (1995) have shown that the nitrogen content of chitosan from various sources ranged from 7.06 to 7.97%. In this study, the nitrogen content of the chitosan products was in the 0.9% - 1.91% range (Table 2, fig.3).

Moisture content: The results of this work demonstrated that there was significant difference in the % moisture (2.37 and 5.4 %) between the five chitosan prepared from crab chitosan (Table 3, fig 3). On a dry basis compared with 2.42% for shrimp chitosan. Since chitosan is a hygroscopic polymer (Khan *et al*., 2002).

Degree of deacetylation (DD): The degree of deacetylation of crab chitosan samples ranged from 23.63% to 52.17% (Table 3, fig.4). Sample DPMCA (52.17%) had the highest DD. According to No and Meyers, (1995) DD of chitosan ranges from 56% to 99% with an average of 80%.

Solubility: In the present study, crab chitosan samples and the commercial chitosan, demonstrated an excellent solubility ranging from 81.78 to 88.78 % (Table 3, Fig. 4) with significant difference, while the DCMPA showed lower solubility (81.78%).

Brine and Austin, (1981) noted that lower solubility values suggest incomplete removal of protein. Since the chemical basis of this method is based on the reaction with the amino group, the presence of protein contaminants remaining in the sample during the analysis process could adversely interfere with the results.

Table 1: Chemical composition of Fresh crab shell.

Source of raw material	Moisture	Protein	Fat	Ash
Crab shell	34.41±2.23	5.3±0.1	0.44±0.11	11.93±0.63

Mean ± standard deviation of triplicate determinations

Table 2: Percentage of ash, and nitrogen of crab chitosan samples.

Chitosan samples	Ash %	N %
DCMPA	2.67 ± 0.49	1.23 ±0.02
DMCPA	2.45 ±0.45	1.24 ± 0.02
DMPCA	1.87 ± 0.26	1.3 ± 0.03
DMPAC	2.55 ±0.17	0.9 ± 0.03
DPMCA	3.02 ± 0.22	1.91 ± 0.02

Mean ± standard deviation of triplicate determinations

Table 3: Percentage of Moisture (%,) Solubility(%) and Degree of deacetylation (%) of crab chitosan samples.

Chitosan samples	Moisture%	Solubility (%)	Degree of deacetylation (%)
DCMPA	4.6±0.21	81.78±0.50	23.6375±0.12
DMCPA	5.42±0.33	87.13±0.42	40.1775±0.05
DMPCA	5.37±0.21	87.21±1.25	23.645±0.25
DMPAC	2.37±0.33	86.56±2.16	40.7325±0.54
DPMCA(CONTROL)	3.27±0.17	86.43±1.40	52.175±0.17

Mean ± standard deviation of triplicate determinations

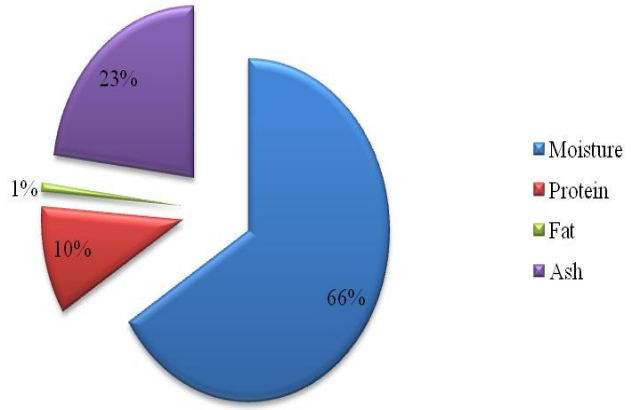


Fig. 2: Chemical composition of crab shell.

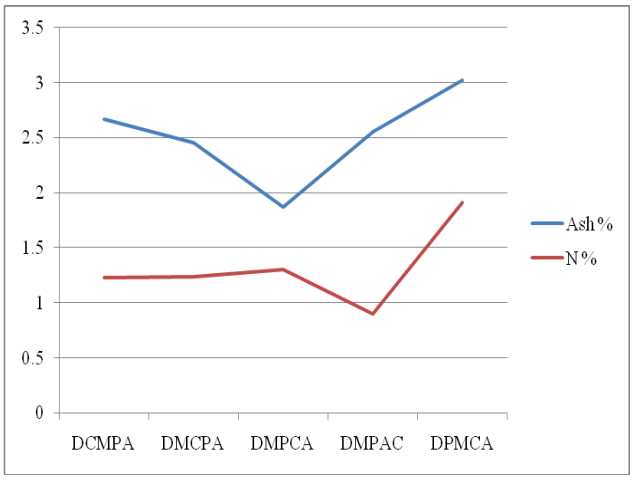


Fig. 3: Percentage of ash and nitrogen of crab chitosan samples.

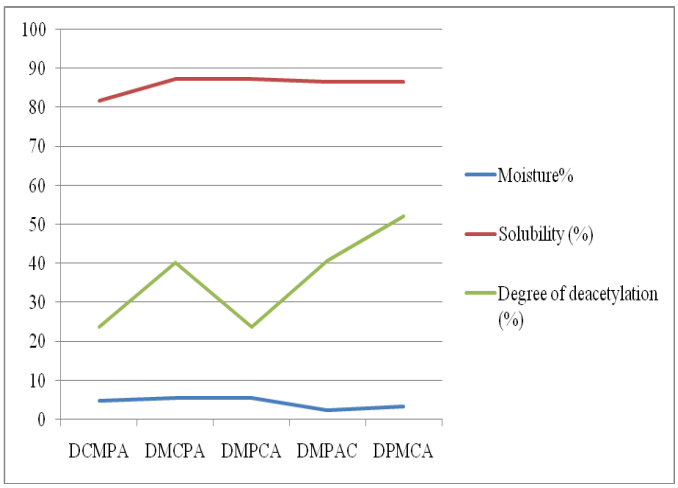


Fig. 4: Percentage of Moisture (%), Solubility (%) and Degree of deacetylation (%) of crab chitosan samples.

Conclusion

On the basis of present study it was concluded that successfully extracted Chitosan from crab shell waste. Result showed that extracted Chitosan is found to be same characteristics as commercial available Chitosan available Fish processing industries produce enormous crustacean shell waste were discarded, is a good waste management practice leading to additional economic benefit by producing chitin and chitosan, for upliftment of socioeconomic status of coastal people. However, chitin and chitosan is a biodegradable product therefore, it helps to maintain the environmental sustainability. Further, there is a tremendous scope of enhancing the functional properties of fish Chitosan extracted particularly from Crustacean waste. Results from this study certainly help to produce the Crab Chitosan suitable for wide range of application especially in the Medicine and industry. These promising findings may contribute to the on-going efforts for using crab chitosan as an alternative to commercial Chitosan available in market.

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