New process for synthesizing chitosan from snail shells

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Abstract. Chitosan is widely known for its unique properties such as nontoxicity, biodegradability, antimicrobial and antifungal properties. It has been used widely in medicine, beverage purification, wastewater treatment, food, and allied industries. The most prevailing method for producing chitosan involve deproteinization, demineralization, decolorization and de-acetylation of chitin. Frequently, during the intermittent intervals of these processes (deproteinization, and demineralization), the products are washed to neutrality, dried before the next phase (discontinuous processes). In this work, we introduce a time and energy saving economic process of demineralization of the deprotenized snail shells through elimination of the drying stage. The yield, moisture content and degree of deacetylation of chitosan produced from the two processes were examined. The chitosan produced from the two processes had similar surface structure and matching FTIR bands. The higher chitosan yield of this energy saving process makes it more economic.

Keywords: Chitosan, snail shells, FTIR, degree of de-acetylation

1. Introduction
Chitosan is a naturally occurring linear polysaccharide. Often times it is produced from chitin by the chemical process which involves deproteinization, demineralization, decolorization and de-acetylation processes. It has notably received growing attention due to its unique properties, apart from the anti-fungicidal effects possessed by chitosan in plant tissue defence systems, it has been considerably used as a molecular polymer compound that is highly biodegradable and can act as a bioactive and nontoxic agent. Chitin and its derivative chitosan are polysaccharides, having a similar chemical structure to cellulose besides nitrogen presence in chitosan and its absence in cellulose [1].

Snail meat has been consumed locally and internationally due to its high protein content and low cholesterol levels. The demand for snail meat has increased in various Nigerians and African restaurants overseas. Countries such as France produce a good delicacy of edible land snails known as escargots. France, Portugal, Sardinia, and Spain serve this delicacy as “hors-d’oeuvre” (meaning appetizer). Protein is an essential constituent of snails. Although various species of snails exists in nature, not all species are good and healthy for human consumption. However, the increase in the consumption of snails has resulted in a high rate of snail shells disposed to the environment causing environmental pollution. Although the shells have been used in feeding layer hens due to its high

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calcium content [2], it is also a suitable source of chitosan. The extraction of chitosan from snail shells has been reported by Abdou et al. [3], Kaewboonruang et al. [4], and Akpan et al. [5].

Chitosan is used in food industries as food preservative against microbial deterioration, biodegradable film formation and material recovery from discarded food processing materials. It functions as dietary fiber as well as a functional ingredient in foods. The degree of de-acetylation is one of the most important factors that affect chitosan characteristics like physiochemical properties and its activity in immune systems of organisms [6]. The functional characteristics of chitin and chitosan are affected by the raw material and its preparation process. Different methods of preparation have resulted in variations in chitosan physiochemical characteristics such as; the degree of de-acetylation, yield, solubility, and molecular weight [7].

This present study aims to compare two different processes of producing chitosan from snail shells, and to investigate the differences in yield, moisture content and degree of deacetylation of the chitosan produced. It examines the structural differences in the surface morphology and the FTIR bands of the chitosan produced. It also compares the synthesized chitosan with commercial chitosan.

2. Materials and Method

2.1. Materials
Snail shells were purchased from Ilorin, North-central part of Nigeria. They were washed with hot distilled water, sundried for a week, and grinded to reduce its particle sizes; this was made to pass through 600 μm mesh sizes. The grinded shells were kept away from dust prior to processing.

2.2. Experimental Procedures
Two different methods of chitosan production were compared, the continuous process (involves demineralizing the wet deproteinized shells) while the discontinuous process (involves demineralizing the dried deproteinized shells). The deproteinization process which was the same for the two methods was done by heating 3 mL of 1 M sodium hydroxide with 1 g of ground snail shell for 2 hours. This was followed by washing the deprotenized shell with distilled water to a neutral pH. For the discontinuous method the washed sample was oven dried at 70 °C until a constant weight was achieved. This was followed by demineralization process using 3 mL of 1.2 M HCl for 1 g of deproteinized snail shell. The same demineralization reaction was carried out with the wet deproteinized sample (continuous process) but the water content was adjusted to keep the acid molarity. Since every 50 g of wet deproteincized shell contains approximately 15 mL of water, this information was used to prepare a higher molarity of HCl for the reaction.

2.3. Chitosan yield and Moisture content
The moisture present in the chitosan products were determined according to AOAC [8] standard methods. Bulk density of the chitosan was determined according to the method of Walke et al. [9]. The yield of chitosan was also determined.

2.4. Analytical methods
SEM and FTIR analysis of the chitosan produced were performed. The surface morphologies of chitosan produced from both processes were compared, and the FTIR bands were compared with a commercial chitosan in order to determine the efficiency of the de-acetylation processes.

2.5. Degree of de-acetylation
The degree of de-acetylation (DD) was estimated as stated in equation (1) by Domszy and Roberts [10].
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\[ \text{DD} (\%) = 100 - \left[ \frac{((A_{1658}/A_{3450}) \times 100)}{1.33} \right] \] (1)

Where absorbances at 1658 and 3450 cm\(^{-1}\) are represented by \(A_{1658}\) and \(A_{3450}\) respectively. N-acetyl group present in amide-I band is measured by \(A_{1658}\) while \(A_{3450}\) represents the hydroxyl band as an internal specification required for film thickness [11].

### 3. Results and discussion

The physiochemical properties such as the yield, moisture content, and degree of de-acetylation of chitosan produced from snail shells using the traditional discontinuous and the new continuous processes are given in Table 1.

| Parameters                     | New process | Traditional process |
|-------------------------------|-------------|---------------------|
| Yield (%)                     | 39.69       | 32.66               |
| Moisture content (%)          | 2.24        | 1.73                |
| Degree of de-acetylation      | 54.98       | 52.56               |
| Bulk density (g/cm\(^3\))     | 0.53        | 0.67                |

Table 1 presents the yield of the chitosan extracted, moisture content and its degree of de-acetylation from both processes. The yield was determined as the dry chitosan weight obtained after extraction from pulverized snail shells. It can be inferred that this new process gave a much higher yield of chitosan than the traditional process. High yield of chitosan from these processes, justifies snail shells usage as a feasible economic source for chitosan production on a large industrial scale as favoured by the persisting snail meat consumption resulting into availability of their shells.

The moisture content of the extracted chitosan in this study was displayed in Table 1. The moisture content of the continuous process was considerably higher than the discontinuous process, however, both chitosan products were remarkably lower (p<0.05) than the commercial chitosan [12]. It was reported by Ocloo et al. [13] that lower values of the chitosan moisture content signifies better self-stability and promotes quality. Chitosan is naturally hygroscopic, therefore, properties of chitosan samples are influenced by amount of moisture absorbed during storage. Although, in application processes, the accepted amount of moisture permitted in chitosan varies from 5.0 % - 15.0 %. The amount depends on the changes in the humidity and the formation of chitosan either in flake or grounded form [14]. The lower values of moisture content from both the new and the traditional methods indicates a better quality of chitosan is obtained using snail shells as raw materials.

#### 3.1. Degree of de-acetylation

De-acetylation process requires the elimination of acetyl groups from chitin molecular chain, resulting into the formation of chitosan having a high degree of amino group that are chemically reactive (-NH\(_2\)) [15]. DD is an essential factor that affects various properties such as biodegradability, chemical reactivity, and solubility of the chitosan produced [16]. In this study, DD of the two chitosan produced were smaller than that of commercial chitosan (58.4 %) reported by Sarbon et al. [12]. Although, DD for the continuous process is higher than the discontinuous process, which shows that chitosan produced from the continuous process has a more probable potential of obtaining higher DD values compared to the discontinuous process. The process of de-acetylation was carried out for four hours which is an essential factor influencing the DD of chitosan. Higher reaction times should be considered. It has been reported that variation in the DD occurs as a result of the different sources, preparation processes [17] and purification methods of raw materials [18]. It also depend on the kind
of analytical methods used and the nature of instrument employed as well as several other conditions that affects the DD values [12].

3.2. Characterization of the chitosan produced
The surface morphology and the FTIR analysis of the chitosan produced from each processes were compared to literature values. From Figure 1, the surface morphologies of both the new and traditional processes showed that they had similar surface morphologies except that the continuous process gives higher bulk density than that of the discontinuous process as indicated by their respective void spaces.

Table 2 shows the FTIR spectra of chitosan formed from our two processes using snail shells (continuous and discontinuous) and commercial chitosan. The spectra bands show the processes of N-H Amide; C-N Nitrile; N-H Amine groups in the three samples. However, the alcohol and alkene groups are absent in the commercial sample. This might be due to a different source of chitin used for the commercial sample.

![Figure 1](image1.png)

**Figure 1.** Surface morphologies of chitosan produced from the continuous (left) and discontinuous (right) processes at 500x magnification.

| Functional group       | Classification of vibration | Adsorption of chitosan (cm⁻¹)          |
|------------------------|-----------------------------|----------------------------------------|
|                        |                             | Continuous process | Discontinuous process | Commercial chitosan [12] |
| alcohol group (O-H)    | stretch                     | 3718.88            | 3718.88                |                           |
| amide group (N-H)      | stretch                     | 3518.88            | 3525.99                | 3369.11 – 3413.07         |
| nitrile group (C=N)    | stretch                     | 2353.23            | 2314.66-2368.66        | 2344.05 – 2346.50         |
| amine group (N-H)      | bending                     | 1512.24 – 1689.7   | 1512.24 – 1689.7       | 1639.59 – 1655.16         |
| alkene group (C-H)     | bending                     | 1419.66            | 1411.94                | –                         |
| alcohol group (C-O)    | stretch                     | 1080.17 – 1180.47  | 1087.89 – 1180.47      | 1128.21 – 1129.02         |
4. Conclusion
This study investigated production of chitosan from snail shells using two different methods, one that involve the conventional method of drying after deprotenization before proceeding to demineralization (discontinuous process) and newly introduced one that eliminates intermittent drying in between deprotenization and demineralization (continuous process). From the results the higher yield and DD of the continuous process is an advantage. The similarity of the surface morphology and the FTIR band analysis of the chitosan produced by the new continuous method introduced with traditional discontinuous method makes the new method acceptable. The moisture content of the continuous process though higher than discontinuous process, is within the range for moisture content for chitosan. Both the higher yield of chitosan and elimination of drying stage of this newly introduced continuous processing makes it more highly economical.

5. References
[1] Bautista-Baños S., Hernandez-Lauzardo, A. N, Velazquez-del Valle, M. G., Hernandez-Lopez M, Ait Barka, E., Bosquez-Molina, E. (2006). Chitosan as a potential natural compound to control pre and posthar- vest diseases of horticultural commodities. *Crop Prot.* 25:108–118
[2] Houndonougbo, M. F., Chrysostome, C. A. A. M., Odoulami, R. C., and Codjia, J. T. C. (2012). Snail shell as an efficient mineral feedstuff for layer hens: Effects and optimum rate. *Livestock Research for Rural Development.* 24: 162. Retrieved November 19, 2018, from http://www.lrrd.org/lrrd24/9/houn24162.htm
[3] Abdou, E. S., Nagy, K. S. A., & Elsabee, M. Z. (2008). Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology,* 99(5), 1359–1367. https://doi.org/10.1016/j.biortech.2007.01.051
[4] Kaewboonruang, S., Phatrabuddha, N., Sawangwong, P., & Pitaksanurat, S. (2016). Comparative Studies on the Extraction of Chitin – Chitosan from Golden Apple Snail Shells at the Control Field. *IOSR Journal of Polymer and Textile Engineering (IOSR-JPTE),* 3(1), 34–41. https://doi.org/10.9790/019X-03013441
[5] Akpan, E. I., Gbenebor, O. P., & Adeosun, S. O. (2018). Synthesis and characterisation of chitin from periwinkle (Tymanotonus fusatus (L.)) and snail (Lissachatina fulica (Bowlidich)) shells. *International Journal of Biological Macromolecules* 106, 1080–1088. https://doi.org/10.1016/j.ijbiomac.2017.08.106
[6] Mahlous, M., Tahtat, D., Benamer, S., Nacerkhodja, A. (2007). Gamma irradiation-aided chitin/chitosan extraction from prawn shells. *J Nucl Inst Methods Phys Res Sect B: Beam Interact Mater Atoms* 265(1): 414–417
[7] Cho, Y. I., No, H. K., Meyers, S. P. (1998). Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. *J Agric Food Chem* 46:3839–3843
[8] AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemistry, 15th edition. *The Association of Official Analytical Chemistry:* Washington, DC, Inc
[9] Walke, S., Srivastava, G., Nikalje, M., Doshi, J., Kumar, R., Ravetkar, S., Doshi, P. (2014). Physicochemical and Functional Characterization of Chitosan Prepared From Shrimp Shells and Investigation of Its Antibacterial, Antioxidant and Tetanus Toxoid Entrapment Efficiency. *Int. J. Pharm. Sci. Rev. Res.,* 26(2): 215-225.
[10] Domysz, J. G., and Roberts, G. A. F. (1985). Evaluation of infrared spectroscopic techniques for analysing chitosan. *Makromol. Chem.,* 1677, 1671–1677.
[11] Mohammed, M. H., Williams, P. A., & Tverezovskaya, O. (2013). Extraction of chitin from prawn shells and conversion to low molecular mass chitosan. *Food Hydrocolloids,* 31(2), 166–171. https://doi.org/10.1016/j.foodhyd.2012.10.021
[12] Sarbon, N. M., Sandanamsamy, S., Kamaruzaman, S. F. S., & Ahmad, F. (2015). Chitosan extracted from mud crab (Scylla olivacea) shells: physicochemical and antioxidant properties. *J Food Sci. Technol.,* 52(7), 4266–4275. https://doi.org/10.1007/s13197-014-1522-4
[13] Ocloo, F. C. K., Quayson, E. T., Adu-Gyamfi, A., Quarcoo, E. A., Asare, D., Serfor-Armah, Y., Woode BK (2011) Physicochemical and functional characteristics of radiation-processed shrimp chitosan. *J Radiat. Phys. Chem.* 80:837–841

[14] Struszczyk, M., (2006) Global requirements for medical applications of chitin and its derivatives. *Polish Chitin Society*. 98

[15] Fernandez-kim S. O. (2004) Physicochemical and functional properties of crawfish chitosan as affected by different processing protocols. *Graduate Faculty of Seoul National University, Dissertation of MSc.*, p 107

[16] Lamarque, G., Cretenet, M., Viton, C., Domard, A. (2005) New route of deacetylation of a-and b-chitins by means of freeze–pump out–thaw cycles. *Biomacromolecules* 6:1380–1388

[17] Martino, A. D., Sittinger, M, Risbud, M. V. (2005) Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials* 26: 5983–5990

[18] No, H. K., Meyers, S. P., Lee, K. S. (1989) Isolation and characterization of chitin from crawfish shell waste. *J Agric. Food Chem.*, 37(3):575–579