Isolation and characterization of chitosan from Ugandan edible mushrooms, Nile perch scales and banana weevils for biomedical applications.

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Abstract

Background: Of recent, immense attention has been given to chitosan in the biomedical field due to its valuable biochemical and physiological properties such as biodegradability, biocompatibility, non-immunogenicity, reactivity, solubility and non-toxicity. For instance, chitosan has exhibited distinguished bioactivity not limited to only antimicrobial activity but also promotion of wound healing and immune system augmentation in model animals. Therefore, chitosan has attracted application as a nano drug targeted delivery system. Traditionally, the chief source of chitosan is chitin from crab and shrimp shells obtained as waste products in the seafood industry. Chitin is also an important component of fish scales, insects and fungal cell walls, therefore, Uganda's edible mushrooms, Nile perch scales and banana weevils can be used as alternative sources of chitin and obviously chitosan. Thus, the aim of this study was to isolate and characterize chitosan from locally available material for potential use in the biomedical field.

Methods: Chitin was extracted from banana weevils, Nile perch scales and mushrooms powder by demineralization with 1.0M HCl solution, then deproteinization with 1.0M NaOH. Chitosan was prepared by treating chitin with 50% NaOH at 100°C for 8 hrs. Chitosan ash and moisture contents were determined gravimetrically while solubility was computed as percentage dry weight of suspended chitosan. FTIR was used to determine the DD while XRD was used to estimate the crystallinity of chitosan.

Results: Ash and moisture contents ranged from 3.5 to 15% and 3.5 to 6.4% respectively while solubility level varied from 57 to 68%. FTIR spectra reveal high degree of similarity between locally isolated chitosan and commercial chitosan with DD ranging from 77.8 to 79.1%. X-Ray Diffraction (XRD) patterns exhibited peaks at 2θ values of 19.5° for both chitosan extracted from Mushrooms and banana weevils while chitosan from Nile perch scales registered 3 peaks at 2θ angles of 12.3°, 20.1° and 21.3° comparable to the established commercial chitosan (Sigma Aldrich) XRD pattern.

Conclusion: Ash content, moisture content, DD, FTIR spectra and XRD pattern revealed that chitosan isolated from locally available materials has physicochemical properties comparable to conventional chitosan and therefore it can be used in the biomedical field.

Introduction

Chitin the raw material for synthesis of chitosan, is the second most copious organic substance subsequent to cellulose. Like cellulose, this polymer is linear and non-polar with very low chemical reactivity but highly soluble in concentrated acids and a few flouroalcohols [1–3]. Chitin is derived from cellulose by replacement of an acetamido group at position C-2 and found in the exoskeletons of arthropods such as insects, crustaceans, arachnids and myriapods; cell walls of fungi and possibly scales of fish providing tensile strength [1]. Due to its insolubility at physiological conditions, chitin can chemically be modified to its soluble alternatives. Chitosan the commonest derivative of chitin is mainly derived through non-enzymatic *N* deacetylation. This is achieved through cleaving off the acetyl residue (R-NHCOCH$_3$) mediated by strong alkali at high temperatures. Furthermore, chitosan can be synthesized through enzymatic processes. However, owing to the high cost of deacetylases and their low chitosan productivity, enzymatic mediated chitin deacetylation is unpopular [4–5].

Of recent, immense attention has been given to chitosan in the biomedical field due to its valuable biochemical and physiological properties such as biodegradability, biocompatibility, non-immunogenicity, reactivity, solubility and non-toxicity. For instance, chitosan has exhibited distinguished bioactivity not limited to only antimicrobial activity but also promotion of wound healing and immune system augmentation [6–7]. The beneficial biological properties of chitosan can be enhanced when converted to its nano scale form. Chitosan is transformed to its nanoparticles by tripolyphosphate (TPP) ionic gelation. Several studies have revealed that chitosan and chitosan nanoparticles have enhanced antimicrobial activity and are good vehicles of drugs and vaccines to the target body parts [8–9].

Conventionally, the chief sources of chitin are crab and shrimp shells obtained as waste products in the seafood industry [10–11]. Chitin is also an important component of fish scales and cell walls of fungal cells; therefore, Uganda's edible mushrooms and Nile perch scales can be alternative sources of chitin and, obviously non nano-scale and nano-scale chitosan. Production of chitin and its derivatives from renewable resources such as fishery wastes and fungi presents sustainability for the ever increasing demands of this polymer.

Furthermore, antibiotic resistant bacteria are escalating in prevalence globally with consequential infections which are hard and costly to treat [12]. This is supported by emergence of carbapenem resistance in *Enterobacteriaceae* yet Carbapenems such as imipenem, ertapenem, meropenem, and doripenem are the newest synthesized beta lactum antibiotics with the broadest spectrum of activity and consequently considered the first line therapy antibiotics in the treatment of multi resistant gram-negative pathogens [13–14]. Thus, isolation of chitosan from readily available resources with potential future applications as antimicrobials and chitosan-based drug delivery system to combat antimicrobial resistance is necessary.

Therefore, this study was aimed at comparing the physicochemical properties of chitosan extracted from banana weevils, Nile perch scales and edible mushrooms with molecular grade Commercial chitosan (Sigma Aldrich). Furthermore, for application purposes, the antibacterial activity of locally isolated chitosan will be assessed against carbapenem resistant *E. coli* and *K. pneumoniae*.

Methods

Source of materials

Nile perch scale wastes were obtained from a local fish market while Banana Weevils and edible mushroom were collected from National Agricultural Research Laboratories Kawanda. Carbapenem resistant *E. coli* and *K. pneumoniae* were a kind donation from Department of Microbiology, College of Health Sciences, Makerere University.

Study design and site

This was a laboratory-based study conducted from College of Veterinary Medicine Animal Resources and Biosecurity, Makerere University, iThemba LABs, Cape Town and University of South Africa (UNISA). Isolation of chitin and chitosan was performed from the Pharmacology Laboratory while chitosan antibacterial activity was evaluated from the Central Diagnostic Laboratory. Characterization of chitosan was conducted from iThemba LABs and UNISA.

Chitin extraction

Banana weevils, Nile perch scales and mushrooms were cleaned using running tap water and finally rinsed in distilled water. The cleaned weevils, scales and mushrooms were oven dried at 60°C for 1 week and then ground to powder using an electric miller. Chitin was extracted from the resultant powder following Musarrat et al., [15] adjusted procedure. Demineralization was carried out by treatment of the banana weevil, Mushroom and Nile perch scale powders with 1.0M HCl solution at ambient temperature for 24 hours with a solution to solid ratio of 15 mL/g. This step was replicated ten times. The mixture was centrifuged at a speed of 4000 x g for 10 minutes using Thermo Scientific™ Fiberlite™F6-10x1000 LEX roto centrifuge. The resultant sediment was washed with distilled deionized water until neutral pH was achieved. The sediment was deproteinized by adding 15 mL of 1.0 M sodium hydroxide and then heated at 80°C for 8 hours. This treatment was repeated four times. The resultant chitin was then washed with distilled deionized water to neutrality. Finally, chitin was washed by boiling in hot absolute ethanol and later in absolute acetone in a water bath for 10 minutes to remove any impurities. The purified chitin was dried in a vacuum oven at 50°C to constant weight. The chitin content was determined by computing the weight differences between the raw materials and that of the chitin obtained after acid and alkaline treatments.

Chitosan preparation

Chitin was treated with 50% NaOH (15 mL/g) at 90°C for 10 hours with continuous mixing using a magnetic stirrer after which the resultant mixture was centrifuged at 4000 x g for 10 minutes using a Thermo Scientific™ Fiberlite™F6-10x1000 LEX roto centrifuge. The residue was washed with hot distilled deionized water until neutrality. The obtained chitosan was dried in a vacuum oven at 40°C for 48 hours. All the chitosan samples were purified by dissolving in 1% acetic acid and reprecipitated in 20% NaOH solution followed by centrifugation at 6000 x g for 10 minutes using a Thermo Scientific™ Fiberlite™F6-10x1000 LEX roto centrifuge to sediment chitosan. The sedimented chitosan was washed with distilled deionized water until a neutral pH, lyophilized and stored at -20°C until further use. The percentage chitosan yield was computed as a fraction of weight of dry chitosan and dry chitin from which it was generated.

Characterization of chitosan

Estimation of the Ash Content Chitosan

The ash content of each chitosan sample was gravimetrically estimated after the pyrolysis of 1 g in a muffle furnace at 650°C for 5 hours. This procedure was done in triplicates and the mean ash content computed. The ash content was computed as a fraction of mass of the residue (MR) and mass of the sample (MS) using the formula that follows;

$$\text{Percentage ash content} = \frac{MR}{MS} X100;$$

where MS and MR are the weights (in grams) of the initial sample of chitosan and residue respectively [16].

Moisture content of Chitosan

The water content of chitosan samples was assessed by gravimetric technique. This method involved drying of the samples until a constant mass in a vacuum oven at 105°C for 24 hours. This experiment was done thrice and the average moisture content was calculated. The water content was computed as the difference between the wet weight (WW) and dry weight (DW) of samples per gram using the formula that follows:

$$\text{Moisture content percentage} = \left(\frac{WW-DW}{\text{WW}}\right) X100;$$

Where WW is the wet weight of samples and DW is the dry weight of samples after oven drying [16].

Determination of chitosan solubility

A 1% solution of chitosan was constituted by adding 0.1 g (W1) of each chitosan sample previously dried at 105°C for 24 hours into 10 ml of 1% acetic acid in 15 ml falcon tube. The tubes were sealed and placed in an overhead shaker running at 60 rpm for 24 hours. The solution was centrifuged at10,000 x g for 15 minutes using a Thermo Scientific™ Fiberlite™F6-10x1000 LEX roto centrifuge. The liquid phase was poured off and the sedimented residue was washed with 10 ml of distilled deionized water and centrifuged at 10,000 rpm for 15 minutes. The supernatant was decanted and the residue dried at 105°C for 24 hours (W2). This experiment was done three times and mean dry residue calculated. The dry residue was weighed and the percentage of solubility was determined using the formula that follows;

$$\text{Percentage solubility} = \frac{(W1-W2)}{W1} X100;$$

Where; W1 was the initial weight of dry chitosan and W2 was the weight of the dried residue

Fourier Transform Infra-Red Spectroscopy (FTIR)

Three milligrams (3 mg) of each chitosan sample and 5 g of Potassium bromide (KBr) were dried at 60°C and 120°C respectively under reduced pressure for 12 hours. Each dried chitosan sample was homogenized with 100mg of KBr and then compressed to form very thin discs of approximately 0.2 mm thickness. The chitosan samples were examined at 4000–400 cm^{-1} Wavenumber range using a PerkinElmer FT-IR Spectrometer. The spectrometer was set to perform at least 64 scans per sample. A KBr disc was used as reference. Functional group assigning to the generated FTIR spectra bands was done using documented literature [29-35].

Determination of the Degree of Deacetylation (DD%)

The acetylation and deacetylation percentage of chitosan samples was determined by Fourier Transform Infrared Spectroscopy (FTIR). This was done through the correlation of some absorbance bands linked to some of amide, methyl and hydroxyl bands registered by the FTIR spectra. Vilar Junior et al. [17] used the amide I band with a wavenumber of 1655 cm^{-1} and the hydroxyl group band at 3450cm^{-1} using the formula that follows to determine the degree of acetylation (DA) and then the DD [18];

$$\mathrm{DA}\ (\%) = \frac{A1655}{3450}\ X\frac{100}{1.33};$$
$$\mathrm{DD}\ (\%) = 100\text{- DA}$$

where A1655 was the absorbance at 1655 cm^{-1} of the amide-I band which is measure of the N-acetyl group content, A3450 was the absorbance at 3450 cm^{-1} corresponding to the hydroxyl band as an internal standard to correct for disc thickness, factor 1.33 is the ratio of A1655 and A3450 for fully N-acetylated chitosan.

X-Ray diffraction Analysis (XRD)

X-Ray diffraction was used to determine the crystallinity of the isolated chitosan where 500mg of chitosan powder were analyzed employing BRUKER AXS diffractometer, D8 Advance (Germany) fitted with Cu-Kα radiation (λKα1=1.5406Å) from 2θ = 0.5° to 130°, with increment D2J: (0.034°), voltage of 40 kV, current of 40 mA, power of 1.6 kW and counting time of 0.5 sec/step. Generated data was analyzed by OriginPro Version 8.5 and resultant peaks 2θ values were compared with the commercial shrimp chitosan from Sigma Aldrich.

Chitosan susceptibility assay

Antibacterial activity of the chitosan was evaluated using standardized inocula of 1 $X10^7$ CFU/mL with 0.5 McFarland standards streaked onto the surface of sterile agar plates. Carbapenem resistant *E. coli* and *K. pneumoniae* suspended in Brain Heart Infusion Broth were inoculated onto the Mueller Hinton Agar plates and round wells of diameter 6 mm, depth 3 mm were prepared using a sterile cork borer in which 25µl of chitosan solution (0.25, 0.5, 0.75, 1, 1.5 and 2mg/ml in 1% acetic) were pipetted. Meropenem disks were used as the positive control while 1% acetic acid as negative control. The plates were incubated at 37°C for 72 hours. Zones of bacterial growth inhibition were measured and record in millimeter (mm).

Statistical Analysis

Data analysis was done using Graph Pad Prism version 7.01. Comparisons of chitin yield, chitosan yield, ash content, moisture content, solubility and DD among the chitosan samples isolated from BW, MSR and NS were performed using One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A P-value of ≤ 0.05 indicated substantial statistical variance.

Results

Composition by dry weight of chitin and chitosan

The composition of dry weight chitin was determined by using the ratio of the starting dry weight of the raw material (10 g) and the obtained chitin dry weight after demineralization and deproteinization. The dry weight of chitin obtained was 11.8%, 9.9% and 39% for banana weevils (BW), mushrooms (MSR) and Nile perch scales (NS) respectively with P values > 0.05 indicating variability between chitin yield from each raw material. The percentage yield of chitosan from chitin ranged from 70.2% to 82% with P value > 0.05 between BW and MSR chitosan, P values < 0.05 among BW and NS chitosan; MSR and NS chitosan, Table 1.

Table 1
Dry weight chitin and chitosan yield

	Chitin dry weight (g)/10			Chitosan dry weight (g)		
Sample	**BW**	**MSR**	**NS**	**BW**	**MSR**	**NS**
1	1.19	0.99	3.8	0.8	0.74	3.2
2	1.17	0.97	4	0.82	0.68	2.5
3	1.18	1.02	3.9	0.82	0.8	3.7
4	1.18	0.99	3.9	0.8	0.68	3.2
5	1.18	1	3.5	0.83	0.8	3
6	1.17	0.98	4.5	0.83	0.81	3.5
7	1.15	0.99	3.7	0.8	0.73	2.9
8	1.15	0.97	3.9	0.83	0.57	3.3
9	1.21	1.01	3.2	0.85	0.92	3.0
10	1.22	0.98	4.6	0.93	0.67	3.7
Mean ± SD (g)/ -mean percentage weight (%)	**1.18 ± 0.023/11.8^{A}**	**0.99 ± 0.016/9.9^{B}**	**3.9 ± 0.416/39^{C}**	**0.83 ± 0.11/70.2^{A}**	**0.74 ± 0.10/74.0^{A}**	**3.2 ± 0.33/82.1^{B}**

Mean values in each column accompanied by the same letter are not significantly different (P < 0.05) (Tukey Multiple Comparison) and values accompanied by letter (s) which are not similar are significantly different (P > 0.05)

Ash Content And Moisture Content

The ash content of chitosan generated was 3.5%, 8.8% and 15.5% for BW, MSR and NS respectively with P values < 0.05 indicating significant differences between BW, MSR and NS chitosan ash content whereas the moisture content varied from 3.5–6.4%, with P values > 0.05 signifying analogous moisture content within chitosan obtained from different locally available materials, Table 2.

Table 2
Physicochemical properties of chitosan isolated from locally available materials

Raw material	Ash content mean weight (g)/ Percentage (%)	Moisture content mean weight (g)/ Percentage (%)	Mean dry residue weight (g)/ Solubility (%)	DD (%)
Banana weevils	0.035 ± 0.003/3.5^{A}	0.064 ± 0.004/6.4^{A}	0.63 ± 0.014/63^{AB}	77.8
Mushroom	0.088 ± 0.008/8.8^{B}	0.039 ± 0.001/3.9^{B}	0.68 ± 0.003/68^{ABC}	78.1
Nile perch scales	0.15 ± 0.05/15^{C}	0.035 ± 0.003/3.5^{C}	0.57 ± 0.007/57^{ABD}	79.1

Mean values in each column accompanied by the same letter are not significantly different (P < 0.05) (Tukey Multiple Comparison) and values accompanied by letter (s) which are not similar are significantly different (P > 0.05)

Solubility

Chitosan isolated from locally available materials exhibited moderate solubility ranging from 57–68%. MSR chitosan registered the highest solubility (68%), followed by BW chitosan (63%) and the lowest solubility (57%) was registered from NP chitosan, Table 2. Tukey comparison between registered a P value > 0.05 between MSR and BW chitosan; BW and NS chitosan while a P value < 0.05 was recorded between MSR and NS chitosan

Infrared Spectrophotometry Determination Of Chitosan Functional Groups And Dd

Fourier Transform Infrared spectroscopy of chitosan isolated from BW, MSR, NS and commercial chitosan (Sigma Aldrich) yielded spectra with functional groups shown in Table 3 and Figs. 1–4. For estimation of the DD of chitosan, bands 1629.8, 1647.4, 1640.8 and 1652.1 cm^{-1} for BW, MSR, NS and commercial chitosan respectively corresponding to acetylated residues of amide I (NHCOCH3) and 3428.5, 3411.3, 3448.7 and 3321.8 for BW, MSR, NS and commercial chitosan respectively associating to the vibration of the OH molecule were used [24]. Analysis by Infrared spectroscopy estimated the percentage of chitosan deacetylation as 77.8%, 78.1%, 79.1% for BW, MSR and NS chitosan respectively, Table 2.

Table 3
Comparison of FTIR characteristic bands for chitosan isolated from locally available materials and commercial chitosan band patterns

Wavenumber (cm^{-1})					Functional group/Molecule
Functional group range	Banana weevil chitosan	Mushroom chitosan	Nile perch scale Chitosan	Commercial chitosan	
4000 – 3700	3829.2	-	-	-	O-H
4000–7000	3734.6	-	-	-	O-H
3650 – 3400	3428.9	3411.3	3424.3	3321.8	Group tension -OH
2919 – 2868	2863.6	2916.4	2912.6	2869.9	Stretching band C-H
2349	2351.2	2352.2	2356.2	2312.1	Carbon dioxide O = C = O
2140 – 1990	2056.5	2065.3	2060.4	2037.1	Isothiocyanate
1650 – 1550	1629.8	1647.4	1644.0	1652.1	Amide I
1560 – 1500	1559.4	1544.0	1551.4	1550.7	Doubling group NH_2
1390 – 1370	1376.8	1385.6	1377.1	1376.4	Amide III
1310 – 1250	1308.7	-	-	1303.6	Aromatic band C-O
1124 – 1087	1073.3	1064.5	1069.8	1027.9	Stretching band C-O-C
900 – 890	-	-	-	890.7	C—O—C bridge and glucosidic linkage
800 – 600	596.0	596.0	598.1	-	Stretching bond of Alkyl halide C-Cl

XRD analysis

XRD patterns exhibited peaks at 2θ values of 19.5° for both chitosan extracted from BW and MSR, Figs. 5 and 6. Chitosan from NS registered 3 peaks at 2θ values of 12.3°, 20.1° and 21.3°, Fig. 7 whereas commercial chitosan (control) scored 2 peaks at 2θ values of 19.6° and 20.2°, Fig. 8. The intensity of the peaks was highest in fish scale chitosan, followed by mushroom chitosan and banana weevil chitosan at values of 1318a.u, 583a.u and 338a.u respectively. However, the peak intensities were lower than those attained by the control (23061a.u and 41664a.u), Figs. 5–8.

Bactericidal Activity Of Chitosan

Chitosan isolated from locally available materials and commercial chitosan revealed no antibacterial activity against carbapenem resistant and sensitive bacterial even at the highest concentration of 2000 µg/ml, Table 4.

Table 4
Growth inhibition zones of chitosan isolated from different sources. Meropenem was used as a positive control while 1% acetic acid as a negative control

	Zone of growth inhibition (mm)					
Bacteria type	Meropenem disk	1% acetic acid	Commercial chitosan (0.25-2 mg/ml)	BW (0.25-2 mg/ml)	MSR (0.25-2 mg/ml)	NS (0.25-2 mg/ml)
Carbapenem sensitive *E. coli*	40	0	0	0	0	0
Carbapenem resistant *E. coli*	0	0	0	0	0	0
Carbapenem sensitive *K. pneumoniae*	40	0	0	0	0	0
Carbapenem resistant *K. pneumoniae*	0	0	0	0	0	0

Discussion

Composition of chitin and chitosan isolated

The percentage of chitin obtained in previous studies ranged from 2.5–12.2% for insects [19], 7.9–11.4% for mushrooms [20] and 33–45% in fish scales [21]. The dry weight of chitin obtained in this study was 11.8%, 9.9% and 39% for banana weevils (BW), mushrooms (MSR) and Nile perch scales (NS) respectively

falling within the ranges of the previous studies. Furthermore, the percentage yield of chitosan from chitin in this study ranged from 70.2% to 82%. These results corroborate well with the chitosan yield reported by Erdogan et al., [20].

Ash Content Of Chitosan

The residue that remains after complete pyrolysis of the material in the presence of air is termed as ash and is inorganic in nature [22]. Therefore, chitosan ash content was determined gravimetrically and the ratio of chitosan weight burnt to the weight of inorganic residue was computed into percentages. Determination of ash content in chitosan a vital litmus to assess the effectiveness of the demineralization process. Solubility of chitosan is greatly affected by the presence of inorganic minerals as this subsequently lowers viscosity [22]. This greatly affects fabrication of chitosan-based drug delivery systems. Furthermore, level demineralization and deproteination determines the purity of chitosan which in turn affects its biological properties like immunogenicity, biocompatibility and biodegradability [23]. A high-grade chitosan with superior biological properties possesses an ash content lower than 1% [24]. The ash content of chitosan generated in this study was way higher than the recommended level; however, comparable to the ash content of honey bees and shrimp shells as reported by Marei et al., [19].

Moisture Content

Chitosan has a great capacity to form hydrogen bonds with water through both its hydroxyl and amino groups hence its hygroscopic in nature. The quantity of adsorbed moisture relies on the initial moisture content of the raw materials and storage environmental conditions [25]. The moisture value of commercial chitosan powder ranges from 7–11% (w/w) and not influenced by degree of deacetylation or molecular weight [26]. Moisture content is one of the most important factors which influence the usability of chitosan powder during drug carrier and tablet preparations. Moisture content level should be put into consideration when formulating chitosan-based drugs to reduce pharmaceutical powder faults especially after storage as water content above 6% affects powder flow properties, compressibility and tensile strength of the tablets [23]. The moisture content of the chitosan isolated from locally available material was within the recommended range hence suitable for pharmaceutical use.

Chitosan Percentage Solubility

Chitosan isolated from locally available materials exhibited moderate solubility ranging from 57–68%. Contrary to this, Nessa et al., [24] reported excellent solubility ranging from 96.01 to 97.2% of chitosan isolated in-house from prawn shells. Low to moderate solubility values of chitosan are attributed to high protein content and low DD [27]. Nevertheless, this study achieved high DD comparable to levels reported by other studies and commercial chitosan. Therefore, this moderate solubility may be attributed to low deproteination (27) and distribution of the remaining acetyl groups along the polymer chain which is termed as the pattern of deacetylation [28].

FTIR comparison of functional groups and DD of chitosan from different sources

Fourier Transform Infrared spectrophotometric examination of chitosan isolated from BW, MSR, NS and commercial chitosan (Sigma Aldrich) yielded comparable spectra an indication that locally isolated chitosan has similar physicochemical properties due to the presence of almost similar functional groups. The percentage chitosan DD varied from77.8–79.1%. These DD values are consistent with the shrimp commercial chitosan DD value (76%) used as a control in this study and DD values reported by other studies. Liu et al., [36] and Santos et al., [37] reported DD values of 73.1% and 76% respectively. Furthermore, the DD values obtained in this study are within the range between 75 and 90% deacetylation degree in industrial processing [38]. One of the most important factors that should be considered when isolating chitosan in-house for biomedical application is DD. Degree of deacetylation influences several chitosan traits that include biological, physicochemical and mechanical properties. It was reported that chitosan polymer with low DD disintegrated fast and induced an acute inflammatory response while highly deacetylated chitosan induced negligible inflammation hence biocompatible [39]. Thus, cautious isolation and purification of chitosan with appropriate DD specifically for fabrication of chitosan-based formulations for parenteral biomedical application should be of great interest.

Crystallinity of chitosan isolated from locally available materials.

The 2θ values obtained from chitosan isolated from locally available materials were within the same range with the XRD patterns (2θ angles 9.6° and 20.2°) registered by commercial chitosan (control) used in this study. Furthermore, peaks at 2θ = 10° and 2θ = 20° of commercial chitosan (Sigma Aldrich) have been exhibited by X-ray diffraction studies [40]. The results from this study are comparable to the established chitosan XRD pattern as peaks at 2θ values of 12.3°, 20.1° and 21.3° for Nile perch scales chitosan, 19.5° for banana weevil and mushroom chitosan were registered. In banana weevil and mushroom chitosan, the weak peak at 2θ = 10° disappeared. Similar deviations were registered by other studies which attempted to extract chitosan from locally available materials (40–43]. However, high peak intensity for mushroom and Nile perch scale chitosan and a slight shift in 2θ = 20° diffractive angle for all extracted chitosan indicates that this study achieved highly crystalline chitosan.

Variability In The Yield And Physicochemical Properties Of Chitosan

A substantial variation was observed in the yield, ash content, moisture content, solubility and crystallinity of chitosan isolated from locally available materials. The variation in physicochemical properties among chitosan obtained from BW, MSR and NS is in line with other studies. Szymańska and Winnicka [23] observed that a variety of chitosan raw materials lead to considerable dissimilarities in the quality and properties of chitosan and its products. Thus, significant deviations from the pharmacopeial recommendation might be registered by chitosan obtained from different sources.

Antibacterial Activity Of Chitosan

Recently, substantial research attempts have been made to investigate the antimicrobial activity of chitosan. It has been reported that chitosan possesses potent antibacterial and antifungal activity [17, 44–47]. Vilar Junior et al., [17] reported that chitosan exhibited minimum inhibitory concentration ranging from 78 to 625 µg/ml in in vitro studies. However, chitosan isolated from locally available materials and commercial chitosan in this study revealed no antibacterial activity. This may be attributed to high ash content way above the recommended value (1%) which affects the physicochemical properties of chitosan such as solubility which negatively affects its bioavailability. Moreover, Zhao et al., [48] reported that low DD and pH chitosan exhibit enhanced antibacterial activity, though chitosan isolated in this study had a relatively high DD.

Conclusion

The purity level of chitosan and its physicochemical properties affect its biological parameters such as biodegradability, biocompatibility and antimicrobial activity. These physicochemical characteristics are influenced by raw materials and the method used in chitosan isolation. Most literature documented shrimp shells and other crustaceans as the main raw materials for high grade chitosan. Basing on this background, this study isolated chitosan from banana weevils, mushrooms and Nile perch scales. Chitosan isolated from the locally available materials exhibited high DD and other physicochemical properties corroborating with commercial chitosan (Sigma Aldrich) but with moderate solubility and no antibacterial activity. Therefore, attempts should be made to improve the chitosan isolation methods so that the DD and solubility are further increased while protein contaminants are completely eliminated. This should result into optimal chitosan isolation suitable for pharmaceutical and biomedical applications. Furthermore, the cell membrane of bacterial cells is negatively charged. Thus, the zeta potential of chitosan intended for antibacterial application should be determined as only positively charged materials with a pH lower than 6.5 interact with the cell membrane.

Abbreviations

HCL
Hydrochloric acid
NaOH
Sodium Hydroxide
FTIR
Fourier Transform Infrared Spectroscope
XRD
X-Ray Diffraction
DD
Degree of Deacetylation
C-2
Carbon atom in position two
R-NHCOCH3
Acetyl residue
UNISA
University of South Africa
KBR
Potassium Bromide
CFU
Colony forming unit
NS
Nile perch scales
BW
Banana weevils
MSR
Mushroom
2θ
Two theta
ANOVA
Analysis of variance

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Availability of Data and Materials:

All relevant data has been submitted with the manuscript and therefore no supplementary data

Competing interests

The authors declare that they have no competing interests

Authors' contributions

This work was carried out in collaboration between all authors. Denis K. Byarugaba (DKB), Eddie Wampande (EW), Kirabira John Baptist (JB), Edward Nxumalo (EN), Malik Maaza (MM) and Francis Ejobi (FB) conceptualized and designed the study. Kenneth Ssekatawa (KS), Tlou N. Moja (TNM) and Juliet Sackey (JS) executed the laboratory work. KS, JB, TNM and JS analyzed the data. KS and JB wrote the first draft of the manuscript and managed manuscript revisions. All authors read and approved the final manuscript.

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References

1. Rudall KM and Kenchington W. The chitin system. *Rev.* **1973**, *48*, 597–633.
2. Nitschke J, Altenbach H-J, Malolepszy T and M¨olleken H. “A new method for the quantification of chitin and chitosan in edible mushrooms,” *Carbohydrate Research*, vol. 346, no. 11, pp. 1307–1310, 2011.
3. Berger LRR, Cardoso A, Stamford TCM, Cavalcante HMM, Macedo RO and Campos-Takaki GM. “Agro-industrial waste as alternative medium in the production of chitin and chitosan by *Rhizopus arrhizus*—a factorial design,” *Asian Chitin Journal*, vol. 7, pp. 83–90, 2011.
4. Gortari MC and Hours RA. “Biotechnological processes for chitin recovery out of crustacean waste: a mini-review,” *Electronic Journal of Biotechnology*, vol. 16, no. 3, article 14, 2013.
5. Younes I and Rinaudo M. “Chitin and chitosan preparation from marine sources. Structure properties and applications,” *Marine Drugs*, vol. 13, no. 3, pp. 1133–1174, 2015.
6. Ong SY, Wu J, Moochhala SM, Tan MH and Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. *Biomaterials* **2008**, *29*, 4323–4332.
7. Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, Galed G and Heras A. Functional characterization of chitin and chitosan. *Chem. Biol.* **2009**, *3*, 203–230.
8. Zhao L-M, Shi L-E, Zhang Z-L, Chen J-M, Shi D-D2, Yang J. and Tang Z-X. 2011. Preparation and application of chitosan nanoparticles and nanofibers. Brazilian Journal of Chemical Engineering, Vol. 28, No. 03, pp. 353 – 362.
9. Pillai CKS, Paul W and CP. Sharma, “Chitin and chitosan polymers: chemistry, solubility and fiber formation,” *Progress in Polymer Science*, vol. 34, no. 7, pp. 641–678, 2009.
10. Martin A. Fisheries waste biomass: biconversion alternatives, in Bioconversion of Waste Materials to Industrial Products, Springer, 1998, pp.449–479.
11. Simpson BK, Gagne N, Simpson MV. Bioprocessing of chitin and chitosan,in: Fisheries Processing, Springer, 1994, pp. 155–173.
12. Centre for Disease Dynamics, Economics and Policy (CDDEP). 2015. The state of the world’s antibiotics. Washington DC-New Delhi
13. EFSA (2013), Scientific Opinion on Carbapenem resistance in food animal ecosystems. EFSA Journal ;11(12):3501
14. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012; 18(5):263–72.
15. Musarrat HM, Williams PA, Tverezovskaya O. Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. Food Hydrocoll. 2013;31(2):166–71.
16. Rødde RH, Einbu A, and Vårum KM. A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (Pandalus borealis) Carbohydr. Polym. 71 (2008) 388–393. https://doi.org/10.1016/j.carbpol.2007.06.006
17. Vilar Junior J C, Ribeaux DR, Alves da Silva C A, and De Campos-Takaki GM. 2016. Physicochemical and Antibacterial Properties of Chitosan Extracted from Waste Shrimp Shells. International Journal of Microbiology, 2016, 1–7. doi:10.1155/2016/5127515
18. Moore GK and Roberts GAF. (1980). Determination of the degree of N-acetylation of chitosan. International Journal of Biological Macromolecules, 2(2), 115–116.
19. Marei NH, El-Samie EA, Salah T, Saad GR and Elwahy AHM. 2016. Isolation and characterization of chitosan from different local insects in Egypt. International Journal of Biological Macromolecules, 82, 871–877. doi:10.1016/j.ijbiomac.2015.10.024
20. Erdogan S, Kaya M, and Akata I. 2017. Chitin extraction and chitosan production from cell wall of two mushroom species (Lactarius vellereus and Phyllophora ribis). doi:10.1063/1.4975427
21. Rumengan IFM, Suptijah P, Wullur S and Talumepa A. 2017. Characterization of chitin extracted from fish scales of marine fish species purchased from local markets in North Sulawesi, Indonesia. IOP Conference Series: Earth and Environmental Science, 89, 012028. doi:10.1088/1755-1315/89/1/012028

22. Kaya, Baublys V, Can E. et al. Comparison of physicochemical properties of chitins isolated from an insect (Melolontha melolontha) and a crustacean species (Oniscus asellus). Zoomorphology 133, 285–293 (2014). https://doi.org/10.1007/s00435-014-0227-6
23. Szymańska E and Winnicka Stability of Chitosan—A Challenge for Pharmaceutical and Biomedical Applications. Mar. Drugs 2015, 13, 1819-1846; doi:10.3390/md13041819
24. Nessa F, Masum SM, Asaduzzaman M, Roy S, Hossain M and Jahan M. 2010. A Process for the Preparation of Chitin and Chitosan from Prawn Shell Waste. Bangladesh Journal of Scientific and Industrial Research, 45(4). doi:10.3329/bjsir.v45i4.7330
25. Gocho H, Shimizu H, Tanioka A, Chou TJ and Nakajima T. Effect of polymer chain end on sorption isotherm of water by chitosan. Carbohydr. Polym. 2000, 41, 87–90.
26. Rege PR, Shukla DJ and Block LH. Chitinosans as tableting excipients for modified release delivery systems. Int. J. Pharm. 1999, 181, 49–60.
27. Brine CJ and Austin PR. 1981. Chitin variability with species and method of preparation. Comp. Biochem. Physiol. 69B: 283-286.
28. Davydova VN, Yermak IM, Gorbach VI, Krasikova IN and Solov'eva TF. Interaction of bacterial endotoxins with chitosan. Effect of endotoxin structure, chitosan molecular mass, and ionic strength of the solution on the formation of the complex. Biochemistry (Mosc.) 2000, 65, 1082–1090.
29. Kasaai RM. 2008. A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using infrared spectroscopy. Carbohydrate Polymers 71 (2008) 497–508. doi:10.1016/j.carbpol.2007.07.009
30. Pongpiachan S. (2014). FTIR Spectra of Organic Functional Group Compositions in PM2.5 Collected at Chiang-Mai City, Thailand during the Haze Episode in March 2012. Journal of Applied Sciences, 14: 2967-2977. DOI: 10.3923/jas.2014.2967.2977
31. Silverstein RM, Bassler GC. and Morrill TC. (1981). Spectrometric Identification of Organic Compounds. 4th Edn., John Wiley and Sons, New York, USA.
32. https://www.sigmaaldrich.com/technical-documents/articles/biology/ir-spectrum-table.html accessed on 28/11/2019
33. http://www.ifsc.usp.br/~lavfis2/BancoApostilasImagens/ApLuminescencia/IR_spectroscopy_LM.pdf accessed on 28/11/2019
34. https://www.thermofisher.com/blog/materials/a-gift-for-you-an-ftir-basic-organic-functional-group-reference-chart/
35. https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry(Wade)/11%3A_Infrared_Spectroscopy_and_Mass_Spectrometry accessed on 28/11/2019
36. Liu H, Du Y, Yang J, and Zhu H. "Structural characterization and antimicrobial activity of chitosan/betaine derivative complex," Carbohydrate Polymers, vol. 55, no. 3, pp. 291–297, 2004.
37. Santos MC, Cirilo Oliveira AN, and Nunes ML. "Determination of degree of deacetylation of chitosan obtained from "shrimp saburica" (Macrobrachium jelskii)," Scientia Plena, vol.1877, p. 7.9, 2011.
38. Ishii D, Ohashi C, and Hayashi H. "Facile enhancement of the deacetylation degree of chitosan by hydrothermal treatment in an imidazolium-based ionic liquid," Green Chemistry, vol. 16, no. 4, pp. 1764–1767, 2014.
39. Kurita K, Kaji Y, Mori T and Nishiyama Y. Enzymatic degradation of β-chitin: Susceptibility and the influence of deacetylation. Carbohydr. Polym. 2000, 42, 19–21.
40. Kumar S, Dutta PK. and Koh J. 2011. A physiocochemical and biological study of novel chitosan-chloroquinoline derivative for biomedical applications. Int. J. Biol. Macromol. 2011, 49, 356–361.
41. Anand M, Sathyapriya P, Maruthupandy M. and Hameedha-Beevi A. 2018. Synthesis of chitosan nanoparticles by TPP and their potential Mosquito Larvicidal application. Frontier in Laboratory medicine 2(2018); 72-78. http://doi.org/10.1016/j.flm.2018.07.003
42. Kumar S. and Koh J. 2012. Physiochemical, Optical and Biological Activity of Chitosan-Chromone Derivative for Biomedical Applications. Int. J. Mol. Sci. 2012, 13. DOI: 10.3390/ijms13056102
43. Green S, Roldo M, Douroumis D, Bourpoulos N, Lamprou D. and Fatouros DG. 2009. Chitosan derivatives alter release profiles of model compounds from calcium phosphate implants. Carbohydrate Research 344 (2009); 901-907. Doi:10.1016/j.carres.2009.02.022
44. Akncbay H, Senel S and Ay ZY. Application of chitosan gel in the treatment of chronic periodontitis. J. Biomed. Mater. Res. B Appl. Biomater. 2007, 80, 290–296. Mar. Drugs 2015, 13 1841
45. Kong M, Chen XG, Xing K and Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int. J. Food Microbiol. 2010, 144, 51–63.
46. Palmeira-de-Oliveira A, Ribeiro MP, Palmeira-de-Oliveira R, Gaspar C, Costa-de-Oliveira S, Correia IJ, Pina V, Martinez-de-Oliveira J, Queiroz JA and Rodrigues AG. Anti-Candida activity of a chitosan hydrogel: Mechanism of action and cytotoxicity profile. Gynecol. Obstet. Invest. 2010, 70, 322–327.
47. Wang G. "Inhibition and inactivation of five species of foodborne pathogens by chitosan," Journal of Food Protection, vol.55, no. 11, pp. 916–925, 1992.
48. Zhao D, Yu S, Sun B, Gao S, Guo S and Zhao K. 2018. Biomedical Applications of Chitosan and Its Derivative Nanoparticles. Polymers, 10(4), 462. doi:10.3390/polym10040462

Figures

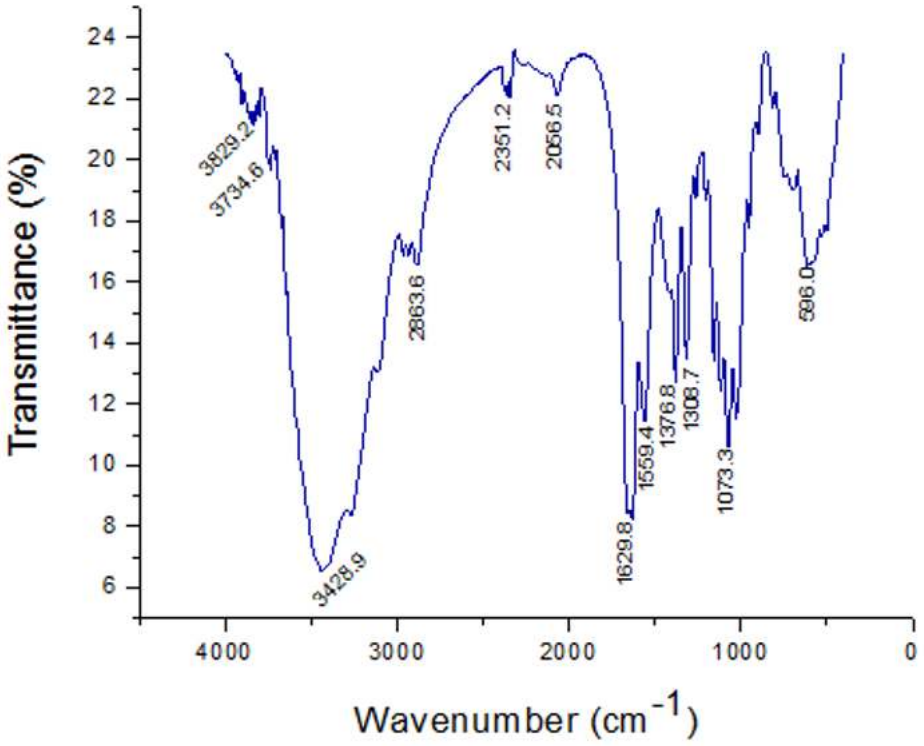

Figure 1

FTIR spectrum for banana weevil chitosan

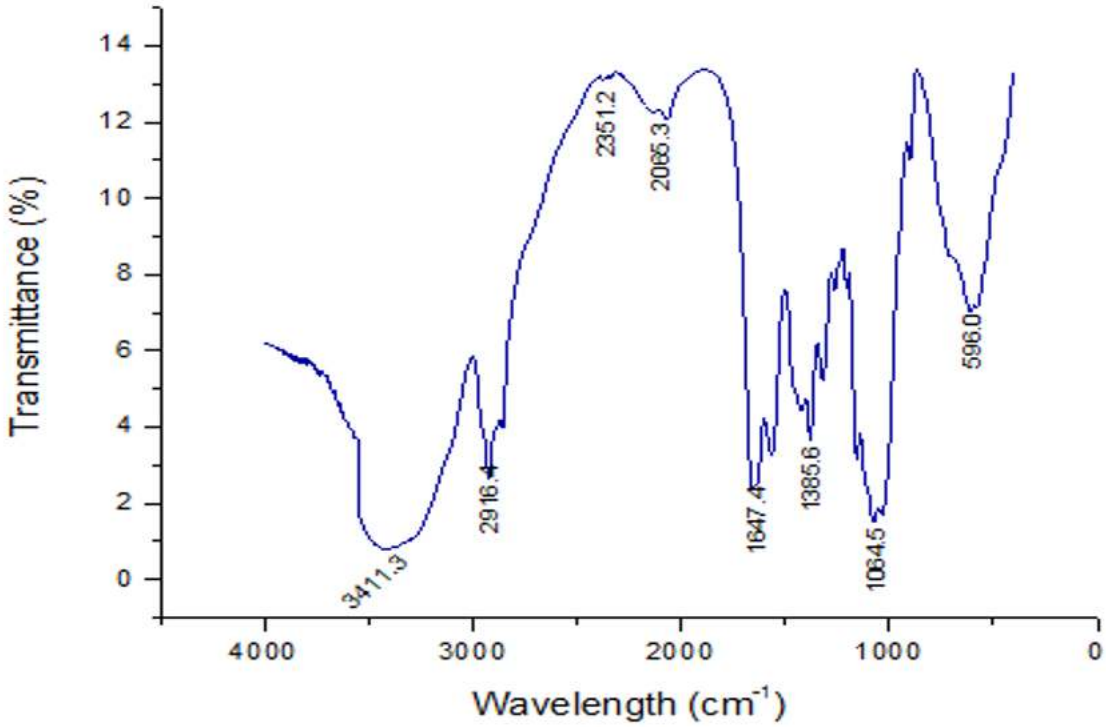

Figure 2

FTIR spectrum for mushroom chitosan

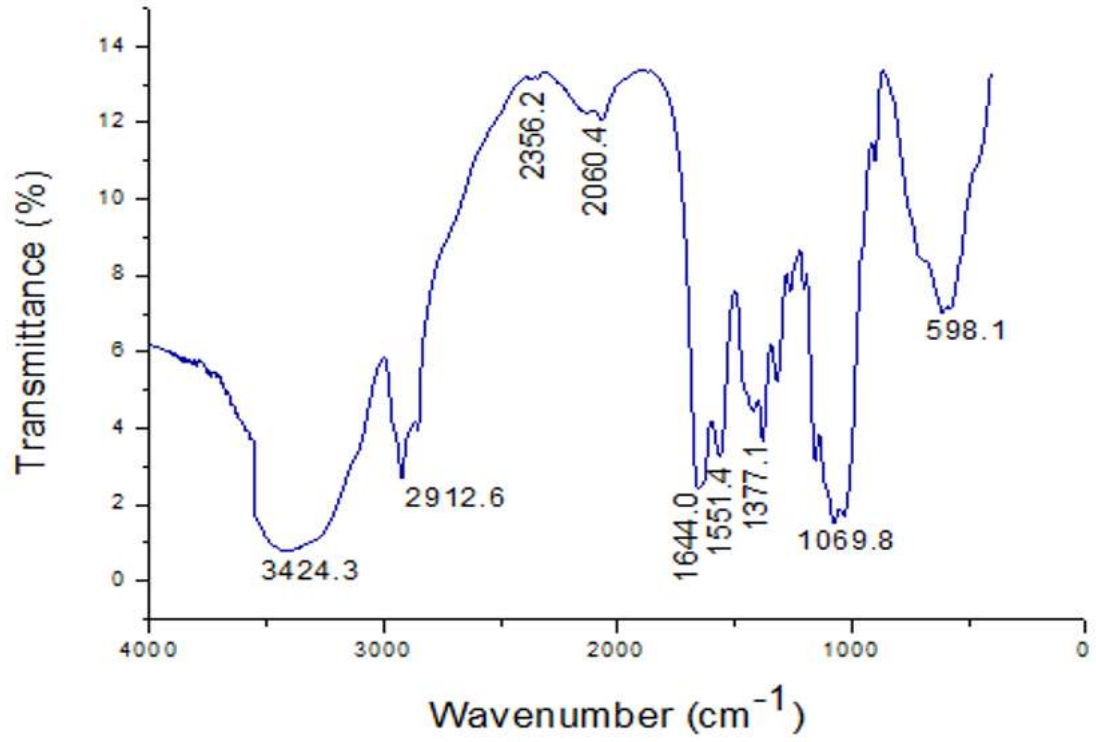

Figure 3

FTIR Spectrum for Nile perch scale chitosan

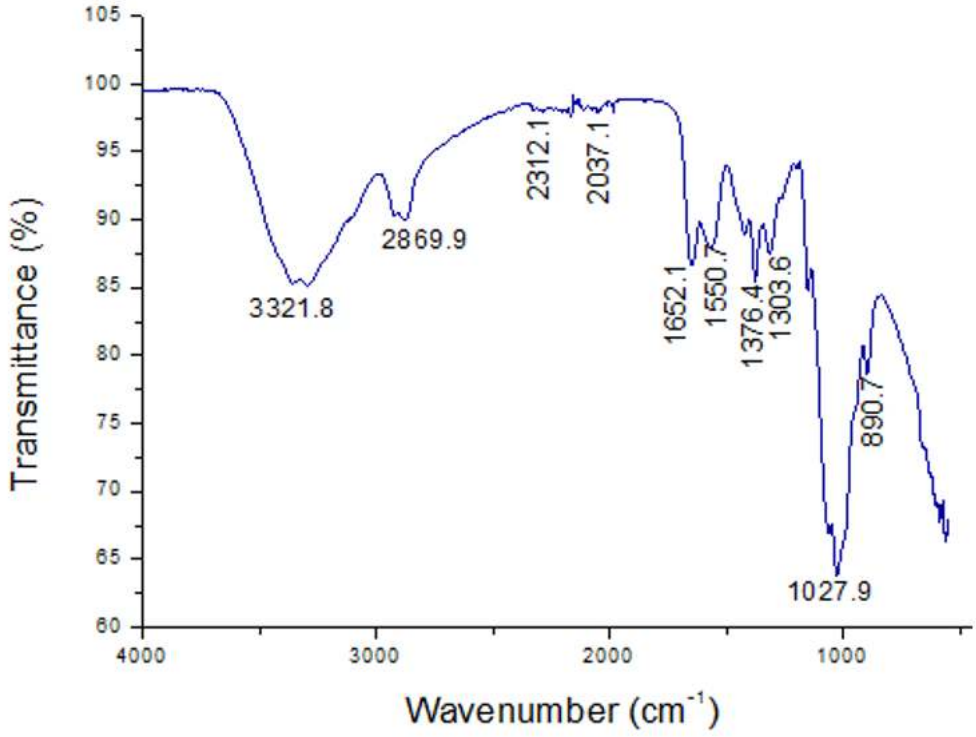

Figure 4

FTIR Spectrum for commercial chitosan

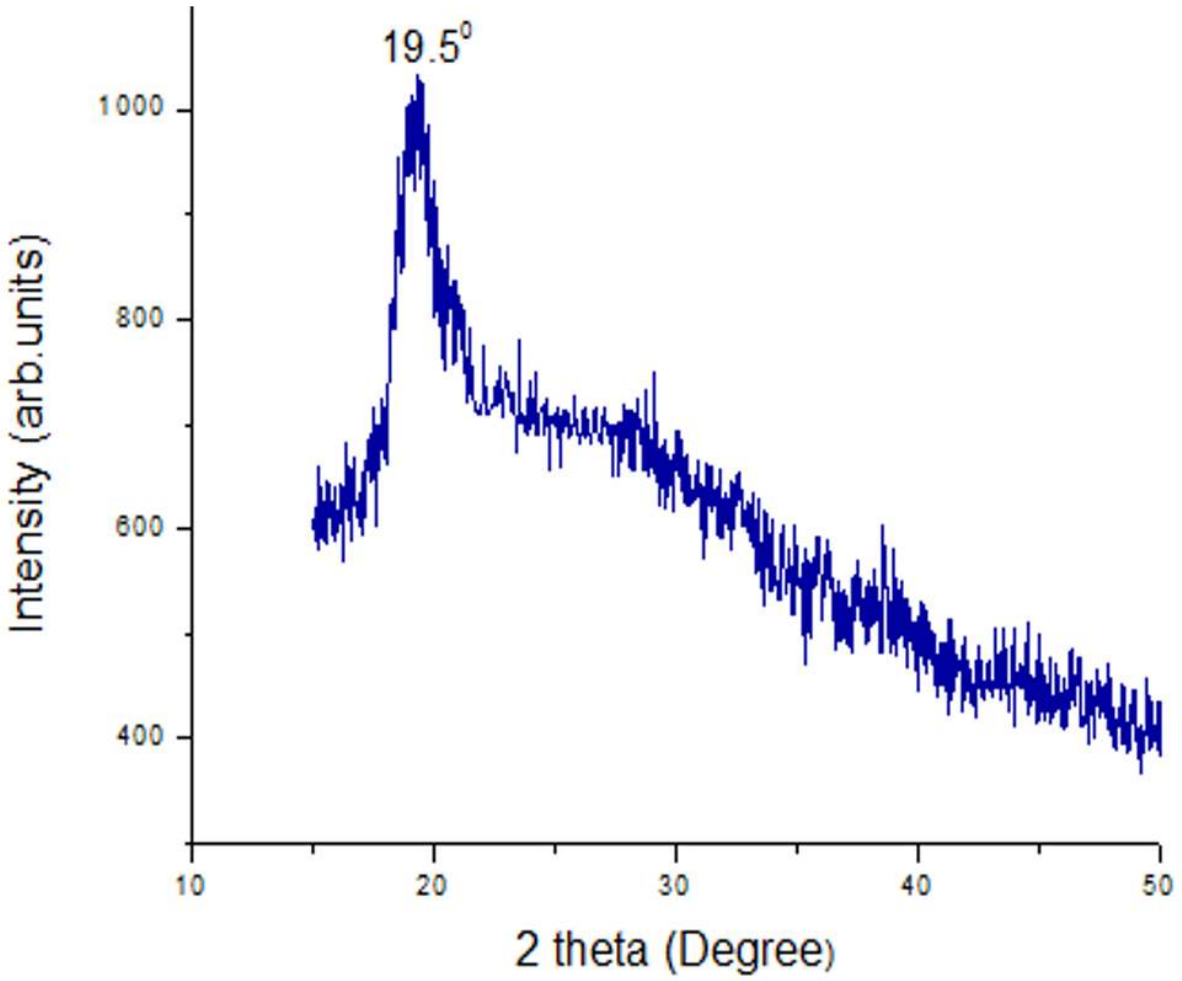

Figure 5

XRD pattern for banana weevil chitosan

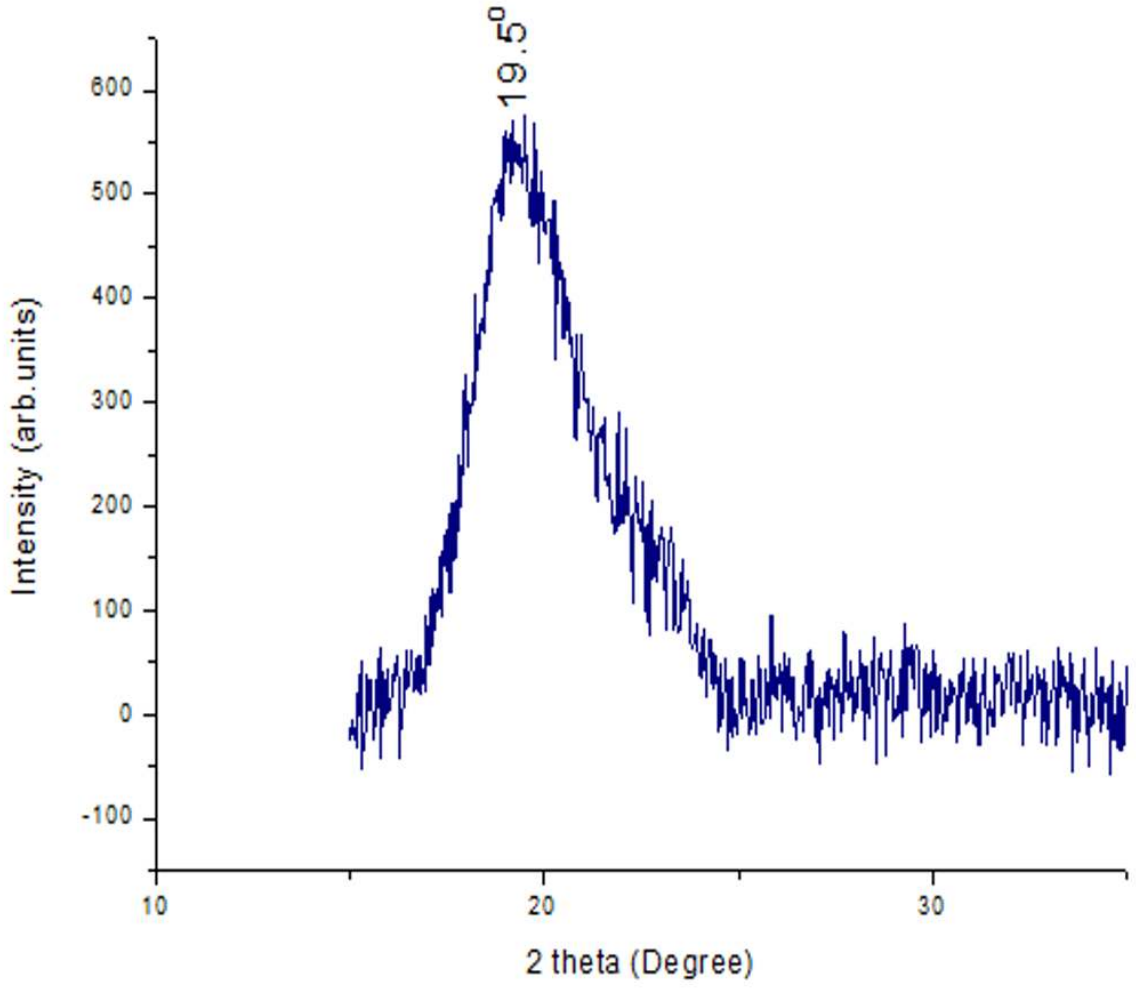

Figure 6

XRD pattern for mushroom chitosan

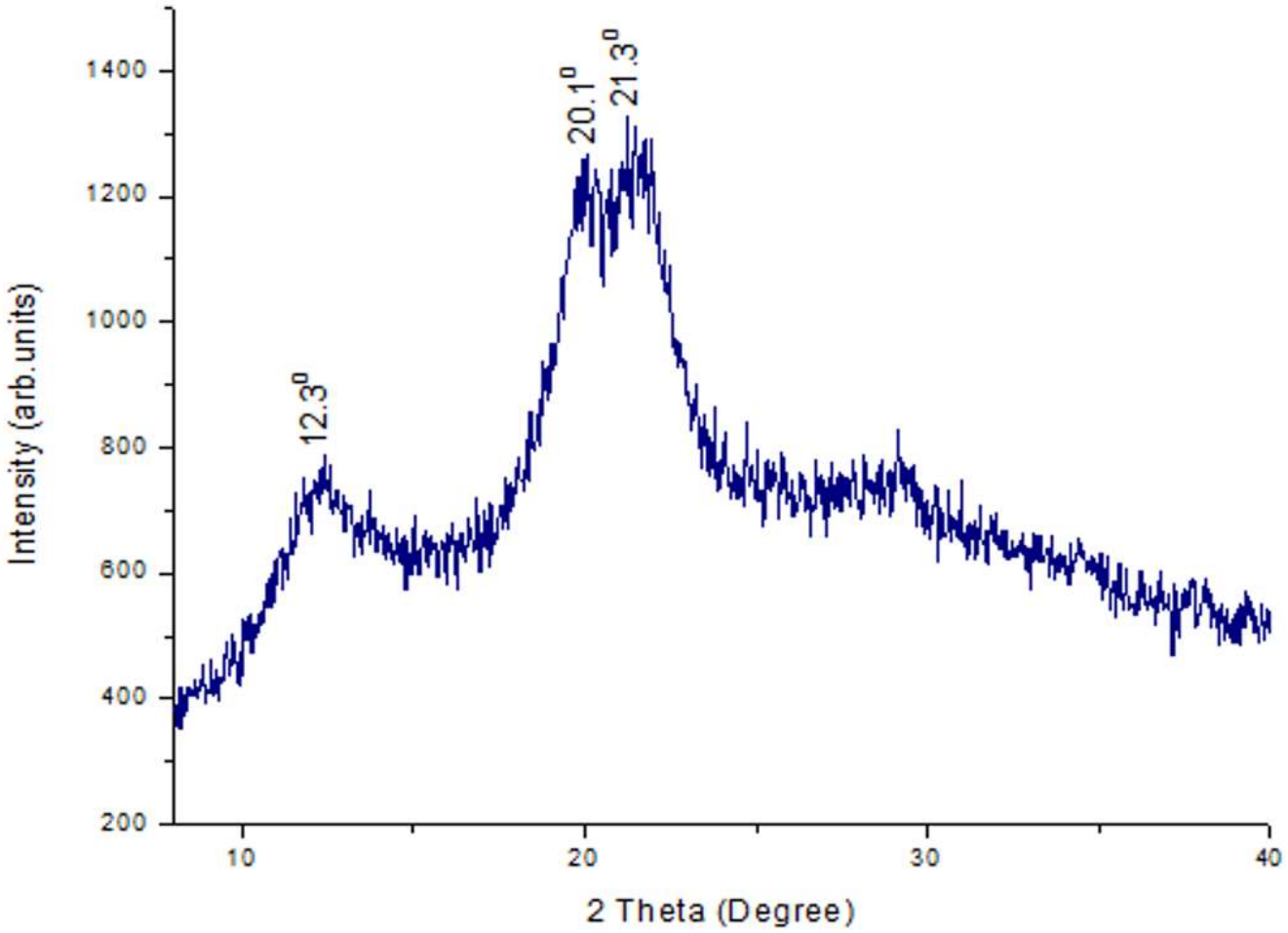

Figure 7

XRD pattern for Nile perch scale chitosan

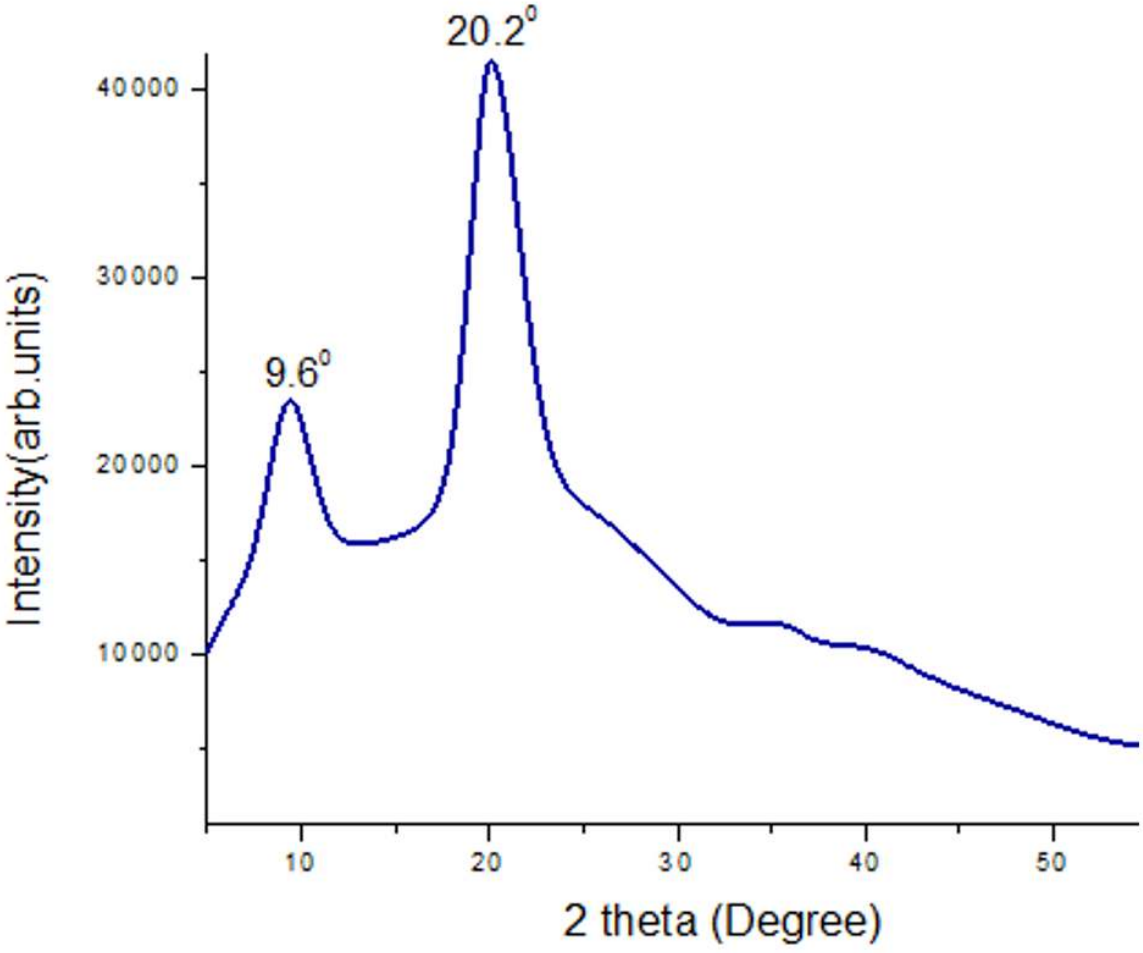

Figure 8

XRD pattern for commercial chitosan