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Extraction and characterization of chitin and chitosan from local sources

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Abstract

Chitin has been extracted from six different local sources in Egypt. The obtained chitin was converted into the more useful soluble chitosan by steeping into solutions of NaOH of various concentrations and for extended periods of time, then the alkali chitin was heated in an auto clave which dramatically reduced the time of deacetylation. Chitin from squid pens did not require steeping in sodium hydroxide solution and showed much higher reactivity towards deacetylation in the autoclave that even after 15 min of heating a degree of deacetylation of 90% was achieved. The obtained chitin and chitosan were characterized by spectral analysis, X-ray diffraction and thermo gravimetric analysis.

Keywords: Chitin extraction; Local sources; Deacetylation conditions; Characterization

1. Introduction

Chitin, a naturally abundant polymer consists of 2-acetamido 2-deoxy-β-D-glucose through a β(1 → 4) linkage. In spite of the presence of nitrogen, it may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it functions as structural polysaccharides. Its natural production is inexhaustible; arthropods, by themselves, count more than 10^6 species from the 1.2×10^6 of total species compiled for animal kingdom, constitute permanent and large biomass source. Chitin is a white, hard, inelastic, nitrogenous polysaccharide and the major source of surface pollution in coastal areas.

Chitin is usually isolated from the exoskeletons of crustaceans and more particularly from shrimps and crabs where α-chitin is produced (Minke and Blackwell, 1978; Austin et al., 1989). Squid is another important source of chitin in which it exists in the β form which was found to be more amenable for deacetylation. It also shows higher solubility, higher reactivity and higher affinity towards solvents and swelling than α-chitin due to much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains (Pawadee et al., 2003; Gardner and Blakwell, 1975; Hunt and Elsherief, 1990; Chandumpaia et al., 2004).

Many authors (Tolaimate et al., 2000, 2003; Pawadee et al., 2003; Gardner and Blakwell, 1975; Hunt and Elsherief, 1990; Chandumpaia et al., 2004; Acosta et al., 1993; Rege and Block, 1999; Galed et al., 2005; Paulino et al., 2006) have tackled the problem of extracting chitin from its natural sources followed by its deacetylation to obtain the much more useful material chitosan.

Potential and usual applications of chitin, chitosan and their derivatives are estimated to be more than 200 (Sandford, 1989; Ravi Kumar, 2000). This wide range of applications include cosmetics, agriculture, food, biomedical, and textile, as chelating agents and refinement industrial effluents (Rathke and Hodson, 1994; Chassarya et al., 2005).

The production of chitosan from crustacean shells obtained as a food waste is economically feasible, especially if it includes the recovery of carotenoids. The shells contain

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considerable amount of astaxanthin, a carotenoids that has so far not been synthesized, and which is marked as a fish food additive in aquaculture.

Chitosan itself was directly extracted from fungi by alkaline and acid treatment (Rane and Hoover, 1993; Cai et al., 2006; Suntornsuk et al., 2002; Chatterjee et al., 2005). Some authors (Wang et al., 2006; Gagne and Simpson, 1993; Oh et al., 2000; Yang et al., 2000) have developed methods to use microorganisms or proteolytic enzymes for the deproteinization of the crustacean chitin wastes in this way a more economic production of chitin and chitosan can be achieved.

The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution. Isolation of chitin itself from different sources is affected by the source. Generally the raw material is crushed, washed with water or detergent and cut into small pieces. The mineral content of the exoskeleton of the different crustaceous is not the same and consequently different treatments may be used. The present work is the first systematic trial to investigate the extraction of chitin and chitosan from different indigenous sources in Egypt.

2. Experimental part

2.1. Isolation of chitin

Chitin was isolated from six sources, two kinds of shrimp (brown and pink) shells, two kinds of squid pens, crabs shells and shells of fresh water lobster (crayfish), *Procambarus clarkii* which is a species of freshwater crayfish, native to the south-eastern United States, but found also on other continents, where it is often an invasive pest. It is known variously as the red swamp crawfish, red swamp crayfish, Louisiana crawfish or Louisiana crayfish. This crayfish is an intriguing species besides being a source for chitin it is also an environmental problem in Egypt, the crayfish has become an abundant resident in the River Nile because of its high reproduction rate and its strong adaptability. This crayfish is a voracious carnivore, preying upon various crustaceans, mollusks and small fish, as well as their eggs and fries causing thus a serious obstacle for aquaculture industry. Other evidence shows that they feed well upon some benthic vector snails, and so, it might be used as a potential biological Schistosome control agent if it is intentionally introduced into the irrigation and drainage canals which are largely infested with these snails.

The raw materials were obtained in solid form from the different sources, washed with water, desiccated at room temperature and cut into small pieces. Demineralization was carried out at room temperature using 1 M hydrochloric acid bathes. The number of bathes and their duration were dependent upon the source; it was observed that the emission of CO_2 gas was more or less an important indicator according to the studied species. It is for example strong in case of crabs and shrimp and crayfish and weak in case of one kind of squid. De-proteinization was performed using alkaline treatments with 1 M sodium hydroxide solutions at 105–110 °C. This treatment was repeated several times. The number of bathes depends on clarity of the solution; the absence of protein was indicated by the absence of color of the medium at the end of the last treatment. Washing with distilled water was then carried out up to neutrality after which the samples were dried.

At this stage, chitin isolated from squid pens is perfectly white unlike those isolated from other sources which were highly pink. Pigment traces responsible for color are removed using a mild oxidizing treatment ($KMnO_4$ + oxalic acid + H_2SO_4). Refluxing in ethanol for 6 h was also used to eliminate traces of protein and coloring materials.

Chitin content was determined from weight difference of the raw material and that of the chitin obtained after acid and alkaline treatments.

2.2. Deacetylation of chitin

Preliminary experiments were carried out by refluxing chitin in strong NaOH solution at normal atmosphere. The experiments took more than 20 h producing low deacetylation content and the reaction was accompanied by drastic degradation of the final chitosan. To avoid long heating times, the refluxing in alkaline solution was tried in an autoclave under two atmospheres pressure. The heating lasted for several hours (10–15 h) and still the resulting chitosan was partially soluble in acetic acid indicating low deacetylation extent.

Kurita (2001) has indicated that deacetylation of chitin can be highly facilitated by steeping in strong sodium hydroxide solution at room temperature before heating. This approach was then adapted and the effect of steeping time on the feasibility of deacetylation was investigated.

3. Determination of the deacetylation percent

3.1. Potentiometric titration

Chitosan (0.5 g) was dissolved in 25 ml of 0.1 M standard HCl aqueous solution. The solution was then toped up to 100 ml with distilled water and calculated amount of KCl was added to adjust the ionic strength to 0.1. The titrant was a solution of 0.05 M NaOH. pH meter was used for pH measurements under continuous stirring. The titrant was added until the pH value reached 2.00, the standard NaOH was then added stepwise and the pH values of solution were recorded and a curve with two inflection points was obtained.

The difference of NaOH solution volumes between these points corresponds to the acid consumed for salification of the amine groups of chitosan and allows the determination of DDA% of the chitosan.

The DA was calculated from the relation (Broussignac, 1968):