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Full Length Research Paper

Categorization and Extraction of chitin; a functional biopolymer derived from scales of common carp fish (*Cyprinus carpio* l.): A stress free source

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Chitin is the most abundant natural amino polysaccharide and is estimated to be produced annually almost as much as cellulose. It has become of great interest not only as an industrialized resource, but also as a new functional material of high potential in various fields. Chitin was successfully extracted from the scales of a common Carp fish and characterized for it functional properties. Structural data of the chitin was determined by FT-IR and the crystalline and surface morphology were also studied by Powder X-Ray diffractometry and scanning electron microscopy (SEM). The proximate analysis determined was 2.12% moisture content, 1.56% ash and 4.18% Nitrogen. The amount of Ca, Mg, Zn, Fe, Cu and Mn present in the chitin was 26.84, 50.49, 21.80, 12.68, 0.22 and 1.17 mg/kg, obtained by atomic absorption spectrometry, respectively.

Key word: Chitin, chitosan, biodegradable, biocompatibility, non-toxic, X-ray diffractormeter, scanning electron microscope (SEM), fourier transform infrared (FTIR).

INTRODUCTION

The use of renewable products to produce valuable and biologically sustainable materials and to minimize waste is a challenge for current research and development. Chitin is the second most abundant polysaccharide in nature after cellulose and it largely exists in wastes from processing of marine food products (crab, shrimp and krill shells as well as fish scales). About 10^{11} ton of chitin is produced annually in the aquatic biosphere alone (Wang et al., 1998).

The waste generated from the worldwide production and processing of shell-fish and fish scales is a serious problem of growing magnitude. This abundant waste may pose environmental hazard due to the easy deterioration (Mejia-Saules et al., 2006). The use of this waste for renewable products such as biopolymers is a dual-purpose opportunity (Gildberg et al., 2001). Chitin is a natural biopolymer with a chemical structure similar to that of cellulose and is a major component of the exoskeleton of invertebrates (Knorr, 1991; Rodde et al., 2008). Therefore, crustacean and fish scale waste is ideal as raw material for chitin production. The extracted chitin can be used to produce chitin-derived products, such as chitosans, chito-oligosaccharides and glucosamine, but also for bioplastic production.

Most of the naturally occurring polysaccharides, for instance, cellulose, pectin, alginic acid, and carragenans are neutral or acidic in nature, whereas chitin and chitosan are examples of highly basic polysccharides (Takeuchi et al., 2001; Kato et al., 2003). This polymer is known to be nontoxic, odourless, biocompatible in animal tissues and enzymatically biodegradable (Zong et al., 2000). The unique properties of chitin and chitosan include polyoxysalt formation, ability to form films, chelation with metal ions and optical structural characteristics (Kumar, 2000). The main parameters influencing the characteristics of chitosan are its molecular weight and degree of deacetylation, which affect the solubility, rheological and physical properties (Khan, 2001).

Chitin is a unique material for versatile applications

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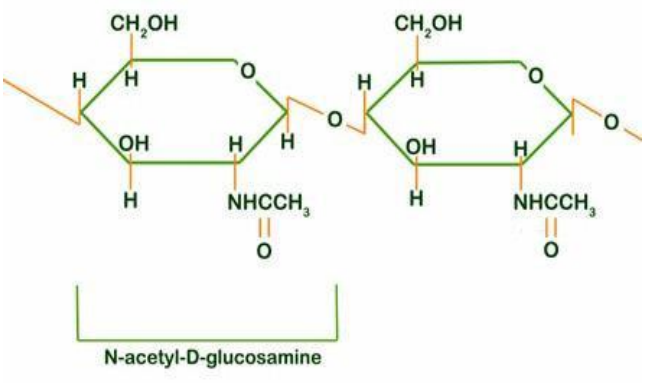

Figure 1. Chemical structure of chitin. (Source:WEB_4 2007).

Figure 2. Fish scale chitin flakes sample.

and it has high commercial interest due to its percentage nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%) (Pastor, 2004). Chitin has already found applications in diverse products that have reached the market. The industrial production and use of chitin has been steadily increasing since the 1970's (Muzzarelli, 1973). In Japan, for example, the production of chitosan from chitin increased an average of 37% each year from 1978 to 1983 (Kumar, 2000). During that time frame, the major applications of chitin were centered on sludge de-watering, food processing and metal ion chelation (Muzzarelli, 1973). The present trend, in industrial applications, however, has expanded toward producing high value products, such as cosmetics, drug carriers, feed additives, agriculture, semi-permeable membranes, and pharmaceutics (Pastor, 2004).

Chitin is expected to have a much higher potential than cellulose in many fields. It is a specialty biopolymer having specific properties and therefore, it is interesting not only as an abundant resource but also as a novel type of functional material (Yadav et al., 2004). The aim of this work was to search for more ways of getting chitin from the waste biomass for industrial and medicinal used.

MATERIALS AND METHODS

Fish-scales waste

The material investigated was common carp fish scales obtained from one of the restaurants at Kwali Town as waste. The fish scale was washed and sun dried for three days and later ground with a milling machine to fine powder. The ground scale was cleaned of mineral remains with 1M HCl (1:5 w/v) for 24h at a temperature of approximately 30°C. It was then rinsed several times with deionized water and deproteinized in 0.5% NaOH (1:1 m/v) for 30 min at a temperature of approximately 95°C.

After deproteinization, the chitin was rinsed several times with deionized water and then dried for 4h at temperature of 80°C. It was then ground in sample milling machine and kept for use. The percentage yield for chitin was calculated from the weight of chitin produced as a percentage of starting dry raw materials and the yield was 20.49%. Figure 1 is the chemical structure of chitin while Figure 2 is the chitin flakes extracted from the scales of the common carp fish, respectively.

Table 1. Physico-chemical parameters.

Parameter	composition
Moisture content (%)	2.12 ± 0.01
Ash content (%)	1.56 ± 0.20
Nitrogen content (%)	4.18 ± 0.03

The results are means triplicate determination ± standard deviation.

Table 2. Minerals contents in mg/kg (DM).

Elements	Composition
Ca	26.84 ± 1.35
Mg	50.50 ± 0.15
Mn	1.17 ± 0.01
Cu	0.02 ± 0.01
Zn	21.80 ± 0.07
pb	Nil
Cd	Nil
Cr	Nil

The results are means triplicate determination ± standard deviation, Nil = not detected.

Characterization of biopolymer chitin

Moisture content

Crude chitin sample was placed in a pre-weighed aluminum dish. The dish and contents were then placed in an oven at 105°C for 24h. The aluminum dish along with the dried sample was first placed in a desicator to cool down and then weighed. The moisture content was then deduced accordingly (Mahmoud, 2007).

Elemental and proximate analysis

The dried chitin samples were analyzed for their mineral content. Quantitative trace element analyses were done using an atomic absorption spectrophotometer. For calcium, magnesium, manganese, iron, chromium, cadmium, zinc, copper and lead analyses, the samples were first digested with hydrochloric and nitric acids (30, 10 mL/g sample, respectively) in a closed vessel at a temperature of 100°C and then the elements were determined by flame atomic absorption with detection limit of 0.001 ppm (Mahmoud, 2007). Ash content of chitin was determined by weight loss of 1g chitin heated at 900°C for 2.5 h, AOAC (1990) and nitrogen was determined by the micro-Kjeldahl method reported by Pearson (1976). All determinations were in triplicates.

Fourier transform infrared (FTIR) spectroscopy

IR spectra of the extracted crude chitin were measured using an FTIR spectroscopy (FTIR-8400S). Samples were diluted 1: 5 with KBr before acquisition.

X-ray diffraction

XRD measurements of crude chitin extracted from fish scales were performed on a XPERT-PRO powder diffractometer (PANANALYTICAL) with CuKα radiation. The tube voltage was set at 40 kV, and the current was set at 30 mA with a wavelength of 1.54060 nm. The XRD diffraction patterns were taken over 11 min in the range of 10° to 60° at a scan speed of 0.0835°/s, the step size was 0.0021°.

Scanning electron microscopy (SEM)

The morphology of the crude chitin extracted was observed using SEM (EVO/MA/10). Before testing, the samples were mounted on the SEM stubs with double side adhesive tapes and then put under vacuum (without coating with Platinum or Gold) to make the sample conductive. Scanning electron photomicrographs were taken at various magnifications to assure clear images.

RESULTS AND DISCUSSION

Proximate and mineral analysis

The mean moisture content of the chitin sample as shown in Table 1 was 2.12%.The sample gave a rather high ash content of 1.56%. The nitrogen content was found to be 4.18% as in Table 1. The mineral contents are presented in Table 2. Expectedly, magnesium and calcium gave the highest contents of 50.50 and 26.84 mg/kg, respectively. A significant amount of zinc (21.80 mg/kg) was also obtained, while on trace amounts of manganese and copper were observed as shown in Table 2. Lead, cadmium and chromium were not detected in the samples studied.

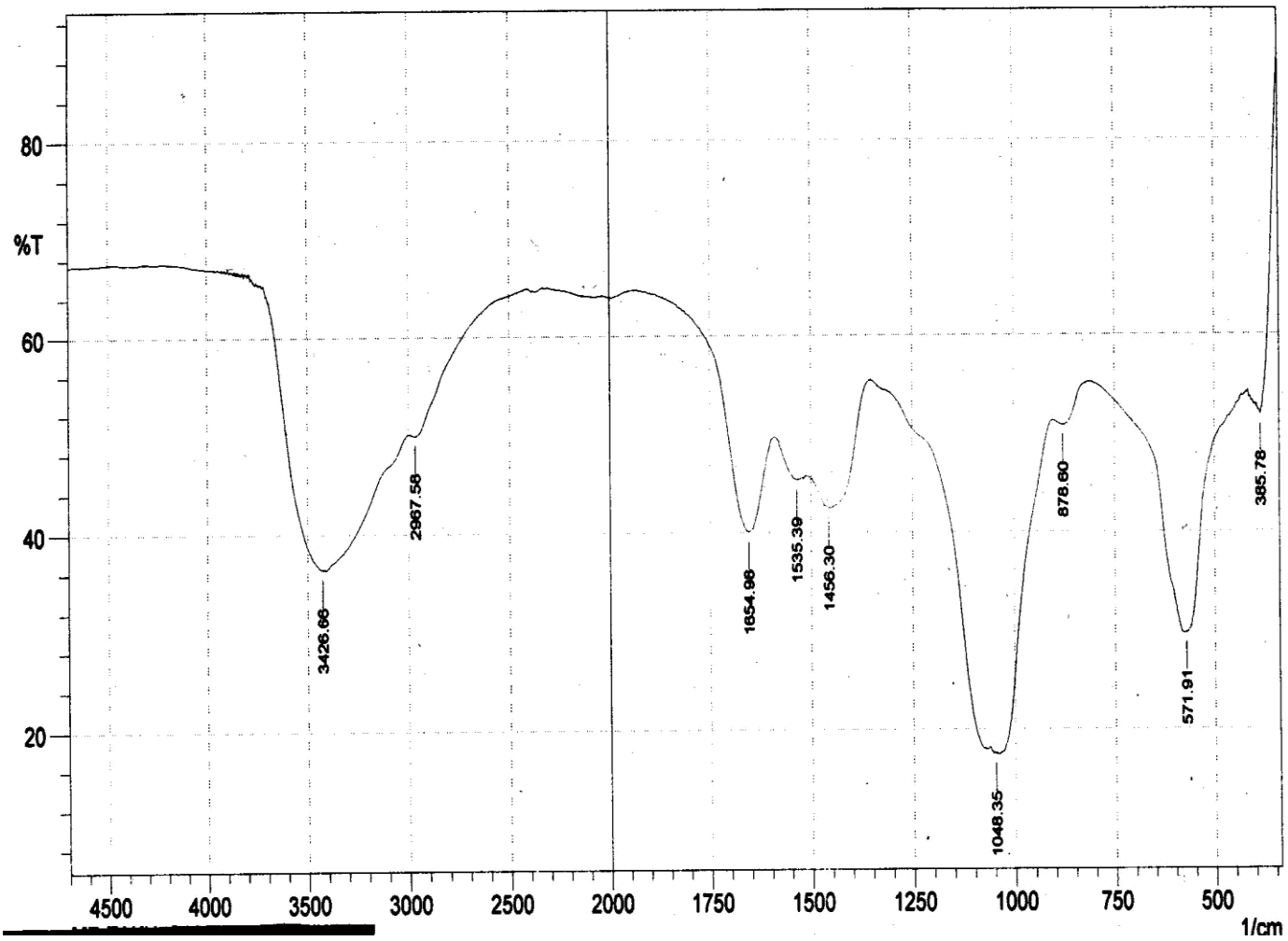

Figure 3. IR- spectra of Chitin.

FTIR measurement

IR characterization of the chitin was performed with FTIR-8400S instrument with a frequency range of 4500 to 500 cm^{-1}. It is clearly seen from Figure 3 that the absorption patterns of the spectrum is similar to that of the literature and suggesting good quality of chitin biopolymers has been obtained. Detailed examination of the spectrum reveals that the spectra have characteristic bands for chitin. The spectra was observed to have a band located at 3426.66 cm^{-1} indicates the stretching vibration of aliphatic O-H, which are more evident in the chitin spectrum (Lima et al., 2004). Absorption peak at 2967.58 cm^{-1} is from C-H vibration of -CH3. Absorption band at 1654.98 cm^{-1} represents the stretching vibration of the carbonyl group, C=O from acetamide (-NHCOCH3). Other characteristic absorptions for chitin are at 1535.39 and 1456.30 cm^{-1} indicating the bending vibration of –NH and stretching vibration of –CN from acetamide group, respectively. Absorption peak at 1048.35 cm^{-1} is the stretching vibration for –C-O-C- of the glucosamine ring. While peak at 878.60 cm^{-1} is ring stretching a characteristic band for β-1, 4 glycosidic bonds. The IR-spectrum agrees with that reported in the literature (Lima et al., 2004) for chitin.

Scanning electron microscope analysis (SEM)

The morphology of the chitin samples was studied using a scanning electron microscope. SEM images with different magnifications and different areas of the chitin sample are presented in Figure 4. It was observed that the biopolymer has porous and fibril structures.

X- ray diffraction (XRD)

The XRD pattern of the sample is shown in Figure 5. Five crystalline reflections were observed in the 2Θ range of

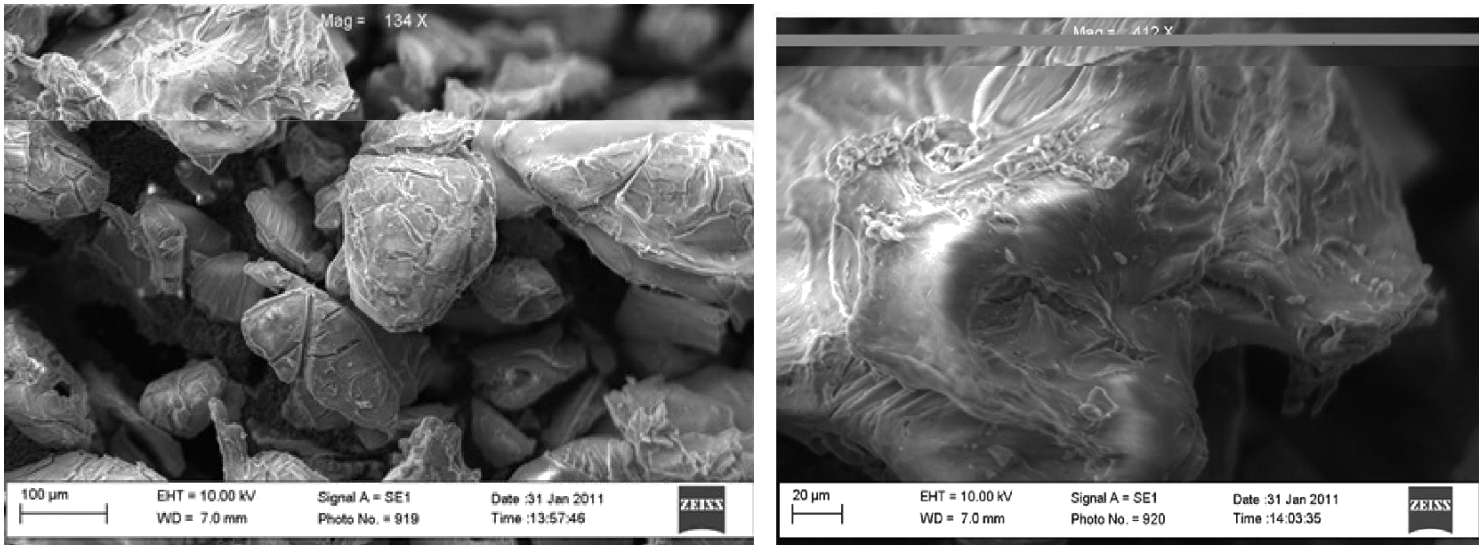

Figure 4. SEM images of chitin (a) x134,(b) x412.

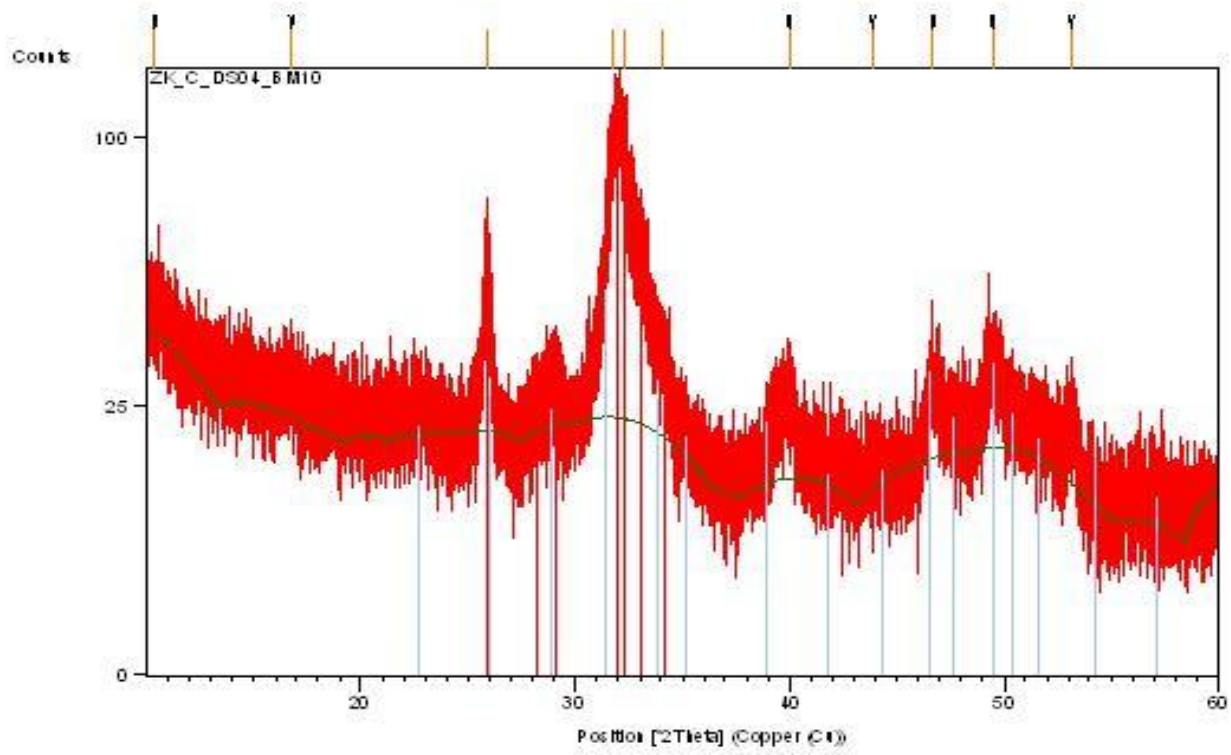

Figure 5. XRD diffractogram of Chitin.

10 to 60^{o}. The peak intensities were 7.81, 50.95, 100.00, 98.52 and 21.22 at 2Θ 16.775, 25.847, 31.772, 32.3102 and 34.1056, respectively (Zhang et al, 2005). Figure 4 shows that chitin has both crystalline and amorphous regions in the structure (Struszczyk, 1987).

Conclusion

The chitin obtained from the scales of the fish (Common carp) could be used in a variety of applications especially when transformed into the more useful compound

chitosan. The increased use of natural chitin (and its derivates) is motivated by the fact that contrary to the petroleum derivatives, the chitin is obtained from a sustainable and renewable source.

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