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Preparation and Characterisation of Shrimp Waste-Derived Chitin, Chitosan and Modified Chitosan Films

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ABSTRACT

Chitin was extracted from shrimp waste through the process of demineralisation and deproteination by dilute HCl solution and dilute NaOH solution respectively. The chitin obtained was subsequently converted to chitosan by the process of deacetylation with the use of concentrated NaOH. The FTIR (Fourier-transform infrared spectroscopy) spectra indicated the amide band split characteristic of chitin and chitosan. Chitosan edible films were prepared with chitosan, starch, glycerol and ginger extract at varying concentrations. The interactions between the major components were assessed by FTIR. The percentage yield of chitin and chitosan with respect to dry weight of shrimp waste were 8.77% and 6.20% respectively. Other parameters like moisture content and ash content of chitosan were 2.81% and 1.32% respectively. The FTIR and viscosity of the chitosan were also analysed. The degree of deacetylation using FTIR method was 77.20% and the intrinsic viscosity and the viscosity average molecular weight of chitosan were 0.3346 dl/g and 8,349.40 g/mol respectively.

Keywords: Chitin, chitosan, chitosan edible films, ginger extract.

INTRODUCTION

Chitin is a biopolymer of N-acetylglucosamine, abundant in invertebrates (crabs, shrimps, crawfish, and snails) and fungi. It is an important structural component of arthropods [1]. It is extracted from natural sources by the process of deminerisation and deproteination. Chitin can be further processed to obtain chitosan. Chitosan, a modified natural carbohydrate polymer, is obtained by the partial deacetylation of chitin. There are more than 200 potential applications of chitin, chitosan and other derivatives [2].

Chitosan is a linear polysaccharide made up of (1-4)-linked 2-amino-2-deoxy-β-D-glucopyranose [3]. The amino ($-NH_2$) group when protonated to $-NH_3^+$ readily forms electrostatic interactions with anionic groups in an acid environment. Chitosan is useful in wide

applications in various spheres such as pharmaceuticals, biochemistry, biotechnology, cosmetic, biomedical, paper industry, food and textile industries and others [4].

In the quest to attain food security and sustainability, there is a growing interest in the development of natural biopolymers. Natural biopolymers as opposed to some synthetic materials used in food preservation are important due to their renewability, sustainability and biodegradability. The desirability to prolong the shelf life and enhance food quality as well as to minimise packaging waste have led to the exploration of new bio-based packaging materials, like edible and biodegradable films from renewable resources [5].

Edible films, according to Mokrejs*et al.*[6], are defined as a thin layer of material which can be consumed and provides a good barrier to moisture, oxygen and solute movement for the food. Edible films are non-toxic, non-polluting biodegradable natural biopolymers, such as polysaccharides (e.g. chitosan, cellulose, starch) and proteins (e.g. paraffin wax, beewax, candelilla wax) [7].

The aim of this research was to produce and characterise chitosan and chitosan ediblefilms from shrimp waste. The aim was achieved through the following objectives:

1. To extract chitin from shrimp waste;
2. To produce chitosan from the extracted chitin;
3. To characterise chitin and chitosan;
4. To produce protective chitosan-derived edible films incorporated with ginger extract; and
5. To characterise chitosan edible films.

MATERIALS AND METHODS

Materials

Some of the major reagents used include: Sodium hydroxide, 97.5%, (BDH Laboratory Supplies, United Kingdom), hydrochloric acid, 36.5% (Sigma-Aldrich Chemical Company, United Kingdom), acetic acid 99.7% (Sigma-Aldrich Chemical Company, United Kingdom), sodium acetate 99.1% (BDH Laboratory Supplies, United Kingdom), potassium bromide 99.2% (Sigma-Aldrich Chemical Company, United Kingdom), glycerol, 99.5% (Sigma-Aldrich Chemical Company, United Kingdom) starch and ginger (locally sourced).

Sample Preparation

The shrimp waste was washed thoroughly with water and dried in an oven at 60^{o}C to a constant weight. The dried shrimp was then ground to fine particles. Then 500 g of the sample was taken out for demineralization and subsequent deproteination according to Abdou*et al.*[8].

Demineralization

The ground sample was treated with 1 M HCl acid bathes (1:15 w/v) at ambient temperature (approximately 25^{o}C). The number of bathes depended on the ceasing of the emission of carbon dioxide gas. The sample was then washed with distilled water to a neutral pH and subsequently dried to a constant weight at 30^{o}C [8].

Deproteination

The dried acid treated shrimp shell was weighed and treated in 1 M NaOH solution bathe (1:15 w/v) at a temperature of 100^{o}C. The number of bathes in this case was dependent on the clarity of the solution; a clear solution indicated the absence ofprotein. This process resulted in the production of chitin. The chitin was then washed thoroughly with distilled water to a neutral pH and was subsequently dried to a constant weight at 30^{o}C [8].

Deacetylation

The chitin obtained from the processes of demineralization and deproteination was treated with 50% NaOH (1:15 w/v) at 100^{o}C with the aid of a heating mantle for 4 hours. After this deacetylation step, the obtained chitosan was washed thoroughly with distilled water to a neutral pH and then dried to a constant weight at 55^{o}C according to Puvvada*et al*. [9] with some modifications.

Extraction of ginger

Fresh ginger rhizomes were thoroughly washed with distilled water to remove any contaminants. The non-edible parts were scraped free from the edible parts. Exactly 200 g of the sample were chopped into small pieces and ground to a smaller size with the aid of an electric blender. The ginger extract was obtained by hydrodistillation for 4 hours using a Clevenger apparatus.

Film Formations

Starch solution of 4 g, 2 mL of glycerol (90% v/v), and ginger extract (0.5 mL, 1 mL, 1.5 mL, 2 mL respectively) were blended as shown in Table 1. Each combination was dissolved in 1% chitosan solution of 1% acetic acid to obtain 100 mL of film forming solutions (FFS). FFS were thoroughly mixed with the aid of magnetic stirrer with the stirring speed of 12000 rpm for 30 minutes. The FFS was then heated on a hot plate with a continuous stirring at the temperature of 95^{o}C until the starch gelatinized and continued for ten more minutes. Some portions of gelatinized FFS were poured into plastic Petri dishes of diameter 8.5 cm and oven dried at 30^{o}C for 24 hours according to Sanyang*et al.*[10] with modifications.

Table 1: Compositions of chitosan/ginger extract/starch/glycerol blends

Formulation	Chitosan (%)	Ginger Extract (mL)	Starch (%)	Glycerol (mL)
Fb1	1	2	4	2
Fb2	1	1.5	4	2
Fb3	1	1	4	2
Fb4	1	0.5	4	2
Fb5	1	0	4	2
Fbc	0	0	4	2

Fbc formulation =control

Yield of chitin and chitosan

The weight of each chitin and chitosan samples were taken before and after deacetylation respectively and the percentage of yields were calculated [11].

$$\text{Yield (\%)} = \frac{\text{Weight of chitin or chitosan sample(g)}}{\text{Weight of raw shell sample(g)}} \times 100$$

Moisture content

Moisture content of chitosan samples was determined by gravimetric method as described by AOAC [12]. Percentage moisture content of chitosan samples were calculated according to the following equation:

$$\text{\% moisture content} = \frac{(\text{Wet weight, g} - \text{dry weight, g})}{\text{Wet weight, g}} \times 100$$

Ash value

Ash content of the chitosan samples were performed using standard ashing method [12]. Percentage ash content of chitosan samples were calculated according to following equation:

$$\text{Ash \%} = \frac{\text{Weight of residue or ash(g)}}{\text{Weight of initial chitosan sample (g)}} \times 100$$

Determination of Average Molar Mass of Chitosan

Average molecular weight of Chitosan was determined by viscometric method using Ostwald's Viscometer. The average molecular weight was obtained from Mark-Houwink-Sakurada equation:

$$[\eta] = KM_v^{\alpha}$$

$$Log\,[\eta] = log\,K + \alpha\, log\, Mv$$

where [η] is the intrinsic viscosity, M_v is the average molecular weight of chitosan, K and α are the Mark-Houwink-Sakurada constants specific for a given polymer. For the solvent mixture (0.5 M AcOH – 0.2 M NaOAc), these constants are 3.5×10^{-4} and 0.76, respectively at 25°C.

Intrinsic viscosity was obtained from linear plots of reduced viscosity against concentration (C, g/mL), extrapolating to zero concentration. The viscosity average molar mass (MW) of chitosan was estimated using the Mark–Houwink-Sakurada relationship.

Functional group analysis

The functional group analysis was carried out using FTIR spectroscopy in the range of 4000 – 650 cm^{-1} using Agilent FTIR spectrometer (CARRY 630, Agilent Technology, USA).

Determination of Degree of Deacetylation (DDA)

The FTIR spectra were measured in KBr pellets in the transmission mode in the range 4000 - 650 cm^{-1} using Agilent FTIR spectrometer. The DDA of the samples were calculated from the IR spectra by the method described by Brugnerottoa*et al.*[13].

% DA = $\%DA = \frac{(A_{1320}/A_{1420}) - 0.3822}{0.03133}$

$$\text{Absorbance; } A = 2 - \log(\%T)$$

$$\%DDA = 100 - \%DA$$

Where, DDA = degree of deacetylation, DA = degree of acetylation, A_{1320} =Absorbance band at 1320 cm^{-1}, A_{1420} = Absorbance band at 1420 cm^{-1}, 0.3822 and 0.03133 = correction factor as a result of using KBr, A = Absorbance, %T = Percentage transmittance.

Fourier Transform Infrared (FTIR) Spectroscopy Spectra of the film

FTIR spectra were used to evaluate the compatibility and uniformity of the films. These were obtained in the range of 4000 – 650 cm^{-1} using Agilent FTIR spectrometer (CARRY 630, Agilent Technology, USA).

RESULTS AND DISCUSSION

Results of some physicochemical characterisation (composition of chitin and chitosan in shrimp, moisture content, ash content, degree of deacetylation, viscosity, and average molecular weight) of chitin and chitosan as well as the FTIR analyses of the chitosan edible films are presented in this section.

Chitin and Chitosan yield

The chitin and chitosan percentage yield of shrimp waste is presented in Table 2, with the yield of chitosan less than that of chitin.

Table 2: Chitin and Chitosan Composition in Shrimp

S/N	Components	Composition (%)
1	Chitin	8.77
2	Chitosan	6.20

According to the results presented in Table 2, the chitin and chitosan yield was found to be 8.77% and 6.20% respectively; this is consistent with what was reported by Isa *et al.* [14]but much lower than the range of 13.12%-17.36% reported by Hossain and Iqbal[15] and Hajji *et al.* [16] using different extraction methods. Abdou*et al.*[8] reported the respective yield of 23.72% and 21.53% for chitin and chitosan. The differences in the yields can be attributed to differences in geographical origin of the shrimp and the extraction methods used [8].

The moisture and ash content of chitosan

These parameters and their compositions are presented in Table 3,

Table 3: Moisture and Ash Content of Chitosan

Parameters	**Composition (%)**
Moisture Content	2.81
Ash Content	1.32

The moisture content of the analysed chitosan was 2.81%. The moisture content ranges depend upon the season, relative humidity and intensity of sunlight. The drying methods also play a role. According to previous study carried out by Alishahi *et al* [17], the chitosan sample was initially sundried for 6 hours before drying in the oven, thus resulting in a lower moisture content of the chitosan sample (2.5%). According to experiments performed by Szymańska and Winnicka [18]

the moisture content of chitosan must be below 10% in order to have a greater hydrogen bonding forming capability.

The ash content value was 1.32%. Lower ash value is an indicator of the efficiency of the demineralisation step, which is the removal of calcium carbonate. High quality chitosan has an ash value of <1% [15].

Functional Group Analysis

The FTIR transmittance spectra of the extracted chitin, chitosan and ginger extract samples are shown in Figure 1, 2 and 3 respectively. The key functional groups and their differences between chitin and chitosan are captured in the Figures.

Chitin FTIR Spectral Analysis

Characterization of extracted chitin by FTIR spectrum (Figure 1) shows the major bands of different bonds as presented in Table 4.

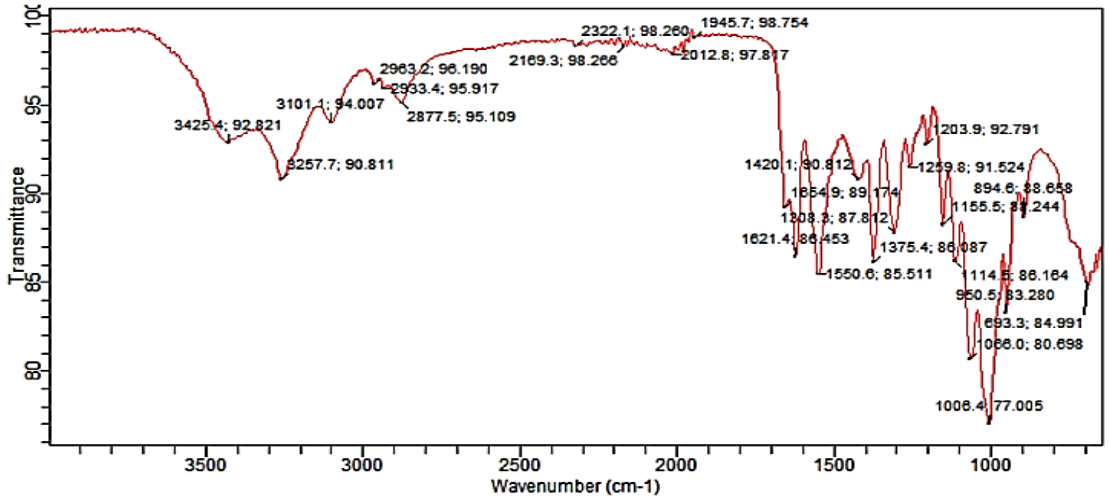

Figure 1: FTIR transmittance spectrum of chitin

Table 4: FTIR Band Assignments for Chitin

Band (cm^{-1})	Assignment
3425.4	vOH
3257.7	$v_{as}NH$
3101.1	$v_{s}NH$
2963.2	$v_{as}CH_3$
2933.4	$v_{s}CH_2$
2877.5	$v_{s}CH_3$
1654.9	vC=O (Amide I)
1621.4	vC=O (Amide I)

1550.6	N-acetyl ester bonds, Amide II
1420.1	OH and CH deformation ring
1375.4	v_sCH_3 symmetrical deformation
1308.3	vC-N + δNH (Amide III)
1259.8	δNH
1203.9	vC-O
1155.5	v_{as}C-O-C (ring)
1114.5	vC-O
1066.0	vC-O
1006.4	vC-O
950.5	$ϒCH_3$
894.6	ϒCH (C1 axial) (β bond)
693.3	ϒNH (Amide V)

In-plane (δ) and out-of-plane modes (ϒ) (bending or deformation vibrations); vibration (v), asymmetric (as), symmetric (s)

As presented in Figure 1 and Table 4, the amide I band is split into two components at 1654.9 cm^{-1} and 1621.4 cm^{-1} which shows α-chitin characteristic bands. 1550.6 cm^{-1} represents the amide II band. The CH deformation of the β-glycosidic absorption band is presented at 894.6 cm^{-1}[11]. In Table 4, 3425.4 cm^{-1} (O-H stretching), 2963.2 cm^{-1}, 2933.4 cm^{-1}, 2877.5 cm^{-1} (CH-stretching), 1654.9 cm^{-1} (Amide I), 1550.6 cm^{-1} (Amide II) and 1308.3 cm^{-1} (Amide III) were assigned. The bands at 1155.5 cm^{-1} (anti-symmetric stretching of the C-O-C bridge), 1114.5 cm^{-1}, 1066.0 cm^{-1} and 1006.4 cm^{-1} (skeletal vibrations involving the C-O stretching) are characteristic of its saccharide structure [19].

Chitosan FTIR Spectral Analysis

Characterization of extracted chitosan by FTIR analysis (Figure 2) shows the major bands assigned to different bonds as presented in Table 5.

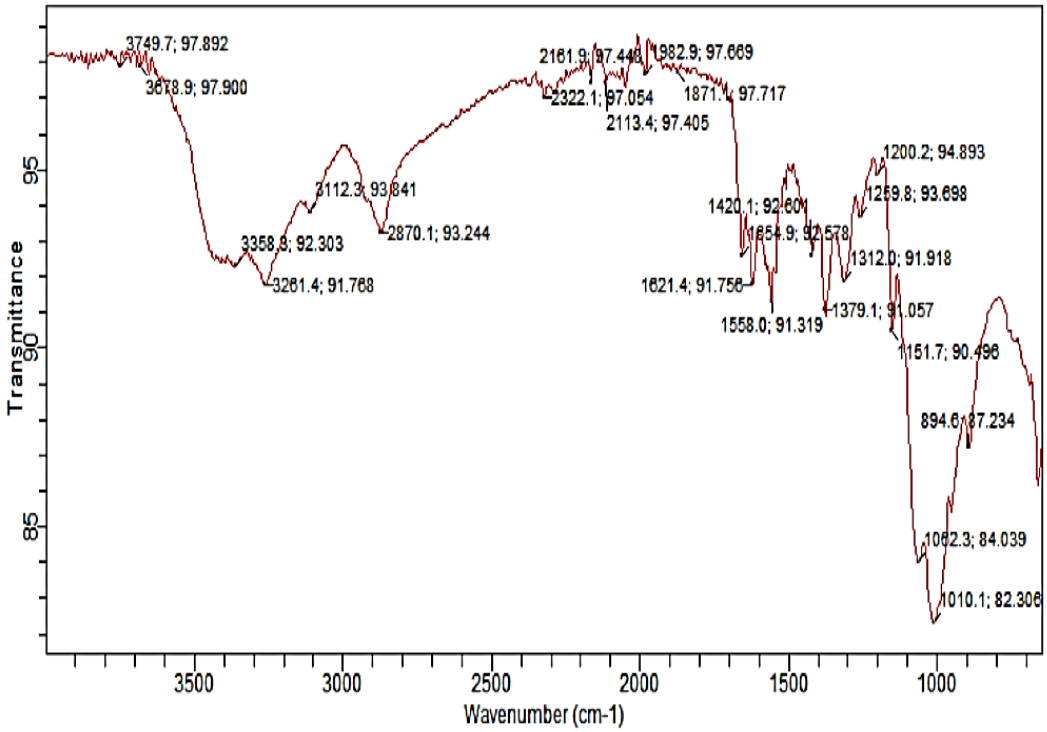

Figure 2: FTIR transmittance spectrum of chitosan

Table 5: FTIR Band Assignments for Chitosan

Band (cm^{-1})	Assignment
3358.3	O-H and N-H stretching
3261.4	NH stretching
3112.3	NH stretching
2870.1	C-H stretch
1654.9	Amide I band
1621.4	C=O stretching amide I
1558.0	Amide II band
1420.1	CH_2 bending and CH_3 deformation
1379.1	CH_3 C-H bend
1312.0	amide III band
1269.8	C-N vibration
1151.7	C-O stretch Alcohols
1062.3	C-O stretch Alcohols
1010.1	=C-O-C symmetric & asymmetric stretch
894.6	=C-H bend Alkenes

The NH stretching bands at 3261.4 cm^{-1} and 3112.3 cm^{-1} shown in Table 5 are associated with CO-NH intermolecular bonding and H bonded NH group. 1654.9 cm^{-1}, 1558.0 cm^{-1} and 1312 cm^{-1} are for amide I, amide II and amide III bands respectively. These observations agree with

the findings ofPuvvada*et al* [9]. The band observed between 1220 cm^{-1} and 1020 cm^{-1} represents the free amino group (-NH_2) at C2 position of glucosamine, a major group present in chitosan. The bands observed at 3358.3, 3261.4, 3112.3, 2870.1, 1654.9, 1621.4, 1558.0, 1420.1, 1379.1, 1312.0, 1269.8, 1151.7, 1062.3, 1010.1 and 894.6 cm^{-1} are in agreement with reports by Ahing & Wid [20] and Puvvada *et al* [9].

FTIR Spectral Analysis of Ginger Extract

The FTIR spectrum of ginger extract (Figure 3) shows the major bands assigned to different bonds as presented in Table 6.

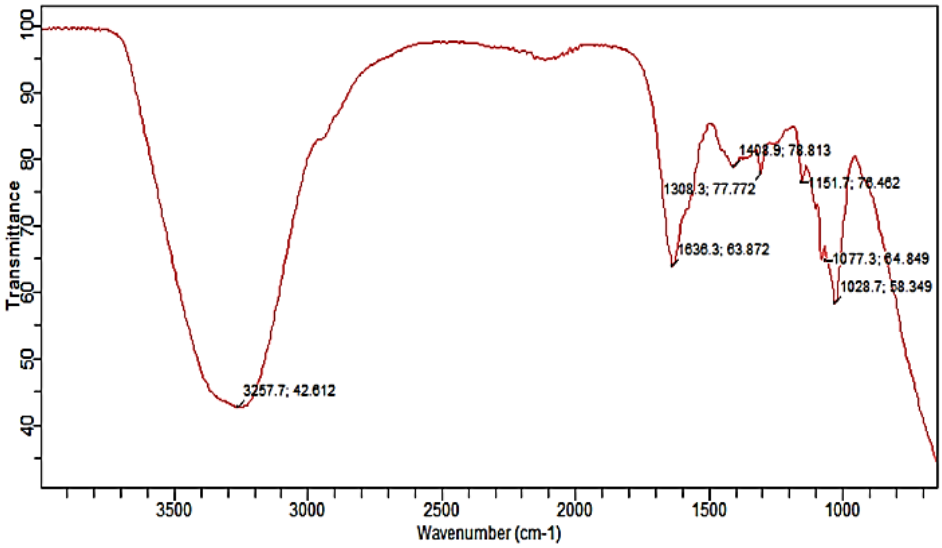

Figure 3: FTIR transmittance spectrum of ginger extract

Table 6: FTIR Band Assignments for Ginger Extract

Band (cm^{-1})	Assignment
3257.7	υ (O-H)
1636.3	δ (H_2O), amide I, υ (C = O) aromatic ring
1408.9	Aromatic skeletal joint with C–H in-plane deformation and stretching
1308.3	aromatic O=C-O-C stretch
1151.7	υ (C = O), υ (C-O-C)
1077.3	υ (C-O-C)
1028.7	Amino acid, δ (C–H) , υ (C-C)

δ, rocking; υ, stretching

Based on Figure 3 and Table 6, the broad band at 3257.7 cm^{-1} is attributed to O-H stretching of hydrogen-bonded hydroxyl groups in ginger structure [21], and could also be attributed to O-H of the residual water left in the extract.

The band appearing at 1636.3 cm^{-1} assigned to amide I band (C-O) was as a result of both a random coil and α-helix conformation [22]. The bands at 1636.3 cm^{-1} (bending), 1408.9 cm^{-1} (aromatic skeletal joint with C-H in-plane deformation and stretching) and 1308.3 cm^{-1} (O=C-O-C stretching) are shown in the spectrum.

Supplementarily, the characteristic deformation band of aliphatic methylene groups of CH_2 was shown at 1408.9 cm^{-1}[23], while the band at 1151.7 cm^{-1} was also typical of the symmetrical vibration of C–O–C [24]; the band at 1028.7 cm^{-1} was attributable to the vibration of the glucose unit ring involving the stretching of C-H and C-C [25].

The different groups for lignin and cellulose with the characteristic bending or stretching vibrations appeared at 1408.9, 1151.7, 1077.5, and 1028.7 cm^{-1}[26].

Determination of Degree of Deacetylation of Chitosan

The FTIR spectrum of chitosan (Figure 2) measured in KBr pellets in the transmission mode in the range 650 – 4000 cm^{-1} was used in the calculation of degree of deacetylation (DDA).

$$\% \text{ DA} = \frac{(A_{1320}/A_{1420}) - 0.3822}{0.03133}$$

Absorbance; A = 2 – log (%T)

Transmittance (%)	Absorbance
91.992	0.03660
92.601	0.03338

$$\% \text{ DA} = \frac{(0.03660/0.03338) - 0.3822}{0.03133}$$

$$\%\text{DDA} = 77.20$$

The degree of deacetylation was calculated using the baseline equation [13]. The wavelengths 1420 cm^{-1} and 1320 cm^{-1} were the baseline bands used in calculating the degree of deacetylation. Using the FTIR spectrum in Figure 3, the DDA obtained from the ratio A_{1320}/A_{1420} was reported to be in agreement with DDA determined using ^{1}H NMR and ^{13}C NMR and positions at 1320 $cm^{-1}$and 1420 cm^{-1} are not affected by humidity [13]. DDA obtained from this study (77.20%) was higher than 50.64% reported by Isa *et al.*[14], 75% by Islam *et al.*[27], but lower than 81.24% and 88% reported by Hossain and Iqbal [15], and Hajji *et al.*[16] respectively.

Abdou *et al* [8] has reported DDA values of 87-97% at varying deacetylation conditions, while Kalut[28] achieved 98.38-98.79%.

Temperature and processing time were found to significantly affect the DDA and MW according to Rege and Block [29]. Chitosan DDA was greatly affected by temperature and repetition of alkaline steps [30]. Effects of time and NaOH concentration on DDA were investigated by Bough *et al.*[31] and Tsaih and Chen [32] worked on the effect of reaction time and temperature. From these reports, MW and DDA of chitosan were significantly affected by NaOH concentration, reaction time, temperature and repetition of alkaline steps.

Average Molar Mass of Chitosan

Viscosity average molar mass of chitosan, which is a measure of the magnitude of solute-solvent attractive interaction was calculated using Mark-Houwink-Sakurada equation.

Table 7: Parameters for Intrinsic Viscosity Measurements

Chitosan Concentration (C) ($x10^3 g/mL^{-1}$)	Time (s)	η_r	η_{sp}	η_{sp}/C (dL/g)
Pure Solvent				
0	267.67	1.000	0.0000	0.00
Solution				
10	459	1.715	0.715	0.7150
6.67	375	1.401	0.401	0.6012
5.00	338.5	1.265	0.265	0.5300
3.33	308.33	1.152	0.152	0.4565

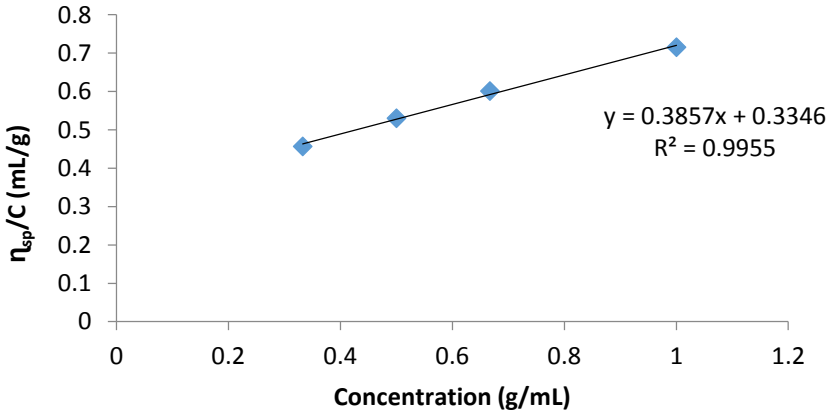

Figure 4: Reduced viscosity and concentration plot for intrinsic viscosity determination

Relative viscosity (η_r) : $\eta_r = t_{solution}/t_{solvent}$

Specific viscosity (η_{sp}) ; $\eta_{sp} = \eta_r - 1$

Intrinsic viscosity ([η]) ; $[\eta] = \eta_{sp}$ at C = 0

$t_{solution}$: flow time of solution

$t_{solvent}$: flow time of solvent

Intrinsic viscosity of chitosan obtained by plotting η_{sp}/C (dL/g) against Concentration (g/mL), and at zero concentration is 0.3346 dl/g (Figure 4).

The average molar mass of chitosan was found to be 8,349.40 g/mol.

The viscosity average molecular weight of chitosan varied with the source, method of preparation, and the purity of the chitosan sample [16]. In the present study, intrinsic viscosity and viscosity average molecular weight of chitosan was 0.3346dl/g and 9748.17 g/mol respectively. These values were lower than those obtained by Hajji *et al.*[16]for shrimp chitosan (1.75 dl/g and 17,030 g/mol for intrinsic viscosity and viscosity average molecular weight respectively). High temperature, concentration of alkali, reaction time, previous treatment of chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress are some of the factors during preparation that may influence the MW of chitosan [15, 33].

FTIR Spectra of Modified Chitosan Films

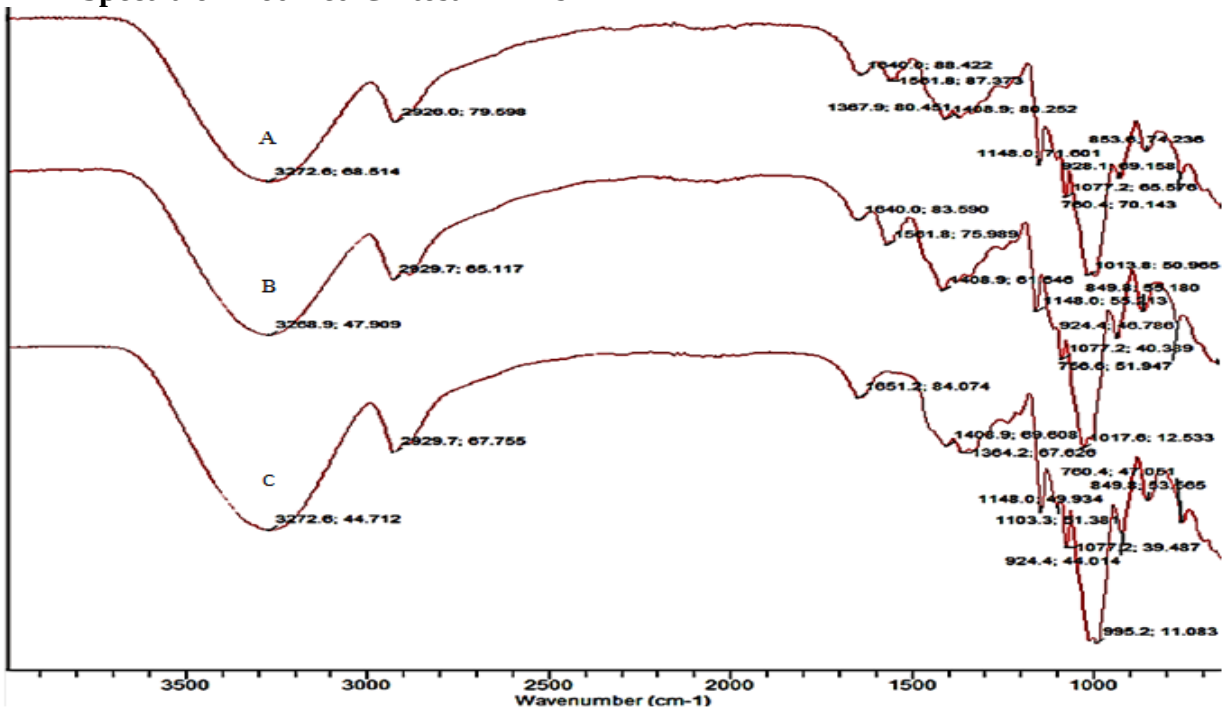

Figure 5: FTIR spectra of chitosan/starch/ginger extract/glycerol blend films ("A" contained all constituents; "B" contained no ginger extract;"C"contained no chitosan or ginger extract)

To investigate the interactions of the constituents of the blend, FTIR spectroscopy was used. In Figure 5, "A" represents the film composed of chitosan, starch, glycerol and ginger extract, and "B" is the spectrum of film comprising of all the constituents except ginger extract, while C shows the spectrum of the film without chitosan and ginger extract.

Pure chitosan has four main distinctive bands. Firstly, broad band ranging from around 3500–3100 cm^{-1} which is attributed to N–H and OH–O stretching vibration. The intermolecular hydrogen bonding of chitosan molecules also, to a certain degree, plays a role in the absorption at this band [34]. Secondly, band located at 2877.5 cm^{-1} is attributed to CH stretching [35]. Thirdly, the band at 1654.9 cm^{-1} is assigned to amide-I band [36]. Lastly, the band around 1580.4 cm^{-1} is the amide-NH_2 band [37].

N-H and OH….O stretching vibrations, and intermolecular hydrogen bonding of chitosan molecules were detected as a broad band around 3500-3100 cm^{-1}[34, 38]. Band between 2929.7 – 2922.2 cm^{-1} was from CH stretching; the bands at 1632.6 cm^{-1} and 1640 cm^{-1} were assigned to amide I [39]. Band at 1408.9 is associated with CH_2 bending and CH_3 deformation, and band at 1543.1 cm^{-1} is assigned to amide II which is noticeably absent in the spectra "C", due to the absence of chitosan in the films.

The observable changes in the spectral peak wavenumbers can be attributed to the interaction taking place in a definite system [40]. As shown in Figure 5, compared to the IR spectra of reference samples (pure chitosan film), the shifts from lower to higher wavenumber and vice versa, indicates that interactions have taken place.

In assessing the contribution of ginger extract to the films, the major percentage transmittance bands highlighted in the ginger FTIR spectrum (Figure 3) at 3257.7, 1636.3 1408.9, 1308.3, 1151.7, 1077.3 and 1028.7 cm^{-1}, seem to correspond to the signal peaks of the other components of the blend as seen in the FTIR of other films without ginger extract; as such, signal peaks of ginger extract blended seamlessly into the signal peaks of the other constituents of the films.

CONCLUSION

Chemical method was used to extract chitin and chitosan from shrimp waste sourced locally. The physicochemical properties (moisture content, ash content, the intrinsic viscosity and the viscosity average molecular weight of chitosan) were 2.81%, 1.32%, 0.3346 dl/g and 8,349.40 g/molrespectively. The FTIR spectra of the prepared chitin and chitosan gave characteristic bands of amide I and II associated to chitin and chitosan; with degree of

deacetylation of 77.20%.Edible films comprising of chitosan, starch, glycerol and ginger extract mixed at varying proportions were prepared and analysed. FTIR was used to determine the functional group interactions between the matrix and the added agents.

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