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Comparative Studies on the Extraction of Chitin – Chitosan from Golden Apple Snail Shells at the Control Field

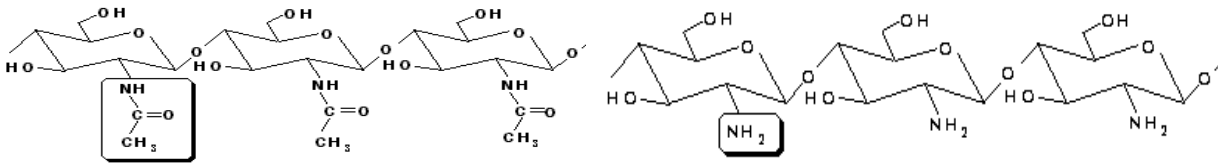
S. Kaewboonruang[1], N. Phatrabuddha[2], P. Sawangwong[3] and S. Pitaksanurat[4]
[1] *Environmental Science Program, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[2] *Department of Industrial Hygiene and Safety, Faculty of Public Health, Burapha University, Chonburi 20131, Thailand*
[3] *Environmental Science Program, Faculty of Science, Burapha University, Chonburi 20131, Thailand*
[4] *Department of Environmental Health Science, Faculty of Public Health, Khon Kaen University, Khon Kaen 40002, Thailand*

Abstract: *Chitin and chitosan are the second most available biopolymers after cellulose. Chitosan as a result of N-deactylation of chitin and has linear chain of β-(1, 4)-linked 2-acetamino-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-Dglucopyranose. The chitosan extraction consists of four common steps such as demineralisation, deproteinisation, decolourisation and N-deacetylation. This study on the extraction of chitin and chitosan from the shells of with is golden apple snail shells collection in the control field. the extraction methods, by Method 1 is the use of a mechanical stirrer rotating at all times, Method 2 to boil at high temperatures and Method 3 is to soak a long time in the static reactor, to compare the productivity of chitin and chitosan extraction from a chemical process in each Method* 1 *Method* 2 *and Method* 3 *to compare the specific properties of chitin and chitosan extracted in different method. All the resulting products were then characterized and confirmed using the preliminary solubility test, XRD, FT-IR, SEM and EDS and tested with 1H NMR. The resulted from first method 1.99% of chitin and 42.56% of chitosan while the Method 2 and Method 3 was 0.55% of chitin and 0.03% of chitosan, 23.02% of chitosan and 15.06% of chitosan respectiy.*
Keywords: *Chitin, Chitosan, Extraction, Golden apple snail.*

I. Introduction

Chitin is the chemical name of a poly [β-(1->4)-2-acetamido-2-deoxy-D-glucopyranose]. Chitin is a substance found naturally in the exoskeletons of insects, in the shells of crustaceans, such as crab, shrimp and crawsh and in fungal cell walls. Chitin is obtained from the shells by removing calcium carbonate, pigments, proteins and lipids immediately after peeling the shrimps. [1]

Chitosan Chemical linear polysaccharide composed of randomly distributed β-(1->4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), a derivative of chitin extracted, through a process in which methyl among native of chitin with alkali. This process is called deacetylation Chitosan products are high quality and different features depending on the technical and production process. [2]



Chitin Chitosan

Figure 1 Chemical structure of chitin and chitosan [Adapted from Rinaudo, M. 2006 [3]

The golden apple snails (*Pomacea canaliculata* Lamarck), the farmers' enemies, can be used as animal feed, fertilizer and others. [4]. Chitin and chitosan extracted from the golden apple snail shell is by deproteinization using dilute alkali and by mineral removal process using dilute hydrochloric acid. The snail chitin is obtained by rinsing the extraction with clean water and filtered until the pH of the solution becomes neutral. The chitin extraced is deacetylation of chitin extract was then washed and filtered water until a neutral pH alkaline out the final stage will be dried chitosan. [5] the deacetylation of chitin of the chitin extracted by using 50% alkaline solution the product is then was is water and fiter utni final pH of the solotion is nature. The

dry chitosan is obtained by deacetylation of the chitin extrac. If you can find ways to exploitation by extraction of chitin - chitosan as compared to the best performance. [6]

II. Materials and Methods

2.1 Sample Preparation

Sizing snail samples of various sizes, number of live weight per 1 kg, washed with water, desiccated at room temperature, boiled to separate the meat from the shells of shellfish and dried in the sun, then bake in a hot air oven. at 100 $\pm$ 10^{o}C for 1 hour.

2.2 Experimental procedures

The golden apple snail shells were scraped free of loose tissue, washed and dried. The product was then blended with blender and crushed with mortar to create golden apple snail shells powder. After the snail shells powder was obtained, the effort to extract chitosan from Golden Apple Snail shells was attempted with three methods naming as Method 1 (M1), Method 2 (M2) and Method 3 (M3). The sequence of process, chemical concentration and treatment times were varied among the three methods.

2.2.1 Method 1 (M1) for Chitosan Preparation

First, prepared golden apple snail shells powder was weighed with an analytical balance to a specific mass. Then, deproteinisation was carried out by using 2M NaOH with a ratio of 20 ml : 1 g (w/v) at a room temperature. The treatment was carried out for duration of 2 hours. The use of a mechanical stirrer rotating at all times. washed with water and filtered water until it has a neutral pH.

The residue from deproteinisation was weighed with analytical balance. Demineralisation was carried out with diluted 2 M HCl solution. The sample was treated with 2 M HCl with a ratio of 20 ml : 1g (w/v) at a room temperature. The treatment was carried out for a duration of 2 hours. The use of a mechanical stirrer rotating at all times. washed with water and filtered water until it has a neutral pH.

The product from demineralisation was measured with analytical balance. Then, it underwent deacetylation with 50 % NaOH with ratio of 20 ml : 1g (w/v) at high temperature 140$\pm$10^{o}C. The treatment was carried out for duration of 2 hours. The use of a mechanical stirrer rotating at all times. Rinse with water and filtered water until it has a neutral pH, Boil in boiling water bath again for another 4 hours, final stage filter to drying oven at 100$\pm$10^{o}C for 1 hour.

2.2.2 Method 2 (M2) for Chitosan Preparation

First, prepared Golden Apple Snail Shells powder was weighed by using an analytical balance to specific mass. Then, deproteinisation was carried out in using 2M NaOH with ratio of 20 ml : 1 g (w/v) using heat to boil, the high temperatures at 120^{o}C. The treatment was carried out for duration of 4 hours. washed with water and filtered water until it has a neutral pH.

The residue from deproteinisation was weighed an analytical balance. Demineralisation was carried out with diluted 2 M HCl solution. The sample was treated with 2 M HCl with a ratio of 20 ml : 1g (w/v) using heat to boil, the high temperatures at 100^{o}C. The treatment was carried out for a duration of 24 hours, using heat to boil at all times. washed with water and filtered water until it has a neutral pH.

The product from demineralisation was measured with analytical balance. Then, it underwent deacetylation with 50 % NaOH with ratio of 20 ml : 1g (w/v) at high temperature 90-120^{o}C. The treatment was carried out for duration of 4 hours, using heat to boil at all times. washed with water and filtered water until it has a neutral pH, Boil in boiling water bath again for another 4 hours, final stage filter to drying oven at 100$\pm$10^{o}C for 1 hour.

2.2.3 Method 3 (M3) for Chitosan Preparation

First, prepared golden apple snail shells powder was weighed an analytical balance to specific mass. Then, deproteinisation was carried out in using 2M NaOH with ratio of 20 ml : 1 g (w/v) using soak it up a long time in the static reactor at room temperature. The treatment was carried out for duration of 48 hours. washed with water and filtered water until it has a neutral pH.

The residue from deproteinisation was weighed with analytical balance. Demineralisation was carried out with diluted 2 M HCl solution. The sample was treated with 2 M HCl with ratio of 20 ml : 1g (w/v) using soak it up a long time in the static reactor at room temperature. The treatment was carried out for duration of 72 hours, washed with water and filtered water until it has a neutral pH.

The product from demineralisation was measured using an analytical balance. Then, it underwent deacetylation with 50 % NaOH with ratio of 20 ml : 1g (w/v) at room temperature. The treatment was carried out for duration of 48 hours, Rinse with water and filtered water until it has a neutral pH, Boil in boiling water bath again for another 4 hours, final stage filter to drying oven at 100$\pm$10^{o}C for 1 hour.

Table 1 : Sample name, chemical, time and parameters for the stages of each method

Method	M1	M2	M3
Stage 1	**Deproteinisation** 2 M NaOH 1g:20ml RT Stirrer 2 hrs	**Deproteinisation** 2 M NaOH 1g:20ml 120°C Boil 4 hrs	**Deproteinisation** 2 M NaOH 1g:20ml RT Soak it up 48 hrs
Stage 2	**Demineralisation** 2 M HCl 1 g: 20ml RT Stirrer 2 hrs	**Demineralisation** 2 M HCl 1 g: 20ml 100°C Boil 24 hrs	**Demineralisation** 2 M HCl 1 g: 20ml RT Soak it up 72 hrs
Stage 3	**Deacetylation** 50% NaOH 1g:20 ml 140±10°C Stirrer 2 hrs	**Deacetylation** 50% NaOH 1g:20 ml 90-120°C Boil 4 hrs	**Deacetylation** 50% NaOH 1g:20 ml RT Soak it up 48 hrs
Final stage	washed with water and filtered water until it has a neutral pH. Boil in boiling water bath again for another 4 hours, final stage filter to dried oven at 100±10°C for 1 hour.		
Total Time at treatment (drying and preparation time neglected)	11 hrs	37 hrs	173 hrs

RT = Room Temperature, 25-30°C
Method 1 (M1) = the use of a mechanical stirrer rotating at all times
Method 2 (M2) = using heat to boil, the high temperatures
Method 3 (M3) = by soak it up a long time in the static reactor

2.3 Characterisation of chitin and chitosan

The produced chitosan was tested with Proton Nuclear Magnetic Resonance (1H NMR) (Bruker, Avance III 500 Mhz, Germany),and Fourier Transform Infrared Spectroscopy (FT-IR) (Bruker, Model tensor 27, Germany), to identify its degree of deacetylation (%DD); X-ray Diffractometry (XRD) (Bruker, Model D2 phaser, Germany), for its lattice parameters, Scanning Electron Microscopy (SEM) (LEO, Model 1450 VP, UK) for its morphology by testing the laboratory center for science and technology, Suranaree University of Technology and laboratory faculty of science, Burapha University.

III. Results and Discussions

3.1 Chitin and Chitosan Yields & Efficiency of Methods

The yielding proportions of various products obtained at various stages of extraction by using three different methods are tabulated in Table 2

Table 2 : Experimental yield of each stage for extraction of chitin and chitosan from golden apple snail shells

Method	M1		M2		M3	
	g	%	g	%	g	%
Golden apple anail shells powder	25.0		25.0		25.0	
Stage 1+2 %wt of Product Remained (Chitin) Based On Dried Shells	0.5007	1.99	0.1363	0.55	0.0835	0.03
Chitin Powder	1.0		1.0		1.0	
Stage 3 %wt of Final Product (Chitosan) Based On Dried Shells	0.4501	42.56	0.2382	23.02	0.1591	15.06

Table 3 : Physicochemical and functional properties of chitin and chitosan extracted

Method	% Moisture	% Ash	% DD	pH
Chitin M1	4.63	1.97	-	6.50
Chitin M2	6.18	1.74	-	6.43

Chitin M3	9.51	2.04	-	6.40
Chitosan M1	6.03	1.74	61.73	7.43
Chitosan M2	2.88	1.64	60.55	7.21
Chitosan M3	7.01	1.92	60.91	7.07

% DD = Degree of deacetylation

3.2 The tests feature of chitin and chitosan extracted

3.2.1 Solubility Test

In common, it is justified that main physical differences between chitin and chitosan is the ability of chitosan to be soluble in organic acid such as acetic acid. Chitosan with higher content of protonated amino group are readily to form well ordered arrangement in Van der Waals force and hydrogen bond which exceed its tendency for intramolecular chemical bonds [7,8]. This explains its solubility in acidic chemical and partial solubility in hydrogen containing solvent.

Test the solubility of chitosan by dissolved chitosan 1 g in 0.5% (w/v) acetic acid 100 ml to shake at 100 rpm for 12 hours, then filtered chitin . Chitosan is insoluble and then dried at a temperature of 50^{o}C for 24 hours and weighed chitosan is insoluble residue.

Table 4 : Experimental analysis of the percentage of solubility test

Method	Chitosan **M1**		Chitosan **M2**		Chitosan **M3**	
	g	**%**	**g**	**%**	**g**	**%**
Chitosan Powder	1.0		1.0		1.0	
Percent Solubility (%)	0.9393	92.19	0.9329	90.87	0.9220	90.70

3.2.2 Scanning Electron Microscopy (SEM)

After the preliminary test, the potential of the product to be chitosan is understood. Next, the product of each method was brought towards to microscopy scale on the surface to understanding its morphology using SEM microscope.

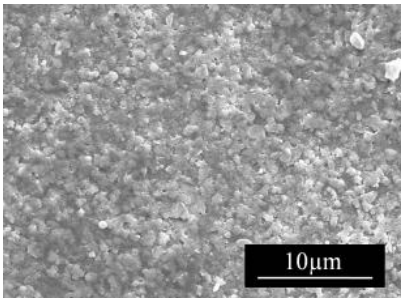
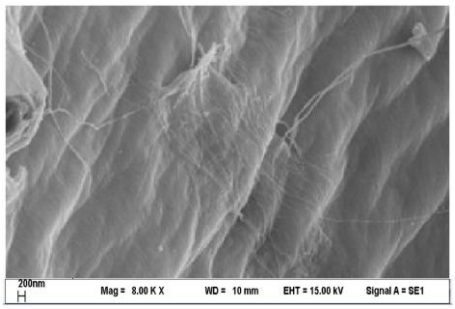


Figure 2 : SEM Micrograph of Golden Apple Snail Shells Powder



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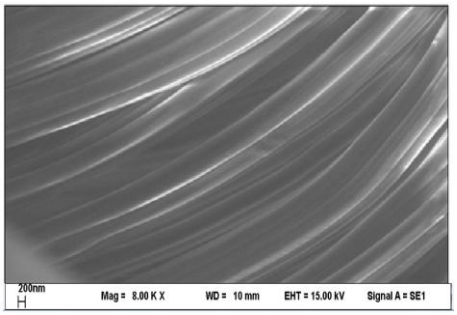

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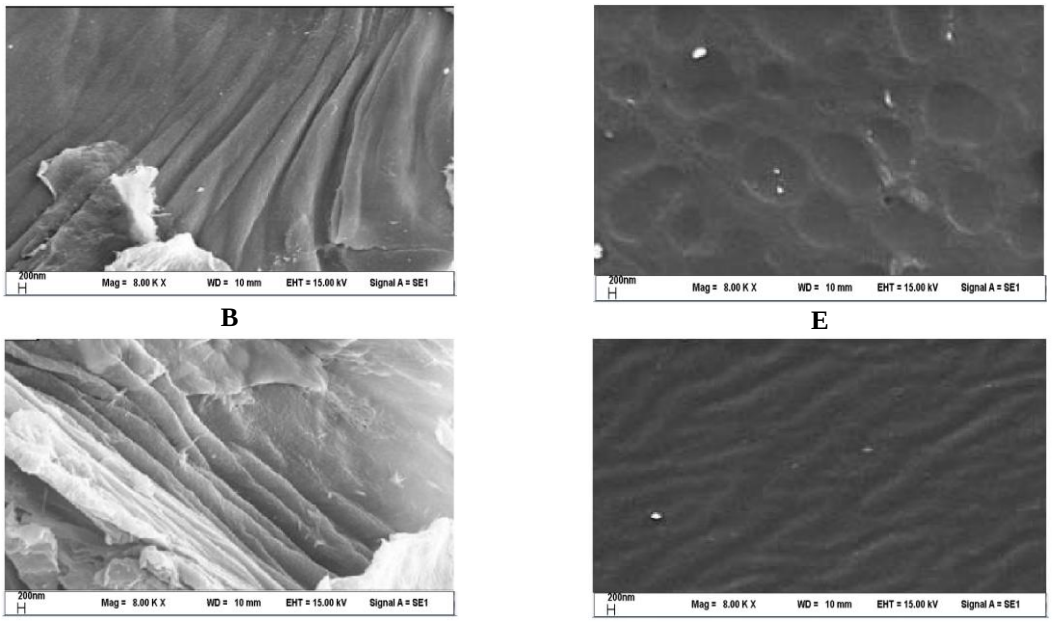
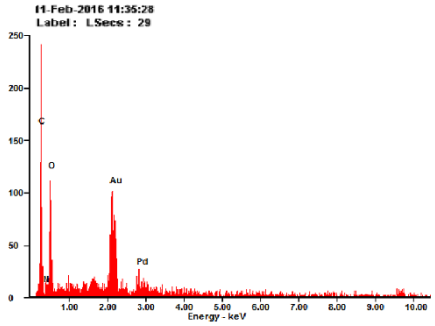


Figure 3 : SEM Micrographs of Chitin M1(A), M2(B), M3(C) and Chitosan M1(D), M2(E), M3(F)

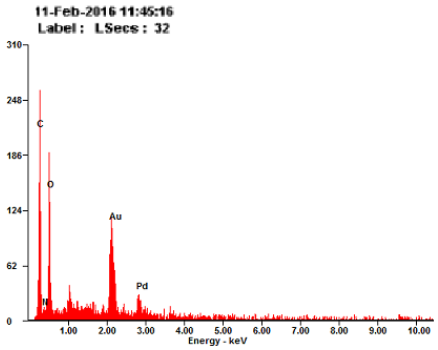
3.2.3 Electron Dispersive Spectroscopy (EDS)

Apart from the morphology, EDS as an add-on function of SEM is a common spectroscopy to identify chemical elements distributed on the surface of the sample. This test provides further understanding on the substances contained on the surface.



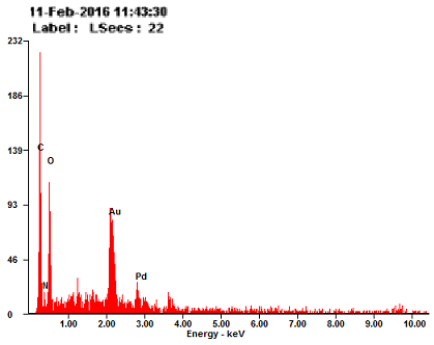
Element	Wt%	At%
CK	08.31	39.83
NK	01.27	05.24
OK	08.16	29.39
AuM	76.24	22.29
PdL	06.01	03.25
Matrix	Correction	MThin

Figure 4: EDS Spectrum and Distribution of Element for Chitosan M1



Element	Wt%	At%
CK	09.64	40.56
NK	00.82	02.98
OK	11.06	34.94
AuM	72.06	18.48
PdL	06.41	03.04
Matrix	Correction	MThin

Figure 5: EDS Spectrum and Distribution of Element for Chitosan M2



Element	Wt%	At%
CK	09.00	39.86
NK	01.33	05.05
OK	09.67	32.14
AuM	74.17	20.02
PdL	05.83	02.91
Matrix	Correction	MThin

Figure 6: EDS Spectrum and Distribution of Element for Chitosan M3

3.2.4 X-ray Diffactometry Analysis (XRD)

X-ray diffraction (XRD) also known as x-ray crystallography is defined as an analytical technique used to indentify phase of a material and provided information on and unit cell dimensions. A key component of all diffraction is the angle between the incident and diffracted rays. Powder and single crystal diffraction vary in instrumentation beyond this. Generally, XRD is based on diffracted ray of monochromatic x-ray and a crystalline sample where crystalline structure will act as three-dimensional diffraction gratings for the x-ray wavelengths. This is similar to the spacing of planes in a crystal lattice [9,10,11].

X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing. Its application are 1) characterisation of crystalline materials, 2) identification of fine-grained minerals that are difficult to determine optically, 3) determination of unit cell dimensions and 4) measurement of sample purity. The analyzed materials were usually finely ground and homogenized before being tested [9,10,11].

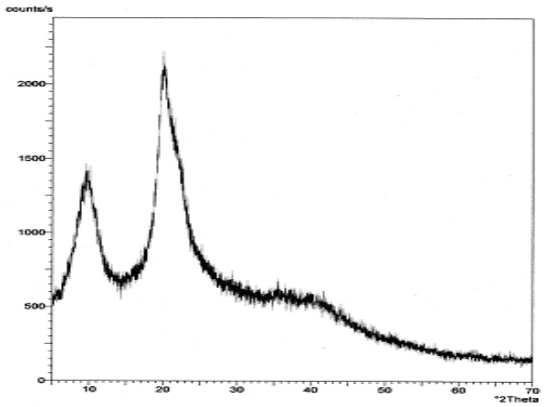


Figure 7 : XRD diffractogram of prepared chitosan

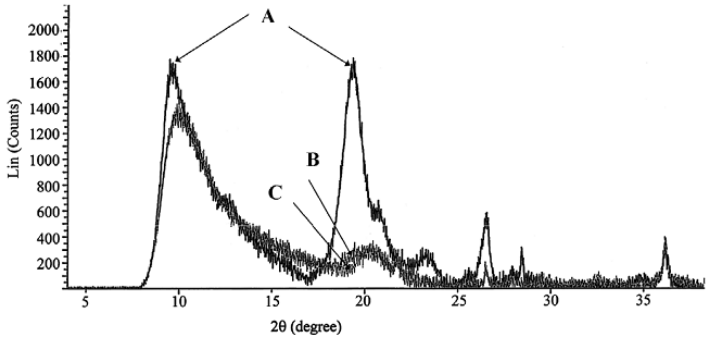


Figure 8 : XRD Pattern of (A) α-chitin of Tiger Prawn, (B) Corresponding Chitosan Prepared under Microwave Heating, (C) Chitosan Prepared under Traditional Heating [Adapted from Al Sagheer, Al-Sughayer, Muslim and Elsabee (2009)]

3.2.5 Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectroscopy (FT-IR) is one of commonly used method of infrared spectroscopy. Infrared spectroscopy has been an effective technique for materials analysis in the laboratory for

over few decades. In infrared Spectroscopy, IR radiation in different wavelength is released on to the sample where certain degree of radiation will be absorbed by the sample while the remains will transmit through. An infrared spectrum represents a fingerprint of a sample with absorption peaks. This will then form a spectrum showing the absorption and transmission of the sample molecule which correspond to the frequencies of vibrations between the bonds of the atoms which compile the material. The spectrum is unique for the material as it has the unique combination of atoms and no other compound can produce the same spectrum. Therefore infrared spectroscopy can result in a positive qualitative analysis of every different kind of material. In addition, the size of the peaks in the spectrum directly indicates the density of material present [12,13,14].

Chitin and chitosan contain three characteristic band which are 1577 cm-1, 1654 cm-1 and 2932 cm-1 corresponding to vibration of -NH, -C-O and –CO-CH3 group. Meanwhile, the content of polysaccharide is represented by bands between 890 and 1156 cm-1 [14]. Futhermore, chitin has more intense band for 2932 and 1577 cm-1 than commercial chitosan, this difference is the evidence of deacetylation [7].

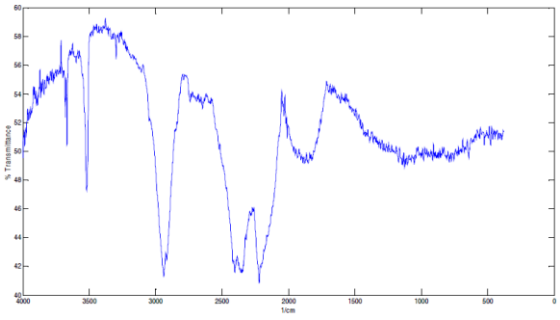


Figure 9 : FT-IR spectra of chitin

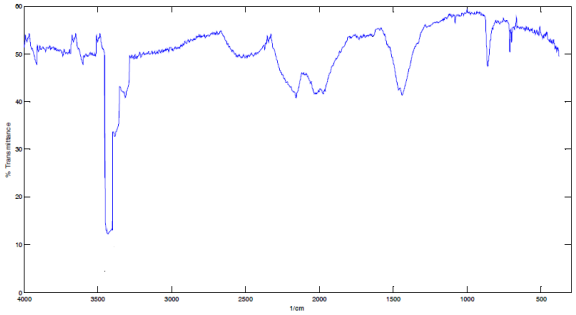


Figure 10 : FT-IR spectra of chitosan

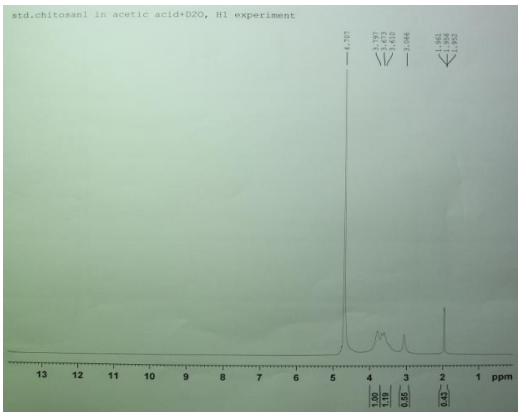
FT-IR analysis of chitosan was based on the identification of bands and its vibrations. The bands wave numbers are as Table 5 : Wavenumbers and chemical group of FT-IR absorption bands for chitosan [17].

Table 5 : Wavenumbers and chemical group of FT-IR absorption bands for chitosan [Adapted by [17].

Wave number (cm^{-1})	Chemical group
3450	OH hydroxyl group
3360 NH	group-stretching vibration
2920, 2880, 1430, 1320,	Symmetric or asymmetric CH_2 stretching vibration
1275,1245	Attributed to pyranose ring
1730	Carbonyl group vibration
1660	C=O in amide group (amide I band)
1560	NH-bending vibration in amide group
1590	NH_2 in amino group
1415, 1320	Vibrations of OH, CH in the ring
1380	CH_3 in amide group
1255	C–O group
1150–1040	–C–O–C– in glycosidic linkage
850, 838	CH_3COH group

3.2.6 Proton Nuclear Magnetic Resonance (1H NMR)

The degree of deacetylation of chitosan salts is an important characterization parameter since the charge density of the chitosan molecule is responsible for potential biological and functional effects. The degree of deacetylation (% DD) of water-soluble chitosan salts can be determined by 1H nuclear magnetic resonance spectroscopy (1H NMR). Several workers have reported on the NMR determination of chemical composition and sequential arrangement of monomer units in chitin and chitosan. The test method described is primarily based on the work of [18] which represents the first publication on routine determination of chemical composition in chitosans by solution state 1H NMR spectroscopy. This test method is applicable for determining the % DA of chitosan chloride and chitosan glutamate salts. It is a simple, rapid, and suitable method for routine use. Quantitative 1H NMR spectroscopy reports directly on the relative concentration of chemically distinct protons in the sample, consequently, no assumptions, calibration curves or calculations other than determination of relative signal intensity ratios are necessary.



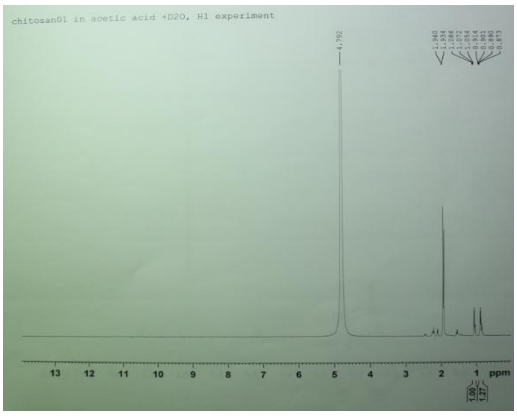


Figure 10 : 1H NMR Spectrum of Commercial Chitosan **Figure 11 :** 1H NMR Spectrum of Chitosan extracted

III. Conclusion

Chitin and chitosan extracted from golden apple snail shells at the control field by chemical processes extraction, the resulting products were chitin pH 6.40-6.50 and chitosan pH 7.07-7.43, Method 1 (M1) using mechanical. stirring rotation time yield is best chitin 1.99% and chitosan, 42.56%, and take time to extract a minimum 11 hour, Method 2 (M2) by heating in boiling high temperatures. Yield 0.55% chitin and chitosan 23.02% and extraction time 37 hour and the 3 (M3) by soaking for a long time. It takes up to 173 hours. Yield least chitin 0.03% and chitosan 15.06% and chitosan from the , Method 1 (M1) has dissolved the best is 92.19%, followed by the Method. 2 (M2) 90.87%, and the Method 3 (M3) 90.70% respectively Percent DD (%) of chitosan extracted by the, Method 1 (M1) 61, 73%, method 2 (M2) 60.55%. and , Method 3 (M3) 60.91%.

Acknowledgements

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