

STUDIES ON EXTRACTION METHODS OF CHITIN FROM CRAB SHELL AND INVESTIGATION OF ITS MECHANICAL PROPERTIES

Kishore Kumar Gadgye

Head, Department of Mechanical Engineering,
Government Polytechnic College, Sanawad, MP, India

Dr. Amit Bahekar

Head and Associate Professor,
Department of Mechanical Engineering,
Oriental University, Indore, MP, India

ABSTRACT

This paper describes the most common methods for recovery of chitin from crab shell. Deproteinization, demineralization and deacetylation are the main processes for the extraction of chitin and chitosan. The mechanical properties were investigated to recognize their mechanical applications. Chitin is the most widespread biopolymer in nature, after cellulose. It has great economic value because of their biological activities and their industrial and biomedical applications. Chitin can be extracted from three sources, namely crustaceans, insects and microorganisms. However, the main commercial sources are shells of shrimps, crabs, lobsters and krill that are supplied in large quantities by the shellfish processing industries. Extraction of chitin involves two steps, demineralization and deproteinisation, which can be processed by two methods, chemical or biological. Acids and bases are required for chemical method, while the biological method involves microorganisms. The mechanical properties of isolated crab chitin are highly susceptible to the effects of hydration. Philippine blue swimming crab were used for the extraction of chitin. The extracted chitin was used to form polymer films at different conditions. Polymer films were also formed from commercially acquired chitin. It was observed that the films prepared at different conditions have greater ultimate tensile strengths as compared to the commercially-available films. The Chitin discussed in the present study is analyzed mechanically. Thus ensuring the extracted Chitin and Chitosan could be considered for further applications. This study therefore, intends to extract and investigate the mechanical performance of chitin from crab shell.

Key words: Biopolymer, Chitin, Chitosan, Chitin Extraction, Crab Shell.

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1. INTRODUCTION

In 1799 A. Hachett, a English scientist discovered a material particularly resistant to usual chemicals. After that Henri Braconnot, a French professor of natural history, discovered chitin in 1811. Then in 1823, Odier found the same material in insects and plants and named it chitine. Since all chitin-based materials are derivatives of chitin, in this paper the word chitin is used generally to describe both chitin and its derivatives unless mentioned otherwise. Chitin is a substance that makes up the exoskeleton of insects and crustaceans, which can also be obtained from other sources like fungi, mushrooms, worms, diatoms, etc. [1-5]. Like cellulose it functions as a structural polysaccharide. Chitin is the second most abundant natural polymer in nature after cellulose [6]. Chitin and its derivatives chitosan have several applications, these include, biomedical, food, emulsifying agent, wastewater treatment, biocatalysts, textile and paper industry and agriculture [7,8]. The isolation of chitin from different sources is depends on source and also the percentage of chitin present in source where it is found varies according to the origin of the source [2-3]. The extraction and characterization of the chitin and its derivatives from different origins have been reported. The extraction and characterization of chitin and chitosan from two species of crustacean of Tunisian origin has been reported by Limam et al. [9]. Also, Al-Sagheer et al. [10] produced chitin from Arabian Gulf crustaceans' sources to determine the protein content in chitin. Abdou et al. in [3], reported The production of chitin and its derivative from crustacean of Egyptian origin was reported by. Abdou et al. [3], Yildiz et al. [11] reported the extraction and characterization of chitin and chitosan from Mediterranean crab. The extraction and characterization of chitin from crustacean of Nigerian origin was also reported. It was reported that sources of chitins are highly available in Nigeria and are abundant in the rural areas of Nigeria [12,13]. These waste materials litter the banks of rivers constituting environmental pollution because they are underutilized. Chitin and Chitosan proved to be a versatile and promising biopolymer. The use of these biopolymers is in various fields. They have an important role as natural alternatives having some biological properties and some specific applications like drug delivery, tissue engineering, functional food, food preservative, biocatalyst immobilization, wastewater treatment, molecular imprinting and metal nanocomposites. The molecular mechanism of the biological properties such as biocompatibility, mucoadhesion, permeation enhancing effect, anticholesterolemic, and antimicrobial has been an area of interest for many researchers [5]. Shellfish including Crab, lobster and crayfish continue to predominate due to at least two factors. The first is the growth of aquaculture, and the second is the large increase in consumption of crustaceans. The objective of the study is to utilize the shell waste of the commercially important crab to produce an important biopolymer. The mechanical properties was investigated to represent the quality of chitin for various mechanical applications. Due to the wide application of chitosan (alkaline hydrolysis of chitin); different methods of chitin extraction have been published. Chitin can be extracted by fermentation and enzymatic methods. While the extraction of chitin by fermentation is very expensive, enzymatic extraction does not denature the chitin. Another method that has been widely reported is the chemical method [14], [15], [16], [17], [18] which make use of plenty alkaline.

2. MATERIALS AND METHODS

2.1. Methods of Chitin Extraction

2.1.1. Chemical methods

In the exoskeleton tissue, protein and chitin combine to form a protein-chitin matrix, which is then extensively calcified to yield hard shells. The waste may also contain lipids from the muscle residues and carotenoids, mainly astaxanthin and its esters [19]. A traditional method for the commercial preparation of chitin from crustacean shell (exoskeleton) consists of two basic steps (A) protein separation, *i.e.* deproteinisation by alkali treatment, and (B) calcium carbonate (and calcium phosphate) separation, *i.e.* demineralization by acidic treatment under high temperature, followed by a bleaching step with chemical reagents to obtain a colourless product[20-22]. Deproteinisation is usually performed by alkaline treatment [23]. Demineralisation is generally performed by acid treatment including HCl, HNO₃, H₂SO₄, CH₃COOH, and HCOOH; however, HCl seems to be the preferred reagent [23]. It was shown that the order of the two steps may be reversed for shrimp waste containing large protein concentrations, which stem primarily from the skeletal tissue and to a lesser extent from the remaining muscle tissue [8]. The major concern in chitin production is the quality of the final product, which is a function of the molecular mass (average and polydispersity) and the degree of acetylation. Harsh acid treatments may cause hydrolysis of the polymer, inconsistent physical properties in chitin and are source of pollution [24]. High NaOH concentrations and high deproteinisation temperatures can cause undesirable deacetylation and depolymerisation of chitin [8]. Percot et al.[25] reported that using inorganic acids such as HCl for the demineralisation of chitin results in detrimental effects on the molecular mass and the degree of acetylation that negatively affect the intrinsic properties of the purified chitin. Similarly, according to Crini et al.[26] this method allows almost complete removal of organic salts, but at the same time reactions of deacetylation and depolymerisation may occur. Quality improvement can be obtained by improving the contact of chemicals with the shrimp waste, for instance by using stirred bioreactors. This would allow reactions to proceed with the same efficiency at shorter exposure time and at lower temperature [27]. Comparing different chitins (degree of acetylation, molecular mass and optical activity), variations of the characteristics of the obtained polymer were observed according to the acid used for the demineralisation [26]. In addition, chemical chitin purification is energy consuming and somewhat damaging to the environment owing to the high mineral acid and base amounts involved [28]. These chemical treatments also create a disposal problem for the wastes, since neutralisation and detoxification of the discharged wastewater may be necessary [29]. Another disadvantage of chemical chitin purification is that the valuable protein components can no longer be used as animal feed [30].

2.1.2. Biological methods

An alternative way to solve chemical extraction problems is to use biological methods. The use of proteases for deproteinisation of crustacean shells would avoid alkali treatment. Besides the application of exoenzymes, proteolytic bacteria were used for deproteinisation of demineralised shells [24]. This approach allows obtaining a liquid fraction rich in proteins, minerals and astaxanthin and a solid chitin fraction. The liquid fraction can be used either as a protein-mineral supplement for human consumption or as an animal feed [31]. Deproteinisation processes have been reported for chitin production mainly from shrimp waste using mechanical [24], enzymatic [32, 33] and microbial processes involving species like *Lactobacillus* [31], *Pseudomonas aeruginosa* K-187 [34] and *Bacillus subtilis* [35]. Biological demineralisation has also been reported for chitin production from crustacean shells; enzymatically, using for instance alcalase, or by microbial process involving species like *L. pentosus* 4023[21] or by a natural probiotic (milk curd) [36]. In these biological processes, demineralization and deproteinisation occur mainly simultaneously but incompletely [24]. Lactic acid is formed from the breakdown

of glucose, creating the low pH, which improves the ensilation that suppresses the growth of spoilage microorganisms. Lactic acid reacts with the calcium carbonate component in the chitin fraction, leading to the formation of calciumlactate, which precipitates and can be removed by washing. The resulting organic salts from the demineralization process could be used as de- and anti-icing agents and/or preservatives [37]. Deproteinisation of the biowaste and simultaneous liquefaction of the shrimp proteins occurs mainly by proteolytic enzymes produced by the added *Lactobacillus*, by gut bacteria present in the intestinal system of the shrimp, or by proteases present in the biowaste. It results in a fairly clean liquid fraction with a high content of soluble peptides and free amino acids [38]. Deproteinisation and demineralization of crab (*Chionoecetes opilio*) shell wastes was carried out by Jo et al. [39] using *Serratia marcescens* FS-3 isolated from environmental samples (seaside soil in the southwestern area of Korea) which exhibited strong protease activity. The demineralization and deproteinisation of natural crab shell wastes with 10 % *Serratia marcescens* FS-3 as inoculums was 84 and 47 % after 7 days of fermentation. When the shell waste was treated with 1% Delvolase® (Gist-Brocades, DSM, Heerlen, The Netherlands) as a reference, deproteinisation rate was 90 %. With a combination of 10 % *Serratia marcescens* FS-3 culture supernatant and 1 % Delvolase®, deproteinisation rate of the shell waste was 85 %, while the rate was 81 % in 10 % *Serratia marcescens* FS-3 culture supernatant only . The effect of crab shell size on biodemineralisation by means of *L. paracasei* ssp. *tolerans* KCTC-3074 was also investigated [40]. Demineralisation was performed using samples with four different particle sizes (0.84–3.35, 3.35– 10, 10–20 and 20–35 mm) with 10 % inoculum, 5 % shell and 10 % glucose at 30 °C and 180 rpm for 7 days. Shell size had a minor effect on demineralisation efficiency [40].

Table 1. Chemical Vs Biological methods for chitin Extraction

	Chemical method	Biological method
Chitin Recovery		
Demineralisation	Mineral solubilisation by acidic treatment including HCl, HNO ₃ , H ₂ SO ₄ , CH ₃ COOH and HCOOH.	Carried out by lactic acid produced by bacteria through the conversion of an added carbon source.
Deproteinisation	Protein solubilisation by alkaline treatment.	Carried out by proteases secreted into the fermentation medium. In addition, deproteinisation can be achieved by adding exo-proteases and/or proteolytic bacteria
	Effluent treatment after acid and alkaline extraction of chitin may cause an increase in the cost of chitin.	Extraction cost of chitin by biological method can be optimized by reducing the cost of the carbon source. Solubilised proteins and minerals may be used as human and animal nutrients.

Chitin Quality		
The major concern in chitin production is the quality of the final product, which is a function of the molecular mass (average and polydispersity) and the degree of acetylation.	<p>A wide range of quality properties of the final product. Using inorganic acids such as HCl for chitin demineralisation results in detrimental effects on the molecular mass and the degree of acetylation that negatively affect the intrinsic properties of the purified chitin [41].</p> <p>This method allows almost complete removal of organic salts, but at the same time the reactions of deacetylation and depolymerisation may occur [42].</p> <p>The comparison of different chitins (degree of acetylation, molecular mass, optical activity) obtained with four different acids showed that the polymer characteristics varied according to the extraction method used [42].</p>	Homogeneous and high quality of the final product.

2.2. MECHANICAL CHARACTERISATION

Hempburn et al [43], investigated mechanical properties of the solid cuticle and the isolated chitin of the crab, *Scylla Serrata*. All test specimens were taken from live crabs caught on the Southern Mozambique coast. Wet and dry specimens of pure crab chitin were tested. These samples were obtained from whole crab legs by treatment in 20% KOH at 293 K for 8 hr. rinsing until neutral pH. followed by 24 hr steeping-in a 5%-HCl solution.

Fernando et al [44], extracted chitin from Philippine blue swimming crab. For the polymer film formation, 5% (w/v) lithium chloride/N,N-dimethylacetamide (LiCl/DMAc) solvent for chitin dissolution was prepared. The covered mixture was stirred at room temperature until all LiCl dissolved. Then, 0.5% (w/v) of extracted chitin was added to the solution, and the solution was agitated until the mixture became homogenous. The solution was then poured into a glass mould, covered with pin-holed aluminum foil, and allowed to set for 24 and 96 hours. The formed gels were soaked in isopropanol and methanol. These were then cold pressed between filter papers, glass plates, and binder clips, then oven-dried overnight. The dried films were again soaked in isopropanol and methanol, then cold-pressed for another 48 hours. The final films were characterized and compared with commercially available plastic film using UTM testing for tensile strength tests. SEM imaging was also conducted to evaluate the weak polymer film's surface morphology.

Ofem et al [45], investigated mechanical properties of Dungeness crab based chitin. Chitin films were made according to a modified published [46,47]. Purified chitin was roughly crushed in a domestic blender, and then vacuum sieved using a Buchner filter funnel having an approximate size of 1.4 mm. The sieved chitin was dispersed in water to make 0.5 wt % content. The pH value was adjusted to 3 by adding few drops of acetic acid. The solution was stirred magnetically over night at room temperature. The suspension was vacuum filtered using Whitman filter paper. Mechanical properties of samples were tested, in tension using a Universal Testing Machine - UTM (Instron 5567). Ten samples were tested at a strain rate of 3mm/min for each gauge length. All specimens were conditioned at a temperature of 23±2 °C and 50±5 % relative humidity for 48 hours before testing. The chitin nanofibre was pressed for up to 60 minutes at a temperature maintained at 80-90°C. The film was dried in an oven at a temperature of 50 °C for 48 hours.

3. RESULTS AND DISCUSSION

The recovery of chitin by chemical method using concentrated acids and bases in order to deproteinise and demineralise shellfish waste (the most industrially exploited) at high temperature can deteriorate the physicochemical properties of this biopolymer and consequently its biological properties which results in products of varying quality that are neither homogeneous nor reproducible.

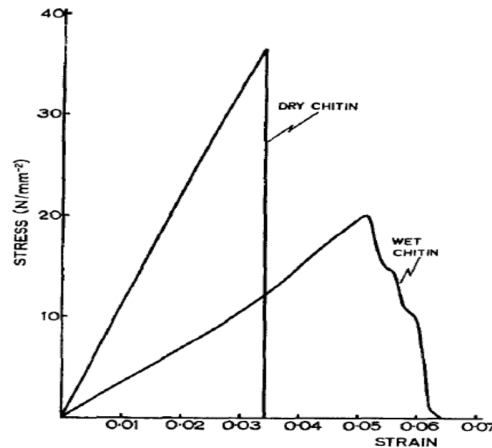


Figure 1 Stress-Strain curves for isolated crab chitin failed in tension [43].

Fig. 1 shows the general tensile stress-strain behavior for wet and dry crab chitin, Hempburn et al [43]. It can be seen that both wet and dry crab chitin are not following the Hooke's Law and that neither exhibits a clear proportional limit. Whereas dry crab chitin shows a sharply defined failure point immediately beyond the ultimate stress, wet chitin does not have a clearly defined failure point. The isolated crab chitin shows markedly different mechanical properties depending upon the state of hydration. While the breaking stress for wet chitin was 19.96 ± 1.6 MPa that of dry chitin was 36.24 ± 2.2 MPa or about twice the wet strength. The elastic modulus increased from a wet chitin value of 330 to 1095 MPa when dry. The strain at breaking decreased from 6.1 per cent in the wet state to 3.4 per cent on drying. These results are consistent with the qualitative observations that wet crab chitin feels "rubbery" while dry chitin is stiffer and somewhat brittle. Dynamic measurements of the torsional rigidity modulus exhibited a similar dependence on the state of hydration. The rigidity modulus for wet crab chitin was 27.84 ± 4.7 MPa which is considerably lower than that of 183 ± 26 MPa obtained for dry chitin. The increased value of the torsional rigidity modulus, the higher elastic modulus, the strength and the decreased value of the breaking strain for dry chitin are results of the considerable influence water has on the general properties of chitin structures. These results are entirely in keeping with those reported for other arthropod chitins [48,49,50]. This water-chitin interaction also manifests itself in large changes in the damping of elastic oscillations of crab chitin strips. The damping coefficient of dry crab chitin (0.023) is an order of magnitude lower than that of wet crab chitin (0.120) and together with the increased strain at breaking suggests a role for water that is more active than merely filling the gaps left behind on removal of the protein and salt phases. This is reminiscent of the role of water in the mechanical behavior of wood as well [51]. As can be seen from Table 1, wet crab chitin is about as strong as both wet prawn and beetle chitin cut in the same plane as well as regenerated prawn chitin. This more or less uniform value for the strength of wet chitin persists despite the grossly different architectural arrangements seen in the examples cited. Regarding the elastic modulus for wet chitin (Table 1), the effects of dehydration are virtually the same and there is a characteristic ratio of about 3:1 for the increase in the elastic modulus on drying. The loose nature of the chitin lamellae has also been observed in another crab species [52]. It was found that the crab exoskeleton is a natural composite consisting of highly

mineralized chitin–protein fibers arranged in a twisted plywood pattern. Gadgy and Bahekar [53] reported the mechanical properties of various crab shells.

Table 1 Mechanical Properties of chitin from different sources

Source	Ultimate Tensile Strength (MPa)	Elastic Moddulus (MPa)	Elongation at breaking (%)	Torsional Rigidity Modulus (MPa)	Damping Coefficient	Reference
Crab						
Wet	20	330	6.1	28±5	0.12	Hempburn et al.[43]
Dry	36	1095	3.4	183±26	0.023	Hempburn et al.[43]
Prawn						
Wet	13	475	2.8	247±28	0.036	Joffe et al.[57]
Dry	21	1220	1.8	682±75	0.023	Joffe et al.[57]
Beetle						
Wet	26	630	2.0			Hempburn and Ball[49]
Dry	80	2900	0.6			Hempburn and Ball[49]
Regenerated Prawn						
Dry	47	2050	10.0			Joffe and Hempburn[58]

Fig.2 shows the extracted chitin from the Philippine blue swimming crab shells Fernando et al.[44],. It can be seen that the powder exhibits a light red to orange color. The polymer films formed from the extracted chitin is also shown in Fig. 2. These films were subjected to tensile loading and compared to the commercially available plastic film.



Figure 2 Image of the extracted chitin and polymer film from Philippin blue swimming crab.

It can be observed from Fig. 3 that both extracted chitin-based films have higher tensile strengths than the film prepared from commercially acquired plastic film with only 18.90 MPa. It can also be observed that a longer forming time increased the tensile strength of the film to 44.22 MPa.

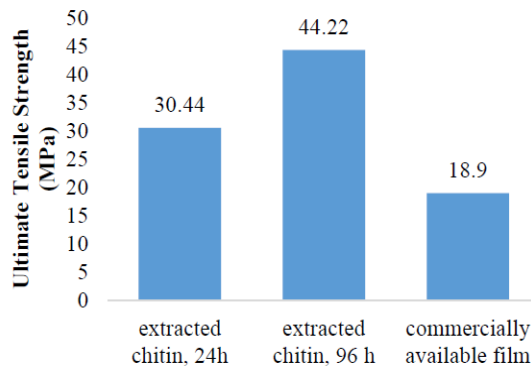


Figure 3 Ultimate tensile strength of extracted and commercially acquired plastic film [44].

Fig.4 show the stress-strain curves for chitin film for different gauge lengths, Ofem et al.[45]. The trend shows that the higher the length of the tested materials the lower the stress at failure, the percentage decrease gradually increases from 6.1 at 10mm gauge length to 15.2 at 50 mm gauge length. This decrease is thought to be due to defects, the larger the specimen size; the more probable it is to have defects. This result is in agreement with Griffith’s theory [54], where a thinner material tends to be close to its theoretical strength. In other words the gradual decrease in tensile strength is an indication of the presence of strength limiting defects, [55] this defects could be voids, minor cuts, non-uniform thickness of films etc. There was an increase in strain as the gauge length decreases. The decrease in strain gradually increases from 14.5% at 10mm gauge length to a maximum of 21.7 % at 30mm and finally drops to 10.7% at 50mm gauge length. Higher ultimate elongation values are associated with increased toughness. It is also observed that the higher the gauge length the smaller the modulus. This may be attributed to strength limiting defects. The mechanical properties obtained here are comparable with various reported properties in the literature. Depending on the method of chitin film preparation, Yusof *et al.*, [56] reported Young’s modulus between 1.2 and 3.7 GPa while the tensile strength ranged between 38.3 and 77.2 MPa and the % strain between 4.7 and 21.3 %. Ifuku *et al.*, [47] however reported a Young’s modulus of 2.5 GPa and a tensile strength of 40 MPa for chitin film.

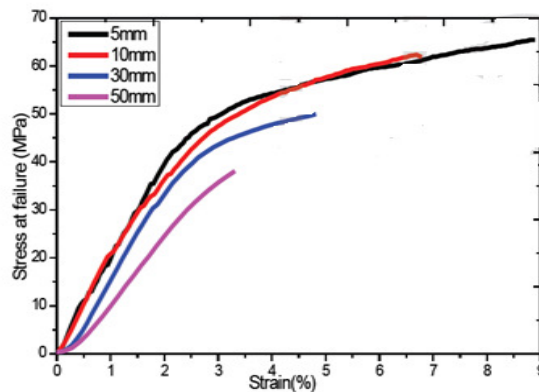


Figure 4 Stress-Strain curve for chitin film sheet at different gauge length [45].

4. CONCLUSION

The importance of chitin and chitosan resides in their biological (biodegradability, biocompatibility and non-toxicity) and physicochemical properties (degree of acetylation and molecular mass). Recently, these properties are widely applied in agriculture, medicine, pharmaceuticals, food processing, environmental protection and biotechnology. The extraction of chitin by chemical method using concentrated acids and bases in order to deproteinise and to demineralise crab shell waste at high temperature can deteriorate the physicochemical properties of this biopolymer and consequently its biological properties, which results in products of varying quality that are neither homogeneous nor reproducible. Nowadays, a new method based on the use of lactic acid bacteria and/or proteolytic bacteria has been used for chitin extraction. This method allows to produce a good quality chitin. Although the biological method seems to be a promising approach for demineralisation and deproteinisation, the use of this method is still limited to laboratory scale because demineralisation and deproteinisation have not yet reached the desired yields if compared to the chemical method. The physicochemical conditions that influence the fermentation are the key factors of this bioprocess. The results of the crabs, *Scylla Serrata* chitin show that the tensile behaviour, elastic modulus and deformation properties of isolated crab chitin are completely consistent with those of other arthropodan chitins. The chitin extracted from the Philippine blue swimming crab was characterized mechanically. After the formation of polymer films from the extracted chitin, it was found that the chitin polymer films have higher tensile strength up to 44.22 MPa as compared to commercially available plastic film's strength 18.90 MPa. It was also found that shorter forming times favor the formation of surface roughness which lowered the tensile strength of the film. The mechanical properties of Dungeness crab based chitin extracted by chemical method could not be observed confirming earlier reported reports. Different gauge lengths give different mechanical properties that are stress and strain at failure and the Young modulus. The variation in mechanical properties was attributed to strength limiting defect some of which are non uniform thickness of film, void and minor cut on the film. While the strain and stress at failure decreases as the gauge length decreases the Young Modulus increases as the gauge length decreases.

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