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Extraction of Chitosan from *L. squarrosulus* (Mushroom) and *C. africana* (Shrimp) Based and Production of Film Polymer

Ovonramwen, O. B., Amoren, E. I.

Department of Chemistry,
Faculty of Physical Sciences,
University of Benin,
Benin city, Nigeria

E mail: oluwaseyi.ovonramwen@uniben.edu

Abstract

The deacetylated form of chitin presents enormous areas of application. The research work was aimed at the production of polymer film by the extraction of chitosan from Lentinus squarrosulus (mushroom) and Caridina africana (shrimp) using a chemical process involving deproteinisation, demineralisation, and deacetylation. The two samples were thoroughly washed with distilled water to remove impurities, then dried at room temperature for three days. The mushroom was grounded to powder, deproteinised at varying concentrations (1, 2, 3, and 4 M) and temperatures (60, 80, and 100 °C). The emanating products were now demineralised and deacetylated at 3 M and 60 °C. Biuret test analysis was carried out on 3 M, 60 °C and 4 M, 100 °C of L. squarrosulus chitosan and C. africana with the later showing a clearer positive result for the application. The physicochemical and mechanical properties of chitosan composite films of C. africana and gelatinous composition (CS30: G70, CS50: G50, and CS70: G30) plasticised with glycerol were studied. The chitosan composite films, L. squarrosulus (3 M, 60 °C), and C. africana were characterised by FT-IR. The FTIR spectra showed the functional groups associated with the various bands, intensities, and stretching established that the samples were chitosan. The results of the flexibility and refractive index analysis indicated that the higher chitosan films were more flexible and refractive, finding a much greater application in the manufacture of lenses of higher magnification. The FTIR spectra of the compact films of the homogeneous structure showed that there was a reaction in C. africana chitosan. The SEM analysis of chitosan in C. africana showed morphological differences of ×300, ×500, ×1000, and ×1500.

Keywords: Extraction, *Lentinus squarrosulus*, *Caridina africana*, Chitosan, Films

INTRODUCTION

Second only to cellulose in terms of abundance is chitin (CHI), the primary component of chitosan (CS) with one hydroxyl group on each monomer replaced with an acetylamine group (Hosney *et al.*, 2022). CHI has a chemical structure that is comparable to cellulose (Figure 1). CHI is sometimes mistaken for a cellulose derivative, but it is not found in cellulose-producing organisms (Lodhi *et al.,* 2014). The word 'chitin' comes from the Greek word '*chiton*,' which means a coat of mail (El-Diasty *et al.,* 2012). For the ever-increasing need for this polymer, sustainability is provided by the production of CHI and its derivatives from renewable resources. According to estimates, CHI is created almost as frequently as cellulose each year. Because of their distinctive characteristics, it has gained a lot of attention as an underutilised resource as well as a new functional biomaterial with tremendous promise in numerous industries. Since it is a linear homopolymer of *N*-acetylglucosamine ($[C_8H_{13}O_5N]_n$, where

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*Author for Correspondence

n>>>1), it has the simplest structure of all the glycosaminoglycans (Feas *et al.,* 2020). Alga (Rahman and Halfar, 2014; Rahman *et al.,* 2019), fungus (Baeva *et al.*, 2019), arthropods like insects, crabs, shrimps, and crayfish (Eddya *et al.*, 2020; Poerio *et al.*, 2020; Jabeen *et al.,* 2021), copepods and mollusks (Duan *et al.,* 2018), are the principal hosts of CHI. The source has an impact on how well CHI can be isolated from various sources (Abdou *et al.,* 2008). Various techniques for extracting and converting CHI into CS have been documented due to its insolubility under physiological circumstances. Thermodynamic, chemical, and biological processes are among them (Abdou *et al.,* 2008; Hahn *et al.,* 2020). The most popular CHI derivative, CS, is mostly produced through non-enzymatic *N*-deacetylation. The acetyl residue (R-$NHCOCH_3$) is removed in order to do this with the use of a powerful alkali and high temperatures (Rujiravanit *et al.,* 2020). Enzymatic techniques can also be used to create CS. However, enzymatic deacetylation of CHI is not widely used because of the high cost of deacetylases and their low production of CS (Gortari and Hours, 2013; Younes and Rinaudo, 2015).

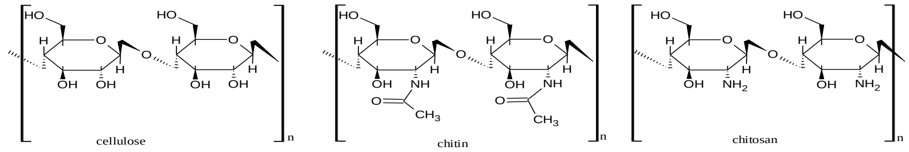


Figure 1: Chemical structure of cellulose, chitin and chitosan.

The useful biochemical and physiological features of CS have recently attracted a lot of interest in the biomedical field. Due to its exceptional qualities when interacting with the human body, CS has a wide range of applications in the field of biomaterials (Feas *et al.*, 2020). These include bioactivity (Sultankulov *et al.*, 2019), antimicrobial and antifungal activity (Deka *et al.*, 2015; Verlee *et al.*,2017), immunestimulation (Torres *et al.*, 2019), chemotactic action, enzymatic biodegradability, muco-adhesion, and epithelial permeability (Ways *et al.*,2018), which promote the adhesion and proliferation of various cell types (Laroche *et al.*, 2018). To expand its potential applications, CS has recently been created in a number of different forms for wastewater treatment, including films (Frantz *et al.*, 2017; Rizzi *et al.* 2018), microcapsules (Tong, 2017), composites (Xie *et al.*, 2013; Duan *et al.*, 2022), nanoparticles (Sivakami *et al.*, 2013), and nanofibers (Nthunya *et al.,* 2017). CS can be utilised efficiently as a film-forming material for food packaging (Wang *et al.,* 2018), wound dressing (Liu *et al.,* 2018), and medication delivery applications (Elassal and El-Manofy, 2019) due to its superior film-forming characteristic.

CS films has wider range of uses, however, were constrained by the fragility of the film. To increase the flexibility of the film, blending is an easy and practical way (Singh *et al.,* 2015). CS has been combined with a number of plasticisers, including fatty acids, polyethylene glycol, glycerol, sorbitol, and erythritol (Jridi *et al.*, 2014; Duan *et al.*, 2022). The flexibility of the chitosan films may differ depending on the types and quantities of plasticisers utilised. However, the other plasticisers for enhancing chitosan film flexibility are remain intriguing (Niamsa and Baimark, 2009). Other distinguishing characteristics of CHI and CS include the ability to create polyoxysalts, biocompatibility, biodegradability, non-toxicity, molecular adsorption capabilities, and so on. CHI and CS are antibacterial and moisturising agents that are biocompatible, biodegradable, and non-toxic biopolymers (Jayakumar *et al.*, 2011).

Therefore, in the present study, we extracted CS from plant and animal based sources, deproteinated the previous at different reaction conditions (concentrations and temperatures) to optimise the reaction. Further steps were carried out and used the purest of the CS from both sources in the production of film of higher quality that could improve the quality of food and safety.

MATERIALS AND METHODS

COLLECTION/PREPARATION OF SAMPLES

The plant *(L. squarrosulus* (Mont Singer)) sample was collected from Afuze market, Owan East Local Government Area, Edo State, Nigeria. The shrimp (*C. africana*) head shell waste was collected from Yanga market located on latitude 6.33594 and longitude 5.618109, Benin City, Edo State in January 2022. The mushroom was identified as *L. squarrosulus* (Mont Singer) by Dr. A. T. Dania, in the Department of Plant Biology and Biotechnology, with the voucher No ACMRTI/002 and the shrimp was identified as *C. africana* (Kingsley 1882) by Dr. S. Ikhuoriah, in the Department of Animal and Environmental Biology both of the Faculty of Life Science, University of Benin, Benin City, Nigeria. Both samples were washed thoroughly with distilled water to remove any impurities and dried at ambient temperature for three days, the mushroom sample was grinded into small size pieces (powder) with a grinding machine at Uselu (postal code 300212) market, Benin City, and packaged in a plastic container.

EXTRACTION OF CHITOSAN FROM SAMPLES

The method of extraction of chitin and chitosan by chemical means implementation involves (Figure 2):

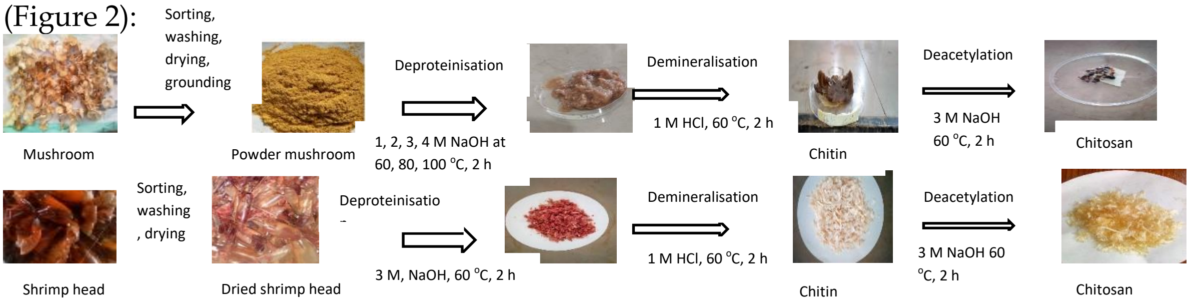


Figure 2: Scheme of extraction of Chitosan from *L. squarrosulus* and *C. africana*

Deproteinisation

The pulverised *L. squarrosulus* (10 g) was immersed successfully in 1, 2, 3, and 4 M NaOH solutions respectively at a solute solvent ratio of 1: 40 (g/mL) and heated for 2 h using a magnetic heater at 60, 80, and 100 °C respectively with constant stirring (Kasongo *et al.*, 2020). The deproteinised results were filtered, washed and filtered several times with distilled water until neutrality and dried for 3 days at room temperature. Deproteinisation was carried out for *C. africana* using 3 M NaOH at 60 °C alone.

Demineralisation

The dried deproteinised samples of *L. squarrosulus* and *C. africana* were weighed, the resulted amount of each sample was immersed in 1 M HCl solution at a ratio of 1: 40 (g/mL), and heated for another 2 h under stirring using a magnetic heater at 60 °C. The resulting CHI was filtered and washed several times with distilled water until neutrality was achieved. The samples were dried at ambient temperature.

Deacetylation

Resulted yield of CHI from *L. squarrosulus* and *C. africana* were immersed separately in 3 M NaOH solutions at a solute solvent ratio of 1:20 (g/mL), heated under reflux at 60 °C for 2 h using a magnetic heater/stirrer. The resulting CS was filtered and washed with distilled water until neutrality. The CS samples were properly dried at 28-30 ^{o}C.

Film Production

Preparation of chitosan/gelatin solution

Chitosan solution was prepared by adding 0.3 g CS in 100 mL of 1% acetic acid, the solution was allowed to stand for 10 days at room temperature. 10 g of gelatin (G) was dissolved in distilled water measuring up to a standard solution. The CS/G solution was prepared at a ratio of CS30: G70, CS50: G50, and CS70: G30 respectively. The CS/G solutions was slightly heated for five minutes to ensure homogenisation of mixture followed by pouring into a Petri dish (Cast method) and allowed to stand for 7 days at ambient temperature.

Proximate Analysis/Characterisation

Percentage yield of samples

The percentage yield ((weight of CHI or CS/weight of dried sample) × 100%) of CHI or CS was calculated by measuring the weight of samples before and after the extraction at varying temperature and concentrations.

Determination of Solubility

The CS of *C. africana*, *L. squarrosulus* of 3 M, 60 ^{o}C and 4 M, 100 ^{o}C were tested for solubility. The oven dried test tube (W_1), CS of 0.2 g (W_2) each was dissolved in 20 mL of 1% acetic acid at room temperature stirred well for 2 h and filtered. The residue (undissolved solid) was dried at room temperature to a constant weigh (W_3). This was repeated in triplicate, and solubility of the chitosan was calculated using equation 1.

$$\%\, solubility = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Biuret test

0.2 g of 4 M, 100 °C, 3 M, 60 °C using CS from *L. squarrosulus* and the sample from *C. africana* respectively were weighed and placed in a conical flask, 0.25 g of the Kjedhal tablet was added into the flasks and 4 mL of sulphuric acid was added and left for some minutes until a black colouration was observed. These samples were heated until they turned colourless. The samples were rinsed into a 100 mL volumetric flask and made to mark using distilled water. 10 mL of each filtrate was pipetted into a volumetric flask to which 2.5 mL of alkaline sodium phenate was added, 1 M of sodium potassium tartrate, 2.5 mL of sodium hypochlorite was also added to the flasks. The flasks were allowed to develop colour for 25 min.

Degree of Deacetylation

The degrees of deacetylation (DD) of *C. africana*, *L. squarrosulus* using 3 M, 60 ^{o}C and 4 M, 100 ^{o}C were determined using Fourier transform infrared (FTIR) spectra method. The absorbance ratio of the CS samples was measured using FTIR spectroscopy. The DD was calculated from the FTIR spectra using equation (2) (El-araby *et al.*, 2022) and 3 (Eddya *et al.*, 2020).

$$\text{DD (\%)} = 100 - \left[\left(\frac{A_{1643}}{A_{3350}}\right) x \left(\frac{100}{1.33}\right)\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$DD(\%) = 100 - \left[31.92\left(\frac{A_{1312}}{A_{1416}}\right) - 12.20\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where, A_{1312} was the degree of absorption of C-N of amide III (CO and NH_2), A_{1412} was the degree of absorption of CH_2 of C-6 and constant of 31.92 and 12.20 showed the value of A_{1312}/A_{1412} of CHI deacetylation. A_{1643} was the absorbance of amide I (acetyl band), A_{3350} was the absorbance of hydroxyl group and a constant of 1.33 showed the value of A_{1643}/A_{3350} of CHI deacetylation, A = 2-log (T%).

Flexibility

The flexibility test was carried out using ASTM D6905. The CS films were applied to the thin metal panel. After the coatings was cured, a standard weight was dropped from a known distance so that it struck the film, which deformed the coating and the film. The percentage elongation was the area of the highest stretched where there was no film broke and can withstood.

Characterisation

The FTIR spectra of CS from *L. squarrosulus*, *C. africana* and films were determined using FTIR (Cary 630 Agilent Technologist) spectrophotometer and the actual topography and composition of CS from *C. africana* was obtained using SEM (Agilent Microlab PC, Zaria, Nigeria.

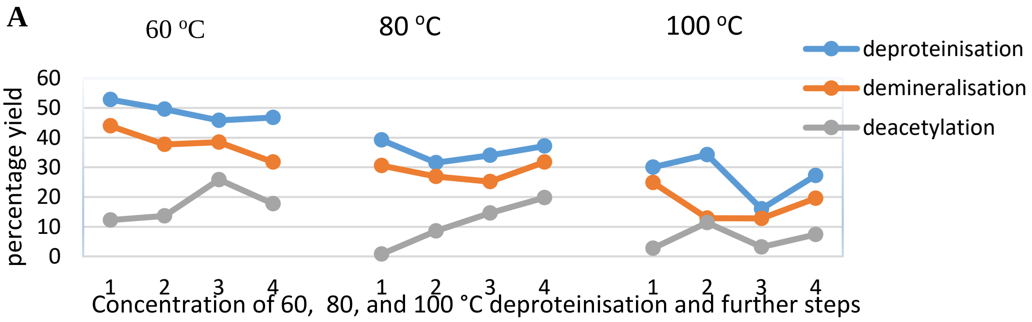
RESULTS AND DISCUSSION

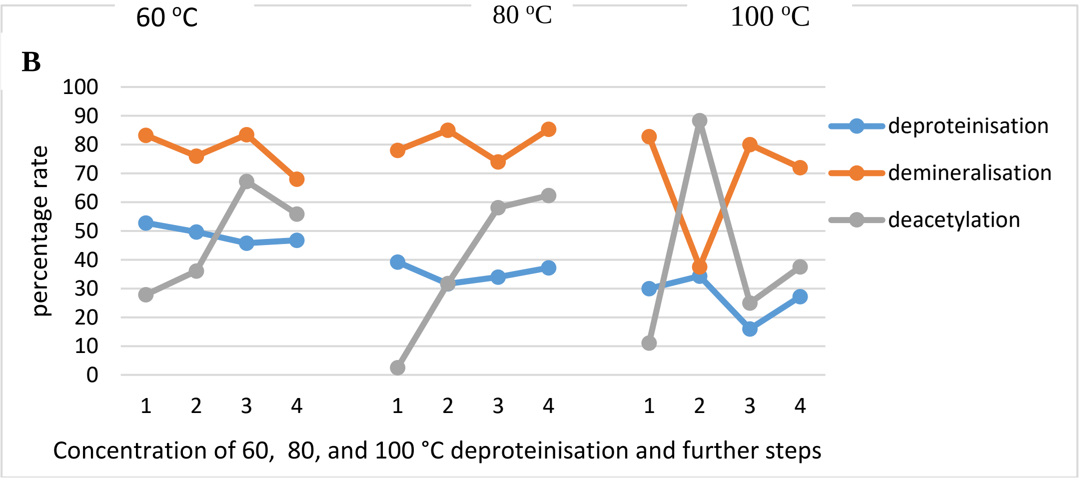
The percentage yield and the rate during chemical processes of the extraction of *L. squarrosulus* chitosan *via* deproteinisation at 60, 80, and 100 °C and various concentrations of NaOH (1, 2, 3, and 4 M) were carried out in order to optimise the yield. Meanwhile, *C. africana* was deproteinated at 3 M, 60 °C. The two samples were demineralised and deacetylated at 1 M, 60 °C with HCl and NaOH respectively (Table 1).

The graphical representation of percentage yields and rate of *L. squarrosulus* and *C. africana* of chitosan at different reaction conditions (Figure 3). The evolution of the extraction yield of CHI, CS and the rate of deproteinisation by varying the concentrations of the NaOH solution are depicted. The yields of CS of mushroom at 60, 80, and 100 °C deproteinisation were 12.25-25.88, 0.78-19.80, and 2.76-11.36% respectively while *C. africana* was 17.30%. The CS samples extracted at 60 °C showed the highest extraction yields due to the lower reaction temperature. There were also, a major decrease in the percentage yield (%) as the concentration increases with a corresponding decrease in percentage yield (%) as the temperature increases, and showed a significant value around 3 M NaOH (Figure 3A). The graph of *C. africana* and *L. squarrosulus* at 60 and 80 °C revealed efficient of demineralisation as more Ca and Mg salts were removed than the other processes. The results obtained in this study are similar to the work of El-araby *et al.* (2022) 15.80-25.33% using different acid during demineralisation. The difference in the results might be as a result of different species, age, reaction conditions (temperature, concentration and time) during the chemical processes (deproteinisation, demineralisation and deacetylation). The temperature and concentration (3 and 4 M, 100 °C) of the deacetylation process can results in depolymerise the CS polymer. This resulted in the mass/weight loss of the sample due to excess removal of acetyl groups from the polymer during deacetylation, which is revealed in the graph of rate of deacetylation of *L. squarrosulus* at 3 and 4 M, 100 °C (Figure 3 A and B).

Table 1: % Extraction yield, rate of deproteinised, deminaralisation and deacetylation of *L. squarrosulus* at varying temperature and molarity

Test no.	Concentration of NaOH	Deproteinised sample (%)		CHI (%)		CS (%)	
				60 °C			
		yield	**rate**	yield	**rate**	yield	**rate**
1	1	52.80	**52.80**	43.93	**83.20**	12.25	**27.89**
2	2	49.60	**49.60**	37.70	**76.01**	13.60	**36.07**
3	3	45.80	**45.80**	38.47	**83.40**	25.88	**67.27**
4	4	46.80	**46.80**	31.82	**67.99**	17.78	**55.88**
				80 °C			
1	1	39.20	**39.20**	30.58	**78.01**	0.78	**2.55**
2	2	31.60	**31.60**	26.86	**85.00**	8.53	**31.76**
3	3	34.00	**34.00**	25.16	**74.00**	14.62	**58.11**
4	4	37.20	**37.20**	31.74	**85.32**	19.80	**62.38**
				100 °C			
1	1	30.00	**30.00**	24.84	**82.80**	2.76	**11.11**
2	2	34.30	**34.30**	12.86	**37.49**	11.36	**88.34**
3	3	16.00	**16.00**	12.80	**80.00**	3.20	**25.00**
4	4	27.20	**27.20**	19.58	**71.99**	7.34	**37.49**
				Percentage yield (%) of *C. africana* (60 °C)			
1	3	32.30	**32.30**	27.80	**86.07**	17.30	**62.23**





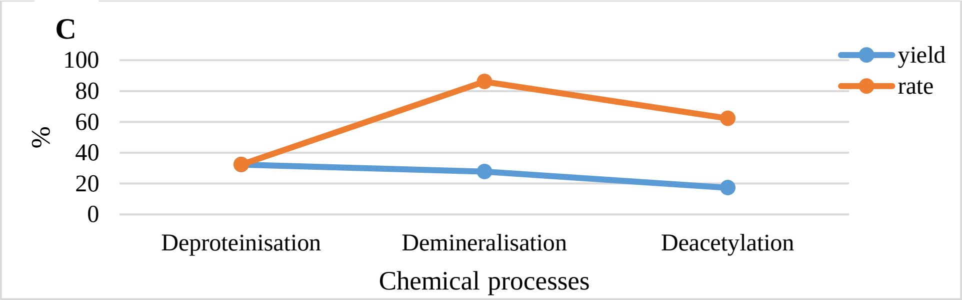


Figure 3: Extraction yield and rate of CHI and CS of *L. squarrosulus* (A and B) and *C. africana* (C)

The solubility and DD are important parameters to determine CS quality in biopolymer applications. The solubility of CS correlated to its biological species, DD (El-araby *et al*., 2022; Hosney *et al*., 2022), and reaction conditions of the deacetylation of CHI (temperature, time, and alkali concentration) (Sogias *et al*., 2010). The presence of protonated free amino groups makes CS more ionic than CHI as the hydrogen bonding of the amine groups in polymeric CS with water molecule is directly proportional to DD. CS solubility values for *L. squarrosulus* were 87.00 and 91.24 at 3 and 4 M, respectively. On the other hand, *C. africana* was 95.55% (Table 2). The solubility of the studied samples at the same reaction conditions during demineralisation and deacetylation but different deproteinisation step indicate that the shrimp head shell was more soluble than mushroom. The solubility values recorded in this current study were higher than the range (60.29-78.45%) reported by El-araby *et al*. (2022) for shrimp wastes using different acids during demineralisation. The values obtained were lower than 98.15% for shrimp waste (Al-Hassan, 2016) (Table 2).

Table 2: Solubility of chitosan extracted from *L. squarrosulus*

Chitosan	*L. squarrosulus*		*C. africana*
	3 M, 60 °C	4 M, 100 °C	
Solubility (%)	87.00 ± 0.01	91.24 ± 0.01	95.55 ± 0.02

The DD is an important factor in determining the biopolymer application as it influences the physical, chemical, mechanical, and biological properties of the polymer (El-araby *et al*., 2022). One of the methods of determining the DD is using an estimation value of % transmittance of FTIR technique (Oyedeko *et al*., 2019; Eddya *et al*., 2020; El-araby *et al*., 2022). The DD of CS in *C. africana* using A1312 and A1416 was 80.37%, similar to the values obtained (75.44-80.85%) by Eddya *et al*., 2020 from shrimp shells (Table 3). The DD (75.18%) was comparable to that reported by El-araby *et al*. (2022) on shrimp waste 83.67, 80.23, 81.47, 77.83, and 69.14 % using different acids during demineralisation (HCl, H_2SO_4, citric, acetic and lactic respectively). The DD is related to the formation of chitosan as > 50% is considered CS. Hence, from the FTIR results, the DD values confirmed the formation of CS. The variation in the values of DD might be as a result of reaction conditions, geographical origin of the species, and instrumentation.

Table 3. DD (%) of chitosan extracted from *L. squarrosulus* (3 M, 60 °C and 4 M, 100 °C)

Chitosan	*L. squarrosulus* 3 M, 60 °C	*C. africana*
$DD(\%) = 100 - \left[31.92\left(\frac{A_{1312}}{A_{1416}}\right) - 12.20\right]$	0.00	80.37
DD (%)= $100 - \left[\left(\frac{A_{1643}}{A_{3350}}\right) x \left(\frac{100}{1.33}\right)\right]$	81.61	75.18

Figure 4A shows a negative biuret test for protein that suggested the absence of protein. Figure (4B and 4C) showed a deviant from the colour obtained when carrying out biuret test. This may be as a result of presence of impurities (in complete deacetylation or depolymerised chitosan) still inherent in the CS samples.

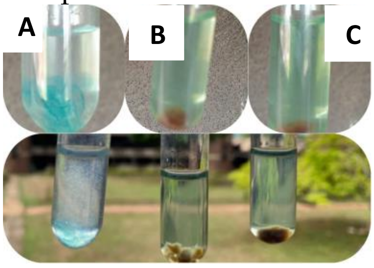
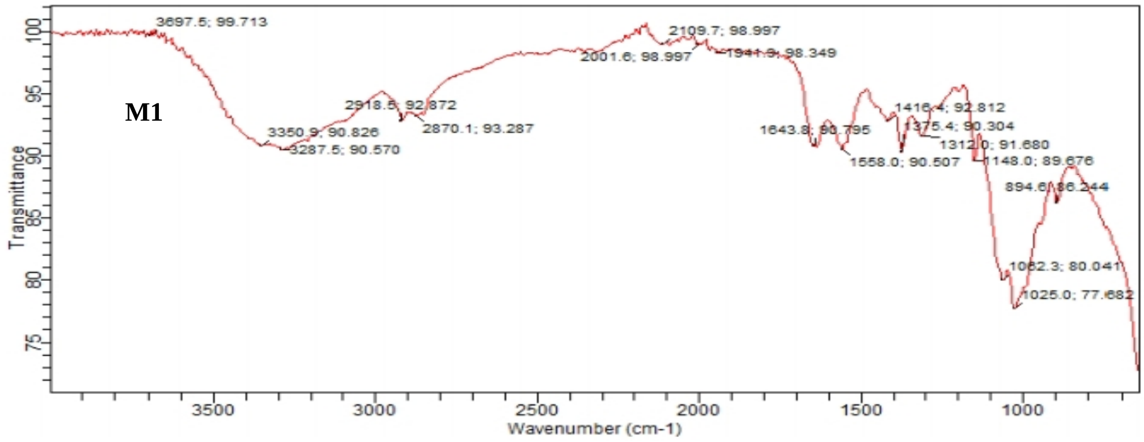


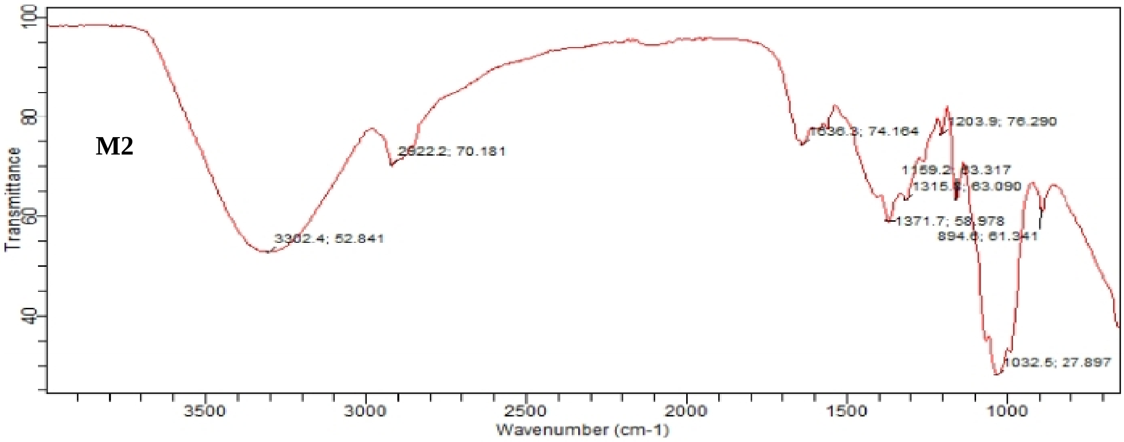
Figure 4: CS Biuret test results from *C. africana* (A), 3 (B), and 4 M (C) *L. squarrosulus*

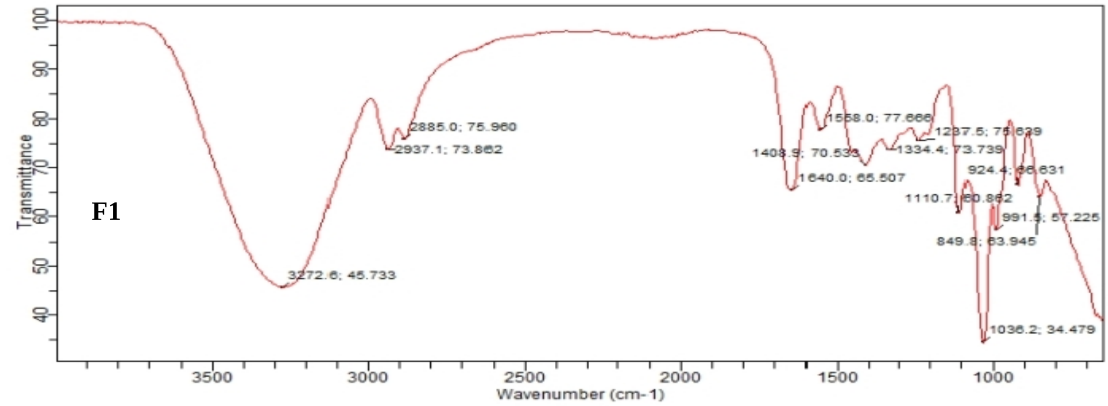
Considering FT-IR results obtained (Figure 5), show the functional groups of CS from *C. africana* and *L. squarrosulus* in variance with the differences in the spectra of the different films (Table 4). The spectra (Figure 5A) show bands at approximately 3350.9 (NH asymmetric stretching and OH stretching vibrations), 3287.5 (NH symmetric stretching) (primary amine N-H bending), 2918.5 and 2870.1 (alkane C-H axial stretching in the polymer chain) cm^{-1}. The amide I and II are 1643.8 and 1558.0 cm^{-1} for C=O and amine N-H bending vibration of NH_2 respectively. The amide III is 1312.0 cm^{-1}. All these characteristic bands are present in CS from *C. africana* with relative similarities with that of films (Figure 5C - 5E), possessing relatively similar peaks at major bands approximately 3272.6 (NH stretching of secondary amine), 2937.1 and 2885.0 (alkane C-H axial stretching in the polymer chain) cm^{-1}. The amide I and II are 1640.0 and 1550.6-1558.0 cm^{-1} respectively. The amide III is 1237.5 cm^{-1}. From the FT-IR results, there are close similarities between the chemical composition of CS from *C. africana* (Figure 5A) and produced films (Figure 5C-5E) deriving its utilisation from CS of *C. africana* with slight shift in the wavenumber of the absorbed functional groups as a result of ionic and hydrogen bonding of cross-linking between the CS and gelatin. The CS FT-IR peaks obtained from *L. squarruosulus* (Figure 5B) show 3302.9 (NH and OH stretching vibrations) and 2922.2 (alkane C-H axial stretching in the polymer chain) cm^{-1}. The amide I and III are 1636.3 and 1316.8 cm^{-1}. The C-O-C of glycosidic bond and C-O stretching in secondary and primary OH groups are 1032.5 and 894.6 cm^{-1} respectively. These characteristic bands of the structure of CS from *C. africana*, its films and *L. squarruosulus* are in line with literature data (Al-Hassan, 2016; Eddya *et al.*, 2020; El-araby *et al.*, 2022). A prominent characteristic band present in Figure 5A when compared to that of Figure 5B at 1558 cm^{-1} (N-H bending vibrations trans-secondary amides II) revealed the complete *N*-deacetylation from CS obtained from *C. africana.* This amide II present two peaks that is not as clear as *C. africana* in *L. squarruosulus* might be overlapping with other bands which supporting the impurities recorded in the biuret test. The FTIR results also revealed the secondary structures of the CS with 1643-1636 (amide I), 1558-1550 (amide II) and 1315-1312 cm^{-1} (amide III) indicate *α*-helices (Gieroba *et al.,* 2020). The presence of free protonated amino groups indicated the production of CS from both samples. This free amino group revealed a bio functional utility of both samples in the medical field.

Table 4. The FTIR bands (cm^{-1}) of 3 M, 60 °C *L. squarrosulus*, *C. africana* chitosan and films.

Samples	OH, NH	CH_2	C=O	NH_2	CH_2	CH_3	CN	C-O-C	C-O
M1	3350.9, 3287.5	2918.5, 2870.1	1643.8	1558.0	1416.4	1375.4	1312.0, 1062.3	1148.0	1025.0
M2	3302.4	2822.2	1636.3			1371.7, 1203.9	1315.8	1159.2	1032.5, 894.6
F1	3272.6	2937.1, 2885.0	1640.0	1558.0	1408.9	1334.4	1237.5, 1062.3	1110.7- 1036.2	1036.2, 991.5
F2	3276.2	2937.1, 2885.0	1640.0	1550.6	1408.9	1338.1	1237.5	1110.7- 1036.2	1036.2, 991.5
F3	3272.6	2937.1, 2885.0	1640.0	1558.0	1408.9	1334.4	1237.5, 1062.3	1110.7- 1036.2	1036.2, 995.2







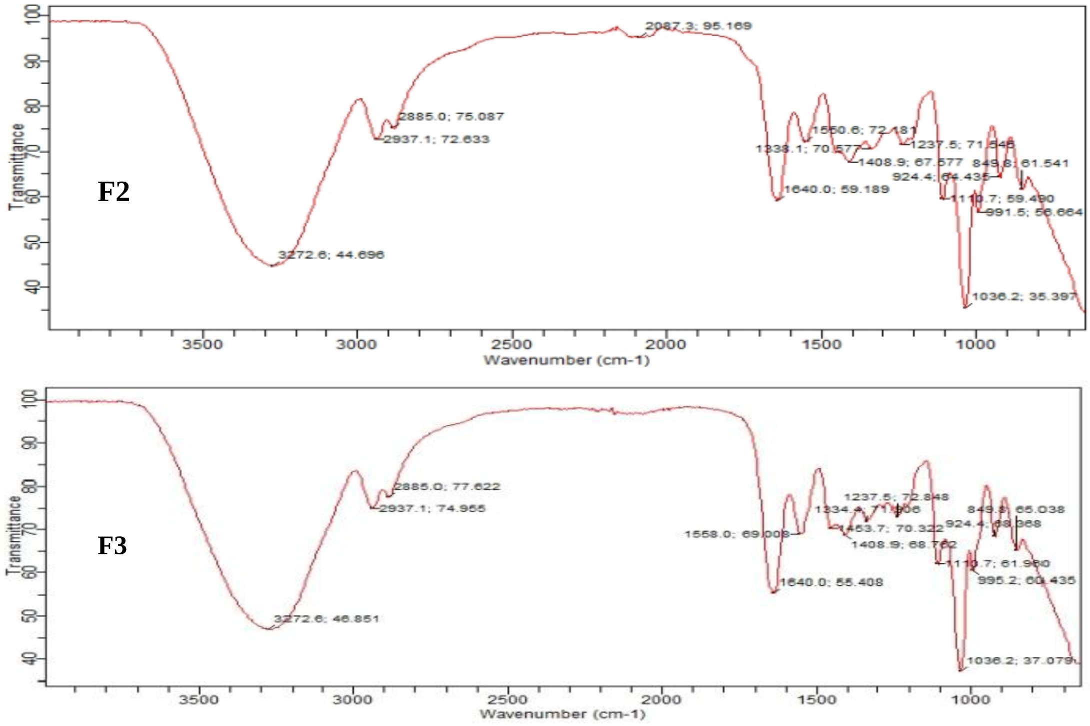


Figure 5: FTIR spectra of CS from *C. africana* (M1), *L. squarrosulus* at 3 M (M2), CS30: G70 (F1), CS50: G50 (F2), and CS70: G30 (F3)

Refractive index results

Refractive index (RI) is an important factor in optical technology and material sciences. RI of the CS films was measured by Abbe refractometer using the principle of transmittance and reflectance of UV–VIS spectrophotometer. Table 5 shows refractive index at CS30: G70, CS50: G50, and CS70: G30 respectively, thus, films of higher number on the index; the slower light travelled through the medium, the more the light was bent and ultimately the more efficient the refraction was. CS70/G30 are more refractive than CS30: G70 when compared to CS50: G50, thus, higher CS solution tends to yield products of higher refraction. The result of this study 1.33-1.51 compared favourably with cross-linked CS film 1.54 (Fen *et al.*, 2011). A higher score shows less materials would be required to achieve the desired effect which definitely is efficient and effective for eyewear production. For higher prescription or thicker glasses in order to have a proper vision, CS50: G50 and CS70: G30 are more preferably which are applicable to trivex, glass, CR- 39 and human eye, within the normal index.

Table 5: Refractive index of films using Abbe refractometer

S/No	CS30/G70	CS50/G50	CS70/G30
Refractive index	1.33 ± 0.02	1.51 ± 0.03	1.49 ± 0.04

When the CS films were applied to thin metal panels for flexibility test, all the film samples passed the test and were not deformed but withstood the stress. This indicates that when the applied stress was removed from all the films, they have the ability to be deformed elastically and returned to their original shape.

The morphology of shrimp shell CS was studied by Scanning electron microscopy (SEM) and the micrograph at different magnification (Figure 6). The product flaws, the elemental makeup of foreign components, coating thickness, and grain and particle size were examined. Generally, at lower magnification (×300) CS showed a combination of rough and smooth surface with dark big pores and rashes (Figure 6A) at 894 µm, similar to this (Figure 6B) at a higher magnification (×500) at 536 µm, although with bigger but fewer number of pores surrounded by white spots and numerous nanoparticles. Also, at higher magnifications (×1000 & ×1500) at 268 and 179 µm respectively, a combination compacts well-defined smooth and rough surfaces with numerous white spots and nanoparticles (Figure 6C and D).

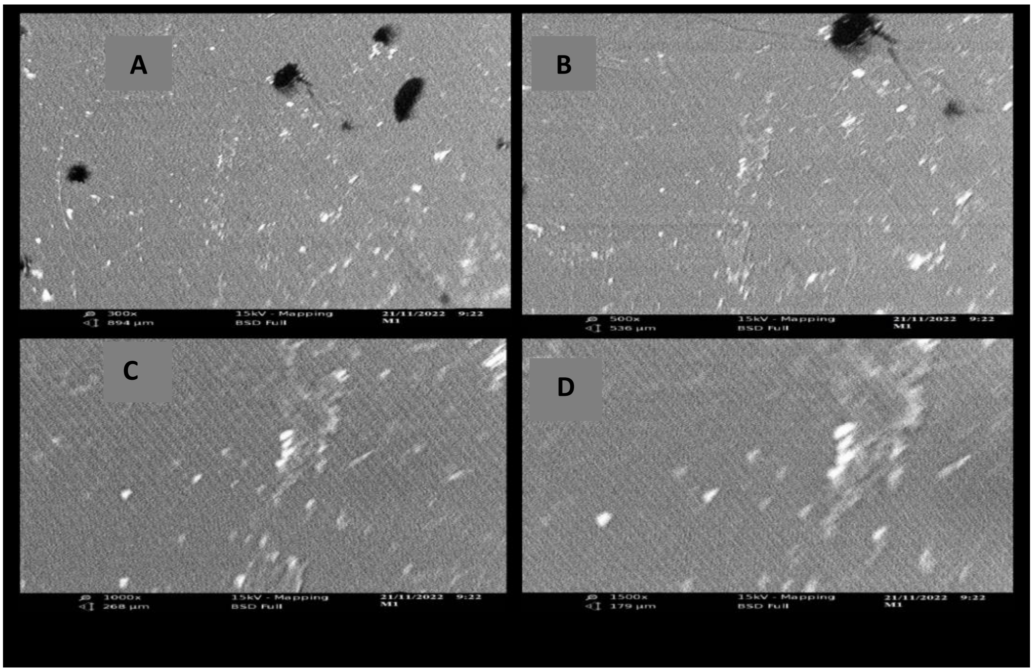


Figure 6: SEM of CS from *C. africana*

CONCLUSION

The research findings indicate that CS a soluble material can be extracted from useful and waste (animal and plant based) products. CS extracted at deproteinised 3 M at 60 °C tend to give higher yield at production at a given time. The films of higher chitosan CS70: G30 and CS50: G50 had higher refractive index which made it more efficient and effective for material of higher quality in the production field. The obtained CS from these products can be turned into other useful materials which have less harm to the environment, are more efficient and effective for economic purpose and products with improve quality.

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