PREPARATION AND CHARACTERIZATION OF CHITOSAN FROM CHICKEN FEET

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Abstract

In view of the increasing importance of chitosan, this study was carried out to investigate the preparation and characterization of chitosan from chicken feet which were economically unevaluated. Chitosan was prepared from the feet of chicken using the conventional methods of pretreatment, demineralization, deproteinization, discoloration (chitin), and deacetylation (chitosan). The yield of crud chitin and chitosan were 17% and 5% respectively. The results obtained revealed that molar mass average of chitosan monomer was 165.62 g/mol with degree of deacetylation (DDA) 89% calculated from mathematical and UV spectrophotometer methods. The FT-IR spectrum of chitosan also determined and characterization was done and compared with standards.

Keywords Chicken Feet; chitosan; Preparation; Characterization; Degree of DE acetylation.

Introduction

Chitosan, a natural polysaccharide biopolymer, has received considerable attention as a functional, nontoxic and biodegradable biopolymer for diverse applications (KeanThanou, 2010). Chemically, it is a high molecular weight linear poly-cationic hetero-polysaccharide consisting of two monosaccharaides, N-acetyl-de-glucosamine and d-glucosamine, linked together by β-(1→4) glycoside bonds. Chitosan derived by partial N-deacetylation of chitin. Chitin is an insoluble linear β-1,4-linked polymer of N-acetyl glucosamine (Hajji et al., 2014; Chatelet et al., 2001). It is the most abundant renewable natural resource after cellulose (Roy et al., 2003; Knorr, 1984), a major constituent of the cell wall of many fungi, insect exoskeletons, and crustacean shells, and it largely exists in wastes from the processing of marine food products (crab, shrimp and krill shells) (Puvvada et al., 2012; Hajji et al., 2014; Yen et al., 2009).

The biopolymer is characterized as either chitosan or chitin according to the degree of deacetylation (DDA) which is determined by the proportion of D-glucosamine and N-acetyl-D-glucosamine. Degree of deacetylation has often been cited as an important parameter that determines many physiochemical and biological properties of chitosan's such as crystallinity, hydrophilicity, degradation and cell response (Dong et al., 2002). The chemical processes of chitin deacetylation are influenced by various parameters, such as time, temperature, concentration and relation of alkali/chitin solution utilized in the deacetylation reaction (Samar et al., 2013). DDA of chitosan is predominantly controlled by processing of the native polymer with alkali, and with increasing time and temperature to obtain the highest DDA (>90) materials [10, 17, 14].Because chitin and chitosan possesses many beneficially biological properties such as antimicrobial activity (Kobayashi et al., 1990; Tokoro et al., 1989), biocompatibility, biodegradability, hemostatic activity, and wound healing property, much attention has been paid to their biomedical applications (Kumar, 2000; FelsePanda, 1999). Furthermore, antioxidant properties of chitosan derivatives have been studied (Xing et al., 2005; LinChou, 2004). Owing to these unique properties, chitosan and its derivatives have been proposed for

applications in biomedical, food, agriculture, biotechnology and pharmaceutical fields (Dutta et al., 2004; Kumar, 2000).

There are many methods for the analysis and physicochemical characterization of chitin and chitosan. These methods is including Fourier transform infrared spectroscopy (FTIR) (Van de VeldeKiekens, 2004), high performance liquid chromatography (HPLC) (Niola et al., 1993), nuclear magnetic resonance (NMR) (Van de VeldeKiekens, 2004), titration and ultraviolet–visible adsorption spectroscopy (Liu et al., 2006; Jiang et al., 2003). However, some of the methods are either too tedious, costly for routine analysis (nuclear magnetic resonance spectroscopy), or destructive to the sample (Ninhydrin Test) (CurottoAros, 1993). On the other hand, FTIR method is offered a simple, accurate and not expensive like other method. Because of chitosan is an important material, there are many papers about preparation of chitosan from different kind of organism such as crustaceans (crabs, shrimp, lobsters, etc.) (Yen et al., 2009), insect (housefly, Honeybees, Beetle) (MiAoWu, 2011), fungi (shiitake stipes) (Yen et al., 2007). Although little work has been recorded on the extraction of chitin from chicken feet which increasingly litters in areas where they aren't consumed, disposal of the chicken feet constitutes an environmental problem. Nobody yet has prepared chitosan from chicken feet, so the aim of this research was to obtain chitosan from chicken feet which are known as a part of the chicken that is eaten in Trinidad, Korea, Indonesia, China, Malaysia and Tobago, Jamaica, South Africa, Peru, Philippines, Mexico and Vietnam. However, this part of the chicken in many countries (as Syria) is not used so we can use it to obtain chitosan.

Materials and Methods

Materials

Chicken feet were collected from Mazzah market (Damascus-Syria). Acetic acid, potassium permanganate, oxalic acid, sodium hydroxide, hydrochloric acid were purchased from panreac quimica SA.(Espan).Chitosan high molecular weight (HMW) was purchased from Sigma Chemical Co.(USA). All chemicals purchased were Analytical grade.

Extraction of chitin from chicken feet

Crude chitin from chicken feet was prepared according to the method of (Ai et al., 2008) with some modification. Chicken feet were rinsed and washed with tap water and deionized water after that dried for 24h at 85°C. The dried feet were grinded using a mill (IKA-A10B-Germany) to obtain crude powder. The powder of feet (~46g) was treated with 1N HCl solution 1:18 (powder/HCl) (w/v) at room temperature for 28 h to remove minerals. Then, the sample was quite squashy and was rinsed with water to remove acid and calcium chloride. The dematerialized sample was then treated with 1.25N sodium hydroxide solution 1:12 (powder/NaOH) (w/v) at 90°C for 24h to remove proteins. The mixture was filtered and washed with deionized water to obtain a crude chitin precipitate. The crude chitin discoloration with 1% potassium permanganate aqueous solution for 2h and then treated with 1% oxalic acid aqueous solution for 2h. Then chitin was collected and washed to neutrality in running tap water, and then it was dried.

Preparation of chitosan from Chicken feet

In order to obtain chitosan from chicken feet many protocols had been tested. The best conditions to obtain chitosan from chicken feet were be used 50% sodium hydroxide solution at 97°C for 8h for N-deacetylate process of crude chitin. After filtration, and neutralization chitosan was dried, and stored until used.

Determination of degree of deacetylation and molar mass average of chitosan monomer

The degree of DE acetylation of chitosan from Chicken feet and Sigma-Aldrich were determined according to the method of (Liu et al., 2006). Amount of chitosan 100±22 mg was heated in 9.8ml of HCl standard solution 37% for 1 min at 60°C with constant stirring. After 1h and 45 min, when chitosan was dissolved completely, the clear solution was diluted to 1L with deionized water. The dilution was necessary to get the chitosan concentration to the range detectable by a spectrophotometer method (spectrophotometer T80). The blank was prepared by the same way. The degree of acetylation (DA) calculated by the formula:

$$DA\ (\%) = (161.1 \times A \times V - 0.0218 \times m\ /\ 3.3615 \times m - 42.1 \times V \times A) \times 100$$

Where

DA– Degree of Acetylation
A – Absorbance of the solution at 207 nm
m – Weight of chitosan in milligram
V – Liters of solution

While the molar mass average of chitosan monomer was measured according to (Aljawish et al., 2012). Using Eq:

$$M = M_d \times DD + M_a \times DA$$

Where M_d is the molar mass average of deacetylate monomer (161 g mol^{-1}), M_a is the molar mass average of acetylated monomer (203 g mol^{-1}), DD is the deacetylation degree and DA is the acetylation degree.

Fourier transform-infrared spectroscopy (FT-IR)

Fourier-Transformed Infra-Red Spectroscopy (FTIR) of the prepared sample was obtained in a spectrometer (JASCO FT/IR-4200). Spectra were registered using attenuated total reflection infra-red spectroscopy (ATR-FTIR). For comparison, a chitosan (HMW-Sigma) have been analyzed as standard.

Results

Preparation of chitin and chitosan from chicken feet

The yield of purified chitin from chicken feet was 17% in the total weight of the dried chicken feet. After N-deacetylation, the yield of purified chitosan was in the range of 5% **(Table 1)**.

Determination of degree of deacetylation and molar mass average of chitosan monomer

The degree of DE acetylation of chitosan from chicken feet was 89%. By comparing the results with chitosan from (Sigma-Aldrich), a degree of

deacetylation was 83%. The Degree of deacetylation and the molar mass average of chitosan from chicken feet and chitosan from (Sigma-Aldrich) were shown in **Table1.**

Table1. Degree of deacetylation (DDA), the molar mass average of chitosan monomer (M_m) of chitosan from chicken feet and the yields of chitin and chitosan by chemical extraction.

	DDA (%)[a]	M_m(g/mol)	Yields [a]		
Chicken Feet	89±0.1	165.62	Powder (g)	Chitin (%)	Chitosan (%)
			46±1	17±0.5	5±0.3
Sigma-Aldrich	83.4±0.7	168.14	--------		

[a] Each value is expressed as mean ± standard error (n = 3).

Characterization of Prepared compound

The FT-IR spectrum of the chitin isolated from the chicken feet was compared with the reference of (Jalal et al., 2012) and prepared chitosan from chitin was compared with the standard chitosan. The FT-IR spectrum of chitin was shown in **Figure 1;** also, the FT-IR spectrum of standard chitosan and chitosan from the chicken feet was shown in **Figure 2**. The major peaks of chitin were compared with the reference was shown in **Table 2** and the major peaks of prepared chitosan were compared with the standard and reference as shown in **Table 3**.

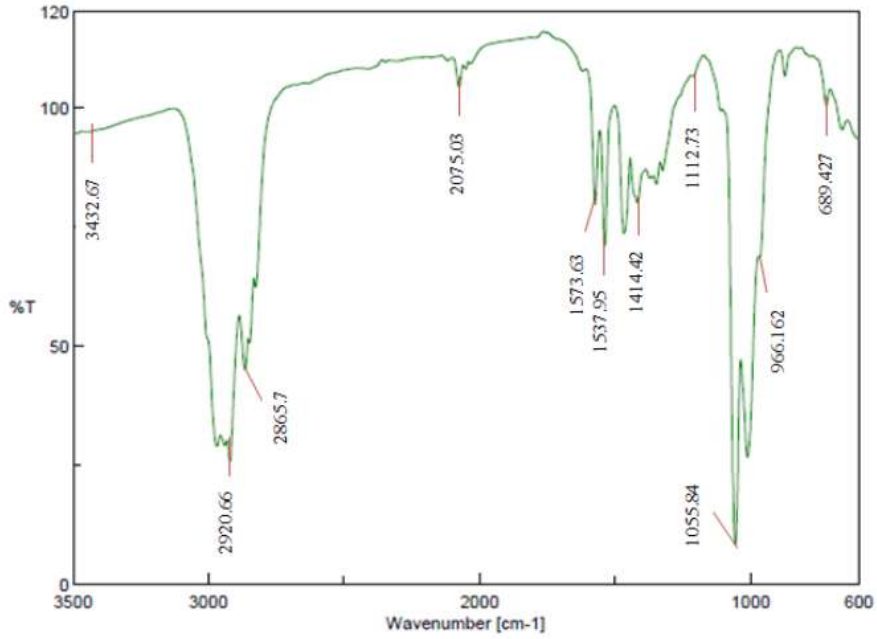

Figure. 1. FT-IR spectrum of chicken feet chitin

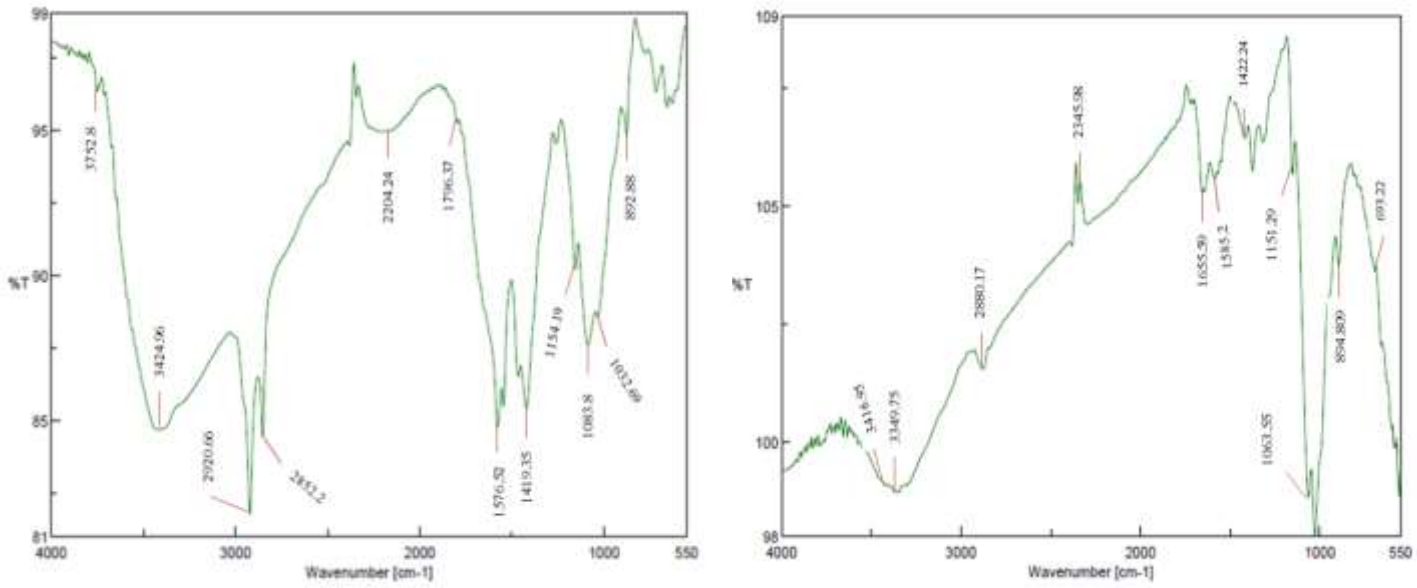

Figure 2. FT-IR spectrum of chicken feet chitosan (a) and standard chitosan (b)

Table 2. FT-IR spectral values of the main bands of chicken feet chitin were compared with the references bands

Vibration modes	Standard chitin (cm^{-1})[r]	Chicken feet chitin[s]

OH stretching	3439.58	3432.67
Symmetric CH3 stretching and asymmetric CH2 stretching	2923.48	2920.66
Amide II band	1558.84	1573.63 – 1537.95
CH2 bending and CH3 deformation	1419.53	1417.42
Amide III band and CH2 wagging	1315.04	1310.39
Asymmetric in phase ring stretching	1115.57	1112.73
CO-stretching	1030.08	1030.77
CH3 wagging alone chain	954.09	966.162
OH– out of Plane bending	697.63	689.427
CH stretching	2990 – 2850	2865.7

(r) References bands based on (Jalal et al., 2012). (s) FTIR spectrum of Sample chicken feet chitin.

Table 3. FT-IR spectral values of the main bands of chicken feet chitosan and the standard chitosan

Vibration modes	**Standard chitosan (cm^{-1})**	**Chicken feet chitosan**
OH– out of Plane bending	3416.95	3424.96
NH (amide II)	1585.2	1576.52
C=O(amide I)	1655.59	----
CH (amide II)	1422.24	1419.35
OH	3450	3446.17

Overlapping (C-H) stretching vibrations of palmitoyl chain (-CH2-, -CH3).	2880.17	2852.2
the anti-symmetric stretching of the C-O-C	1151.29	1154.19
the C-O-C stretching bands	1063.55	1083.8
C-O-C bridge as well as glycosidic linkage	894.809	892.88
free amino group (-NH2)	1026.91	1032.69

Discussion

The main purpose of present study was to prepare chitosan from chicken feet. According to our knowledge, this is the first paper presenting results on the preparation of chitosan from chicken feet samples. The preparation of chitosan involves various chemical steps such as extraction of the chitin from the raw material which will be the initiation of the chitosan synthesis. The extraction of chitin from chicken feet involve two major steps (Jalal et al., 2012), demineralization to remove calcium carbonate using hydrochloride acid and deproteinization to remove proteins with sodium hydroxide and sometime decolored step using potassium permanganate then oxalic acid as bleach material was needed and this like when chitin is extracted from exoskeletons of crustaceans (crab, shrimp) (Hajji et al., 2014; Yen et al., 2009; Yen et al., 2007). The chitin yield from chicken feet was found to be 17% while Yen and Mau (2004) purified the crab chitin using acid and alkaline treatments followed by decolorization with ethanol and got a yield of 64.4%. As well as the obtained yields of fungal chitin from shiitake stipes when Yen and Mau (2006) prepared by using alkaline treatments then followed by decolorization with ethanol or

potassium permanganate was 36.7% or 25.1%. As result, the yields of chitin from the Chicken feet were clearly lower than those of fungal/ crab chitin.

The deacetylation step was needed to obtain chitosan from extracted chitin with using high concentration sodium hydroxide solution. The chitosan yield was found to be 5 % after process of deacetylation by 50% sodium hydroxide solution at 97°C for 8h. Yen (2007) used 40% of sodium hydroxide at 105 °C for N-deacetylation of fungal chitin and found that the yields of fungal chitosan in different time (min) (C_{60}, C_{90}, C_{120}) were 23.7, 21.8 and 24.0% respectively. Puvvada (2012) also used 50% of NaOH at 100°C for 2 h N-deacetylation of crude chitin collected from exoskeleton shrimp of *Triopslongicaudatus* and *Triopscancriformis* specimens and the yield was found to be 34% after purification of the total exoskeleton taken so the chitosan yield of Chicken feet was apparently lower than those of fungal or crab chitosan. However, properties of chitosan as (antioxidant, antimicrobial, antifungal….etc.) affected by the source of chitin and the method of preparation (reaction time prolonged, temperature, concentration of NaOH) effected on important parameters as degree of deacetylation (DDA) (Van de VeldeKiekens, 2004; Jiang et al., 2003; Kumar, 2000). In this study, chitosan from Chicken feet with 89% DDA compared with Sigma-Aldrich (as stander) just 83.4 % (Liu,et al., 2006). Nevertheless, the DDA and the yields of chitosan is depended on the conditions of preparation and the DDA is increased along with reaction time and/or reaction temperature. Alkali treatment at elevated temperatures is a very common practice method to produce chitosan. The higher concentration of NaOH and time reaction were obtained chitosans, which characterized by higher DDA. the elimination of acetyl groups from the molecular series of chitin, leaving behind a complete amino group (–NH2) in the C-2 position, is the process of deacetylation. Chitosan versatility depends mainly on this high degree chemical reactive amino groups (Samar et al., 2013; Muzzarelli, 1997; Rinaudo, 2006). It is well known that DDA affects the physicochemical and functional properties of chitosan considerately and it determines its appropriate applications (Kumar, 2000; TsaihChen, 2003; Chang et al., 2003).

The efficiency of chitosan production by the N-deacetylation of chitin was also investigated by IR and the result is in (Table 2-3). Infrared (IR) spectroscopy

is one of the most important and widely used analytical techniques available to scientists working on chitin and chitosan. However, according to all literature data, appropriate bands measures, appropriate reference bands in IR techniques are required to make sure about that compound product. During the N-deacetylation of chitin, the band at 1655 cm^{-1}gradually decreased, while that at 1590 cm^{-1}(amine) increased, indicating the prevalence of the free amino group (-NH_2) at C_2 position of glucosamine (Kumirska et al., 2010). The band at 1590 cm^{-1} displayed a greater intensity than the one at 1655 cm^{-1}demonstrated the effective deacetylation of chitin. The amide I bands at 1655 cm^{-1} (sometimes together with the amide I band at 1630 cm^{-1}) or the amide II band at 1560 $cm^{-1}$are used as the characteristic band(s) of N-acetylation (Kumirska et al., 2010). Among the postulated internal reference bands are the OH stretching band at 3450 cm^{-1}(DomszyRoberts, 1985), the C-H stretching band at 2920 -2850 cm^{-1}(Dong et al., 2002), the -CH_2 bending centered at 1420 cm^{-1}(Brugnerotto et al., 2001), the amide III band at 1315–1320 cm^{-1}(Qin et al., 2006), the anti-symmetric stretching of the C-O-C bridge at around 1160 cm^{-1}(Miya et al., 1980), the skeletal vibrations involving the C-O-C stretching bands at 1070 or 1030 $cm^{-1}$and the band at 897 cm^{-1} (C-O-C bridge as well as glycoside linkage) (Qu et al., 2000; Baxter et al., 1992).

The FT-IR spectrum of the chicken feet chitosan showed nine major peaks at the ranges of 3425, 1576, 1419, 3446, 1850, 1154, 1084, and 893 cm^{-1}. Because of the difference of the degree of deacetylation between chicken feet chitosan (89%) and standard chitosan (83%), the standard chitosan from sigma (Pure chitosan) had peak was detected at 1659 cm^{-1}corresponds to the C=O for amide I (acetyl groups) and hadn't a peak absorbance at 1560 which corresponds to the N-H band for either primary amines or amide II which indicated that chitosan was not completely deacetylation (OsmanArof, 2003) while the chicken feet chitosan had a peak absorbance at 1577 which corresponds to the N-H band for either primary amines or amiad II so that was indicated about the method was used is good to preparation chitosan from chicken feet.

Conclusion

In conclusion, the method was used in this study for preparation of chitosan from chicken feet was appropriate. However, there are four stages for the

preparation (demineralization, deproteinization, discoloration (chitin) and deacetylation), the discoloration step was not very important but the stage of deacetylation was critical. On another hand, chicken feet are important source of chitosan in the Middle East because it is easy, feasible, simplicity and inexpensive to obtained it from the west food. In addition, it is important to complete study on its activities like antimicrobial, antifungal, anticancer and antioxidant properties for eventual pharmaceutical, cosmetic, medical applications and food industry.

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