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Research Article
Characterization of Chitosan Extracted from Different Crustacean Shell Wastes

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Abstract

Background: Chitosan is basic polysaccharide and partially deacetylated polymer of glucosamine obtained from chitin by alkaline deacetylation. Fish by-products represent a serious threat to environment and disposed of it using simple and inexpensive method are necessary also, the production of natural compounds for food industry used is required. **Methodology:** Chitosan prepared from shrimp and crayfish have a good physiochemical and functional properties when compared with the commercial chitosan. The study was undertaken to extract chitosan from some crustacean shells (shrimp and crayfish wastes) and characterize them using spectral analysis, Fourier transforms infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and energy dispersive analysis of x-ray spectroscopy (EDAX) analysis. Moreover, antioxidant activity, moisture, protein, ash, yields, solubility, degree of deacetylation, water and fat binding capacity were also determined and compared the extracted chitosan with commercial type. **Results:** The study results showed that the extracted chitosan is soluble in 1% acetic acid solution also commercial, shrimp and crayfish chitosan had moisture content (4.1, 0.8 and 1.7), protein (8.5,7.32 and 8.16), ash (1.2, 0.5 and 0.6) and degree of deacetylation (84, 92 and 87), respectively. The FTIR spectra of three chitosan types (commercial, shrimp and crayfish) were observed to have absorption band in the region of (3422.06, 3444.24 and 3446.17 cm^{-1}), respectively which corresponded to the vibrating and of aliphatic O-H and NH stretching vibration of free amino groups. Also, the stretching vibration for glucosamine ring -C-O-C- was indicated by the absorption bands at (1030.77, 1027.87 and 1031.73 cm^{-1}) for the three chitosan types, respectively. Chitosan prepared from shrimp and crayfish have a good physiochemical and functional properties when compared with the commercial chitosan. Also, the antioxidant activity of chitosan gives a great indication for its possible use as natural additives in food industries. The study results are very important to researchers in relevant fields because they can disposed fish wastes in safe method and they can utilized extraction process for producing natural products which can be improve physicochemical, sensorial and shelf life of food products. **Conclusion:** Chitosan prepared from shrimp and crayfish have a good physiochemical and functional properties when compared with the commercial chitosan. Also, the antioxidant activity of chitosan gives a great indication for its possible use as natural additives in food industries.

Key words: Chitosan, shrimp shells, FITR, SEM

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seafood has been considered a healthy food for humans and its by-products could be utilized for production of value-added products like enzymes, xanthophylls, chitin/chitosan and glucosamine[1,2]. Chitosan is basic polysaccharide and partially deacetylated polymer of glucosamine obtained from chitin by alkaline deacetylation[3]. Chitosan consists of β-(1-4-2- acetamido-2-deoxy-D-glucose) units and after cellulose it is the second abundant biopolymer on earth. Chitosan has been used in several agricultural, food protections in biomedical and pharmaceuticals applications as drug delivery systems or in drugs formulations[4].

In Egypt, crayfish and shrimp are the most important crustacean seafood's. Crayfish carapace is by-product from seafood processing with hundreds of thousands of tons it contains 40% calcium carbonate, 30% protein and 30% chitin[5]. Shrimp shell wastes which constitute approximately 40-50% of the total weight of shrimp become an environmental risk in Egypt due to the increase in the total production of shrimp. So, the utilization of crustacean shell wastes could be used in industry, food processing, biomedicine, biotechnology, cosmetics and agriculture and also environmental problems can be solved[6-8]. Fish wastes are one of the most important environmental problems that must attach a growing interest especially in the present time of its harmful effects on the environment, human health and safety and even more importantly, this is how to get rid of them. However, these residues are a source of national income sources by maximizing the benefit from them which requires the development of an integrated management system. Therefore, great attention has been paid to the utilization of crustacean shell wastes so, the objective of this study is to extract and characterize chitosan from shrimp shell wastes and crayfish carapace and compare them with commercial using Scanning Electron Microscopy (SEM), energy dispersive analysis of X-ray spectroscopy (EDAX) analysis and Fourier transform infrared (FTIR) in order to explore possibilities for their utilization.

MATERIALS AND METHODS

Shrimp and crayfish inedible parts including head, body shells and tails were obtained from the local market and they were washed and extracted according to the method explained by Van Toan[9].

Chitosan preparation: Firstly shrimp and crayfish wastes were suspended in 4% HCl at room temperature in the ratio of 1:14 (w/v) for 36 h. Deproteinization of shells was done by treating the demineralized shells with 5% NaOH at 90°C for 24 h with a solvent to solid ratio of 12:1 (v/w). After the incubation time, the shells were washed to neutrality in running tap water and sun dried. The product obtained was chitin which was deacetylatiedby employing 70% NaOH solution with a solid to solvent ratio of 1:14 (w/v) and incubated at room temperature for 72 h, the residues were washed with running tap water to neutrality, rinsed with deionized water then filtered, sun dried and finely grinded to obtained chitosan[10]. Figure 1 shows the different chitosan types (a) commercial, (b) shrimp and (c) crayfish chitosan.

Analysis: Moisture, protein and ash contents of obtained chitosan were determined according to the AOAC[11]. Yield was determined according to Mohanasrinivasan *et al.*[12] water and fat binding capacity were measured according to Wang and Kinsella[13].

FTIR analysis: The samples of prepared chitosan were characterized in KBr pellets by using an infrared spectrophotometer model (4100 Jasco, Japan) in the range of 400-4,000 cm^{-1}.

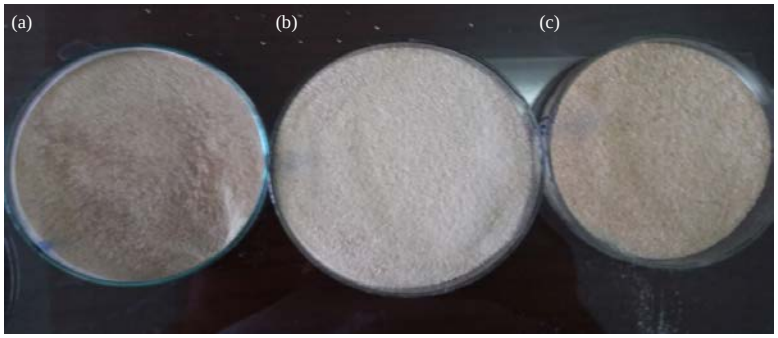

Fig. 1(a-c): Different chitosan types (a) Commercial, (b) Shrimp and (c) Crayfish chitosan

Degree of Deacetylation (DD): The FTIR instrument was used for the determination of DD of the three types of chitosan. The percentage of the acetylated amine group was determined by the following formula:

$$DD\ (\%) = 100\text{-}\left[\frac{A_{1629.85}\text{-}A_{3450.65}\ cm^{-1}}{1.33}\times 100\right]^{14}$$

Scanning Electron Microscopy (SEM): The SEM having a magnification range of 5,000 and accelerating voltage 20 kV were used for characterization of prepared chitosan.

Energy dispersive analysis of X-ray spectroscopy (EDAX) analysis: THE EDAX is used for chitosan characterization by (JED-2300 analysis station,joel).

Antioxidant activity of chitosan: One of the most methods for detecting the antioxidant activity of chitosan is DPPH (2, 2-diphenyl-1-picrylhyorazyl) radical scavenging activity. The antioxidant activity of three different chitosan types on (DPPH) radical were examined according to Tarafdar and Biswas[15] and calculated by the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of blank-Absorbance of sample}}{\text{Absorbance of blank}}\times 100$$

RESULTS AND DISCUSSION

Functional and physicochemical properties of chitosan commercial and extracted are shown in Table 1.

Yield: Yield was obtained by calculated the dry weight of chitosan extracted from 400-600 g of dried shrimp and crawfish shell. Chitosan yield were 18.5% for shrimp and 25.7% for crawfish.

Moisture content: Chitosan types had moisture content 4.1, 0.8 and 1.7% for commercial, shrimp and crawfish chitosan, respectively. According to Khan *et al.*[16] chitosan is hygroscopic in nature so, commercial chitosan during storage may be affected by moisture absorption. Commercial chitosan contain less than 10% moisture content[17].

Protein content: The protein content of commercial, shrimp and crawfish chitosan were 8.50, 7.32 and 8.16%, respectively. These results are in agreement with those obtained by No and Meyers[18].

Table 1: Functional and physicochemical properties of the three chitosan types (commercial, shrimp and crayfish)

Properties	Commercial chitosan	Shrimp chitosan	Crayfish chitosan
Yield	-	18.70	25.70
Moisture	4.10	0.80	1.70
Nitrogen	8.50	7.32	8.16
Ash	1.20	0.50	0.60
WBC	857	1.32	1.25
FBC	539	743.00	698.00
DD	84	92.00	87.00

WBC: Water binding capacity, FBC: Fat binding capacity and DD: Degree of deaylation

Ash: The ash content of the three chitosan types were 1.2, 0.5 and 0.6% for commercial, shrimp and crawfish chitosan types, respectively. No and Meyers[18] found that crab chitosan has less than 1% of ash content. As reported by No *et al.*[19] high quality grade of chitosan should have ash content less than 1%.

Water and fat binding capacity: Water Binding Capacity (WBC) of commercial, shrimp and crayfish chitosan were 857, 1.32 and 1.25%, respectively these results are in agreement with Mohanasrinivasan *et al.*[12] but higher than reported by Cho *et al.*[20] who reported that WBC ranged from 458-805% for five commercial chitosan from shrimp and crab shell.

Fat Binding Capacity (FBC) were 539, 743 and 698% for commercial, shrimp and crayfish chitosan which in agreement with Rout[21] who showed that FBC of crawfish chitosan and commercial crab chitosan for soybean oil was 706 and 587%, respectively.

Degree of Deacetylation (DD): The DD of the three prepared chitosan were (84, 92 and 87%) for commercial, shrimp and crayfish chitosan (Table 1). The DD consider to be an important parameter for the identification of chitosan[16] stated that DD analysis was affected the type of analytical methods employed, type of instruments used and the preparation of sample.

Fourier transforms infrared spectroscopy (FTIR): The structures of the three different chitosan types were confirmed by FTIR spectrum in the range of 400-4000 cm^{-1} (Fig. 2). The a, b and c spectra of three chitosan types were observed to have absorption band in the region of (3422.06, 3444.24 and 3446.17 cm^{-1}) which corresponded to the vibrating and of aliphatic O-H and NH stretching vibration of free amino groups. Another absorption bands were found at (2922.59, 2930.31 and 2924.52 cm^{-1}) for a, b and c, respectively that corresponded to stretching asymmetric of CH_3 and CH_2. Also, the bands of bending vibration of NH_2, the

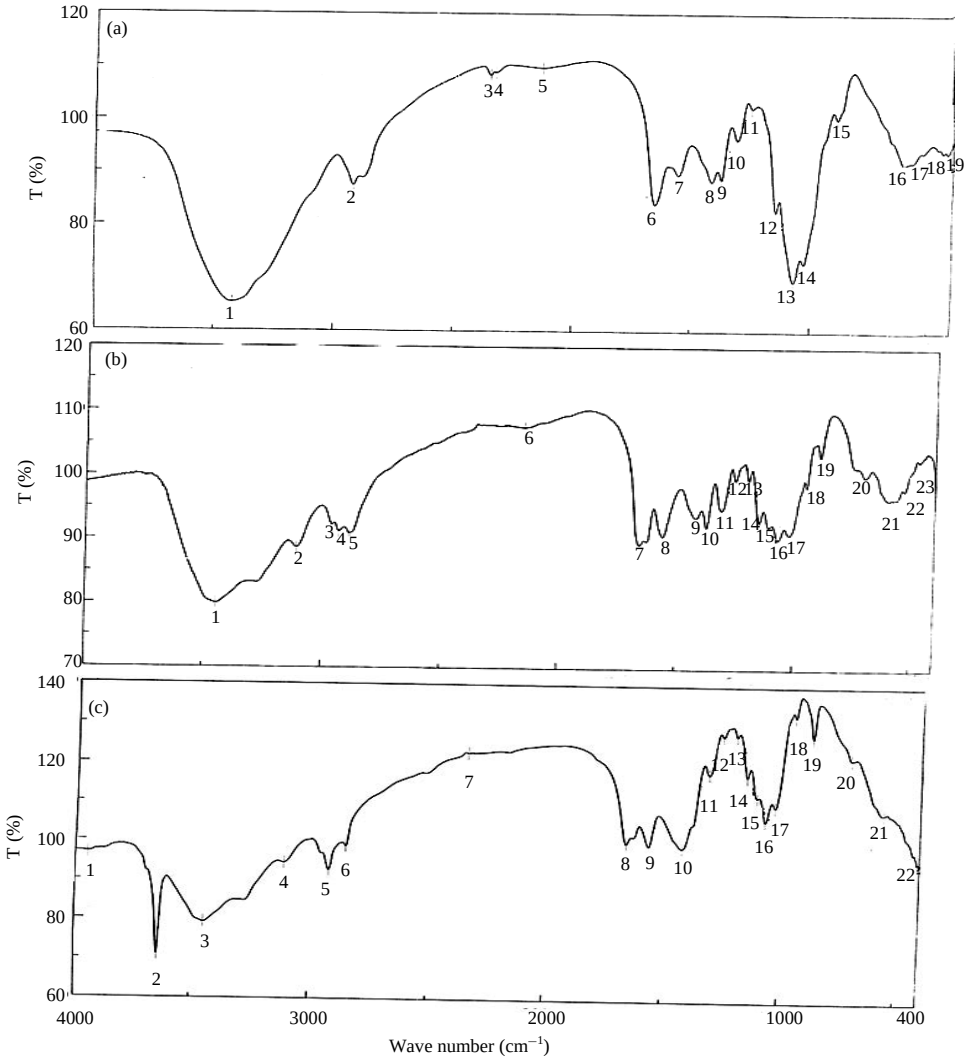

Fig. 2(a-c): FTIR spectra of (a) Commercial chitosan, (b) Shrimp chitosan and (c) Crayfish chitosan

important group in the chitosan were seen at bands (1656.55, 1658.48 and 1660.41 cm^{-1}) for chitosan a, b and c, respectively[22]. The stretching vibration for glucosamine ring-C-O-C-was indicated by the absorption bands at (1030.77, 1027.87 and 1031.73 cm^{-1}) for the three chitosan types a, b and c, respectively. While, the bands (897.70, 895.77 and 876.48 cm^{-1}) were corresponded to ring stretching for β-1,4 glycosidicbonds[23,24].

Scanning Electron Microscopy (SEM) analysis: The structure and morphology of the three types of chitosan were examined by SEM analysis. From Fig. 3, the SEM micrographs showed that there is a big difference in the roughness and surface morphology among the different types of chitosan. The SEM analysis for commercial chitosan (3a) showed non-smooth and non-homogenous surface. For shrimp chitosan (3b) SEM analysis showed that chitosan had a smooth and homogenous surface. The SEM analysis for crayfish chitosan (3c) showed a three-dimensional morphology.

EDAX analysis: Energy dispersive analysis of X-ray spectroscopy (EDAX) spectrum of the three types of chitosan were illustrated in Fig. 4 The elemental composition of the three chitosan types were studied by energy dispersive analysis of X-rays (EDAX). The EDAX analysis confirmed that the main peaks in the three chitosan spectrums are (C) and (O) which is the principle content of chitosan. The intensity of the peaks for (C) and (O) were maximum for crayfish chitosan (Fig. 4c) followed by shrimp chitosan (Fig. 4b) while, the minimum peaks intensity were for the commercial chitosan (Fig. 4a).

Fig. 3(a-c): SEM images of (a) Commercial chitosan, (b) Shrimp chitosan and (c) Crayfish chitosan

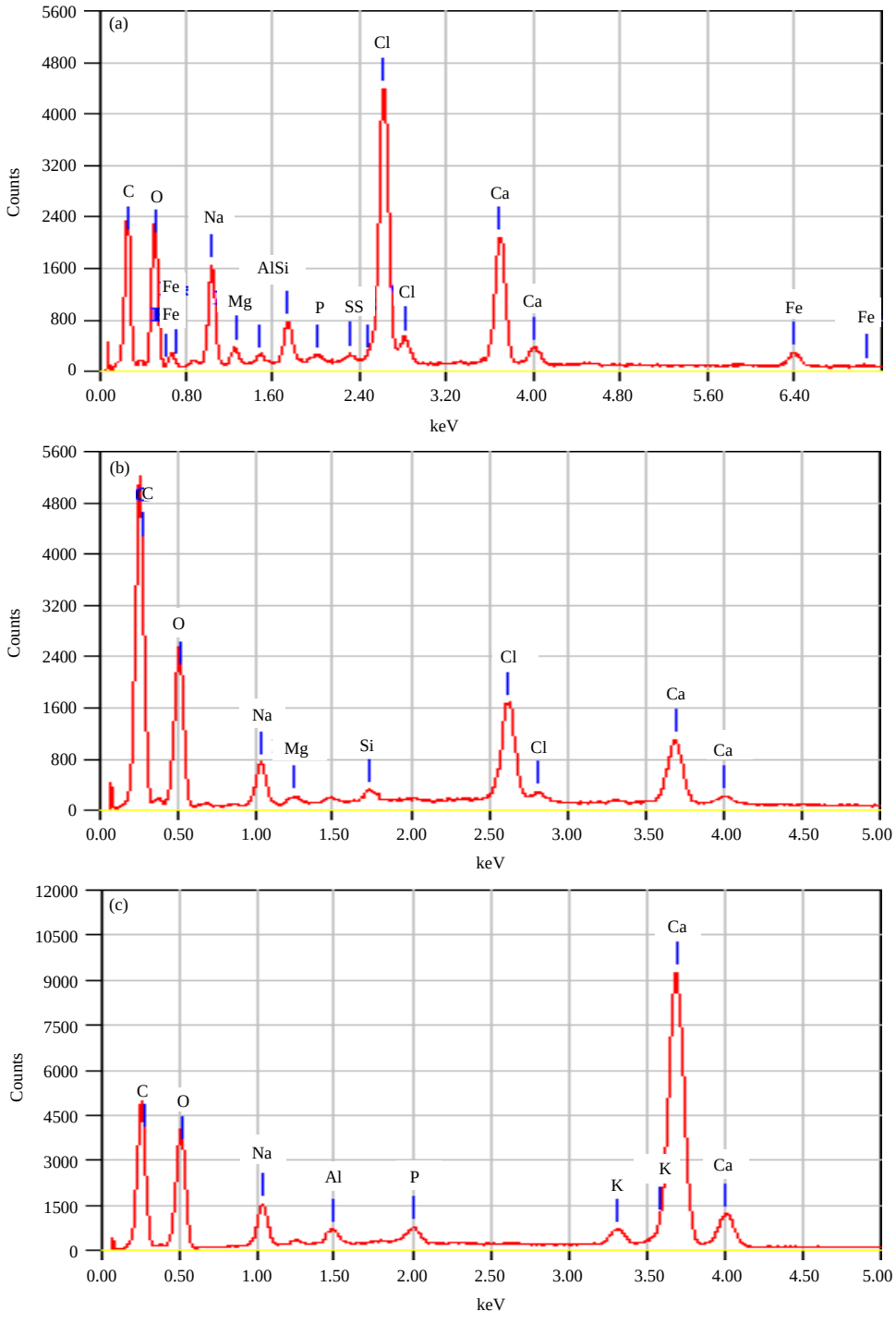

Fig. 4(a-c): EDAX images of (a) Commercial chitosan, (b) Shrimp chitosan and (c) Crayfish chitosan

Antioxidant activity: The antioxidant activity was carried out according to Tarafdar and Biswas[15]. The antioxidant activity of chitosan means its ability in scavenging the DPPH radical, which result from the reaction between the residual free amino group and the free radicals to form stable macromolecule radicals and/or the amino groups can form

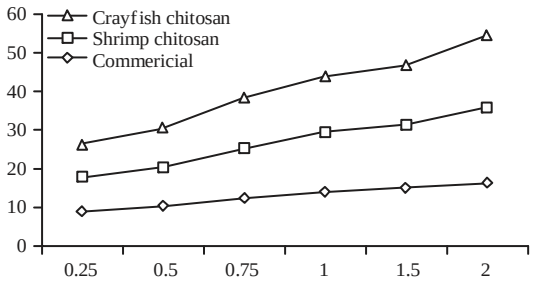

Fig. 5: Scavenging activity of different chitosan types

ammonium groups by absorbing hydrogen ions from the solution and then reacting with radicals through an additional reaction[25].

The comparative study between scavenging activity and concentrations of the commercial, shrimp and crayfish chitosan (Fig. 5) showed that the scavenging activity increased with increase in concentrations of chitosan. Chitosan had the best scavenging ability because of its active amino and hydroxyl groups[26].

CONCLUSION

The present study confirmed the simple extraction method of chitosan is an effective in solving one of the greatest environmental and economic problems in Egypt and producing natural additives for food industries.

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