Extraction of Chitin and Chitosan from Waste Crab Shell for the Application of Wastewater Treatment

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Abstract

Mud crab (*Scylla serrata)* were collected from Sittwe, Rakhine State. The shell and operculum were removed from collected crabs. Only crab shells were thoroughly washed with water. Then washed crab shells were placed in an ice box overnight and dried in sun radiation for (7) days. After drying, crab shells were grounded into smaller pieces using a grinder and sieved with (60) mesh screen with vibratory sieve shaker to get crab shell powder.

Physico-chemical characteristics of crab shell powder such as colour, odour, moisture content, solubility, calcium carbonate content, elemental composition (EDXRF) were then determined.

The crab shell powder was further treated by demineralization with different concentrations of acetic acid, deproteinisation with sodium hydroxide, decolourization with sodium hypochloride for the extraction of chitin and then this extracted chitin was deactylated with sodium hydroxide and acetic acid for the extraction of chitosan.

Characterizations of extracted chitin and chitosan were then analyzed by physico-chemical characteristics such as colour, odour, moisture content, solubility, calcium carbonate content, elemental composition (EDXRF), Fourier Transformed Infrared (FT-IR) Spectroscopy technique, and Thermogravimetric Analysis (TGA) Technique and compared with commercial chitosan powder for the application of textile wastewater treatment

Keywords: crab shell powder, chitin, chitosan, demineralization, deproteinisation, deacetylation

Introduction

Chitosan is partially deacetylated polymer of glucosamine (2 acetamido-2-deoxy b-1, 4-D-glucan). It is essentially a natural water soluble derivative of cellulose with unique properties. Chitosan be used as a flocculent, clarifier, thickener, fiber, film, affinity chromatography column matrix, gas-selective membrane, plant disease resistance promoter, anti-cancer agent, wound healing promoting agent and antimicrobial agent. It is used as processing aid and is being trialed for application in fruit preservation, wound dressing, cosmetics, artificial organs and pharmaceuticals (Brine *et al*., 1991).

Chitosan is usually prepared from chitin and chitin has been found in wide range of natural sources (crustaceans, fungi, insects, annelids, mollusks, coelenterate etc.) (Tharanathan and Kittur, 2003). However chitosan is only manufactured from crustaceans (crab, krill, and crayfish) primarily because a large amount of the crustacean's exoskeleton is available as product of food processing. Crab shell is made up of three basic components.

These are chitin, protein and a calcium salt of which chitin is most important for scientific studies. Chitin is a fairly completely acetylated polysaccharide in nature, being only second after cellulose (Adole and Omogbai, 2012).

Minimization of disposing waste crab and conversion into valuable and biologically sustainable material is the challenge to researchers and scientists.The aim of the present study is to minimize environmental pollution associated with crab shells, for the extraction of chitin and chitosan from crab shell waste and to investigate the treatment of textile wastewater.

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Materials and Methods

Materials

Fresh mud crab (***Scylla serrata***) were collected from Sittwe, Rakhine State, Myanmar to extract chitin and chitosan. The required chemicals such as acetic acid, sodium hydroxide and sodium hypochloride were purchased from Golden Lady Chemical Shop, Pabeldan Township, Yangon Region.

Method of Preparation

The shell and operculum were removed from collected crabs. The shells were then thoroughly washed with water to remove sand and extraneous matter. After washing, the crab shells were sun dried for seven consecutive days at 32-36°C. The dried crab shells were ground into smaller pieces using a grinder and sieved with 60 mesh screen to get crab shell powder. Finally, the crab shell powder was stored in air tight plastic containers for the extraction of chitin and chitosan.

Method of Extraction

1. Demineralization Process

Each crab shell powder of 50 g. was demineralized with 200 ml of different concentrations of acetic acid solution i.e. 0.1M, 0.3M, 0.5M, 0.7M and 0.9M and was allowed to soak for (48) hours at ambient temperature to remove the minerals (mainly calcium carbonate). The solution was then washed with drinking water (4L, 6L, 7L, 8L and 11L) to get neutral solution and filtered with Whatman filter No.1 paper. The residue (demineralized crab shell powder) was further treated with sodium hydroxide solution to remove protein.

2. Deproteinization Process

Demineralized crab shell powder obtained from above process was deprotonized using the same concentration of sodium hydroxide solution. Demineralized crab shell powder was placed in each beaker and treated with (200) ml of sodium hydroxide solution (0.1M) in a ratio of 1:4 (w/v) for 1 hour by constant stirring and heated at 45-50^{o}C in order to dissolve the protein and sugar. The solution was allowed to cool for 30 minutes at room temperature and was also washed in drinking water (6L, 8L, 12L, 15L and 16L) to get neutral solution and then filtered through Whatman filter No.1 paper. The residue (deproteinized crab shell powder) was further treated with sodium hypochloride solution to remove colour.

3. Decolourization Procedure

After deproteinization procedure, deproteinized crab shell powder was bleached with 200 ml of 10% sodium hypochloride solution for 30 minutes under stirring to remove any pigments and also to reduce the odour of the material. Bleached crab shell powder was then repeatedly washed in drinking water 7 L. to get neutral solution and filtered through Whatman filter No.1 paper. After that, the residue(chitin) was dried in an oven at 60-65°C for 24 hours.

4. Deacetylation Procedure

Chitin powder, 50 g. having the suitable condition of 0.7M acetic acid solution was deacetylated using the 250 ml of different concentrations of sodium hydroxide solution (40%, 45% and 50%) with a ratio of (1:5 w/v) at 100°C by constant stirring for 2 hours. After 2 hours, 200 ml of 10% acetic acid solution was further added to this solution, and stored for 12 hours at room temperature. The solution was then washed in drinking water (15L, 18L and 23L) until neutral solution was obtained. Finally, the solution was filtered through Whatman filter No.1 paper and the residue (chitosan) was dried in an oven at 75-80°C for 24 hours.

Methods of Analysis

Physico-chemical characteristics of crab shell powder and chitin such as colour, odour, moisture content, solubility, calcium carbonate content, and elemental composition (EDXRF) were determined. Characterizations of extracted chitin and chitosan were carried out by Fourier Transformed Infrared (FT-IR) spectroscopy technique and Thermogravimetric Analysis (TGA).

Results and Discussion

The present work represents the investigation of various physico-chemical characteristics, elemental composition of fresh crab shell powder; FT-IR and TGA analysis of extracted chitin, chitosan and commercial chitosan. It was found that the protein content of crab shell powder varied according to species and seasons of harvest and was lower than that of the literature value. In the elemental compositions of crab shell powder the highest content was calcium (95.386%) because the compositions of the crab shells could vary from region to region and the results are shown in Table (1), Table(2) and Figure (1).

From the results of Table (3), Table (4) and Figure (2), it was clearly seen that the protein contents of all extracted chitin were rather higher than the literature value. Therefore, higher concentration of sodium hydroxide solution would be required for deproteinization process (www.ijptjournal.com>69-72).The yield percents of extracted chitin from crab shell were between 76.34% and 85.08%. From the observation of qualitative estimation from extracted chitin to chitosan, it was also observed that the colour of chitin was changed on the spot from yellow to brown and then straightly turned dark purple which indicated presence of chitosan.Major peaks in the FT-IR spectrum of chitin lied between 578.64 cm^{-1} and 3668.78 cm^{-1} (https://www.researchgate.net> publication>chitin-chitosan). In this research, the suitable FT-IR peaks for extracted chitin were assigned in the range 3421.75 cm^{-1}, 3269.45 cm^{-1}, 2887.53 cm^{-1}, 1662.69 cm^{-1}, 1484.17 cm^{-1}, 1415.80 cm^{-1}, 1151.54 cm^{-1},1070.53 cm^{-1}, 873.78 $cm^{-1}$and 707.90 cm^{-1} as shown in Table (5) and Figure (6). Thermogravimetric (TGA) analysis of extracted chitin was shown in Figure (8). Stephen et al; 2002 had reported that the temperatures lower than 100°C indicated the loss of water. The temperature above 100°C indicated the decomposition of pyranose ring structure. In this research, the thermal degradation of extracted chitin occurred within nearly 50 to 460°C that could be seen in two-step degradation and also indicated the loss of water and the decomposition of pyranose ring structure. The degradation temperatures were no differences in all extracted chitin.

Characterization of extracted chitosan and commercial chitosan was done through Fourier Transformed Infrared (FT-IR) Spectroscopy and Thermogravimetric Analysis (TGA). It is clearly seen that FT-IR spectra peaks of extracted chitosan were in the range 3277.06 cm^{-1}, 1408.04 cm^{-1}, 1022.27 cm^{-1}, 871.72 cm^{-1}, 711.73 $cm^{-1}$and 567.07 cm^{-1} as shown in Table (6) and Figure (11). The FTIR spectrum of chitosan showed absorption peak was at 3427 cm^{-1}, this attributed to the combined peaks of the NH_2 and OH group stretching vibration (M. Guo, et al., 2005). The FTIR spectrum of chitosan extract showed that characteristic absorption bands of chitosan at 1634 cm^{-1} C=O amide and 1077 cm^{-1} C-O stretching (K. Juntapram et al., 2012).

In this research, the absorption patterns of the spectrum is nearly similar to that of the literature value due to the different regions of collected crab. Therefore 50% concentration of sodium hydroxide was suitable for the extraction of good quality of chitosan.

The FT-IR spectra of commercial chitosan manufactured from Tianjin Tianshi Biological Development Co., Ltd. (Address) No.16 Xinyuan Road, Wuqing Development Area, Tianjin, China (Post Code) 301700 was observed and it was found that the bands were at

3298.28 cm^{-1}, 2864.29, 1585.49 cm^{-1}, 1373.32cm^{-1}, 1022.27 cm^{-1} and 893.04 cm^{-1} as shown in Table (6) and Figure (12). In the present research, the FT-IR peaks of extracted chitosan were a little different from the literature peaks and peaks of commercial chitosan manufactured from Tianjin Tianshi Biological Development Co., Ltd. (Address) No.16 Xinyuan Road, Wuqing Development Area, Tianjin, China (Post Code) 301700.

The thermogravimetric curves were at a heating rate of 20°C min^{-1} under a dynamic atmosphere of nitrogen in the temperature range of 10-500°C. Sania et.al (2012) had observed that the thermograms of chitosan had stage wise weight loss in the range of 50-150°C and 250-300°C, whereas in the case of commercial chitosan sample, decomposition occurred in single stage (250°C-300°C). The initial weight loss in the range of 50-150°C was occured due to the removal of moisture content and the decomposition stage of chitosan occured between temperatures of 250-300°C, which suggested that chitosan had a lower thermal stability (www.agriculturejournals.cz>publicfiles).

In this research, initial weight loss of thermogravimetric analysis of extracted chitosan in the range of ~50-150°C was due to the removal of moisture content and the decomposition stage of chitosan occurred between temperatures of ~250-300°C. But thermal decomposition of commercial chitosan occurred in a single stage (~250-300°C). Those decompositions were nearly similar to the literature value and shown in Figures (13), (14) and (15) for extracted chitosan and Figure (16) for commercial chitosan manufactured from Tianjin Tianshi Biological Development Co., Ltd. (Address) No.16 Xinyuan Road, Wuqing Development Area, Tianjin, China (Post Code) 301700.

Table (1) Physico-chemical Characteristics of Fresh Crab Shell

Sr. No.	Characteristics	Fresh Crab Shell	
		Experimental	Literature
1	Colour	beige	*beige (crab shell powder)
2	Odour	fishy	**fishy (crab shell powder)
3	Moisture (%)	52.7	***34.41±2.23
4	Ash (%)	26.8	***11.93±0.63
5	Solubility (%)	86.6	-
6	Calcium carbonate (%)	85.4	****78.70 (crab shell powder)
7	Protein (%)	10.32	****13.50 (crab shell powder)

* https://www.colour in R.com>pdf

** www.amazon.com

***www.ijsar.in>2015(78-84)

****https://lejpt.academicdirect.org>characterization-of-chitin-from-nigerion-sources

Sr. No.1 to 6 measurement was made at Industrial Chemistry Department's Laboratory, Yadanabon University, Mandalay.
The measurement of protein was made at Union of Myanmar Federation of Chambers of Commerce and Industry.

Table (2) Elemental Composition of Crab Shell Powder Analyzed by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry Technique

Sr. No	Elements	Concentration(%w/w)
1	Calcium, Ca	95.386
2	Strontium, Sr	2.278
3	Potassium, K	0.8
4	Manganese, Mn	0.624
5	Iron, Fe	0.427
6	Bromine, Br	0.276
7	Copper, Cu	0.123
8	Zinc, Zn	0.05
9	Sulfur, S	0.018
10	Rubidium, Rb	0.017

Elemental composition of crab shell powder was determined by EDXRF method at Universities' Research Centre, University of Yangon.

Table (3) Effects of Concentration of Acetic Acid Solution on the Extraction of Chitin from Crab Shell Powder

Weight of crab shell powder – 50 g. Volume of CH_3COOH solution – 200 mL

Soaking time in CH_3COOH solution – 24 hours Volume of NaOH solution – 200 mL

Sr. No	Characteristics	Crude Chitin Extracted with Different Concentrations of Acetic Acid Solution, M					Literature Value
		0.1	0.3	0.5	@ 0.7	0.9	
1	Colour	ivory	ivory	ivory	ivory	ivory	*ivory
2	Moisture,%	5.2	5.4	4.8	5.2	4.4	** 6.10
3	Ash,%	68	68	72.5	67.5	65	** 3.40
4	Solubility,%	50	50	70	80	80	-
5	Protein,%	8.38	8.56	6.3	5.52	7.7	**2.6
6	Yield percent, % (w/w)	85.08	76.34	81.62	82.24	85.04	**16.73
7	Qualitative estimation from chitin to chitosan	Yellow/ Brown to dark purple	Yellow/ Brown	Yellow/ Brown	Yellow/ Brown to dark purple	Yellow/ Brown	***Yellow/Brown to dark purple

@ suitable condition

* www.omicsonline.com>open-acess>effect-of-chitin-and-chitosan

**https://lejpt.academicdirect.org>characterization-of-chitin-and-chitosan

***https://www.worldwidejournals.in>ijar> article>view

Measurement (Sr. No. 1 to 6) was made at Industrial Chemistry Department's Laboratory, Yadanabon University, Mandalay

Measurement (Sr No 7) was made at The Union of Myanmar Federation of Chambers of Commerce and Industry, Yangon.

Table (4) Elemental Composition of Extracted Chitin Analyzed by Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry Technique

Sr. No.	Elements	Concentration(%w/w)
1	Calcium, Ca	20.143
2	Strontium, Sr	1.197
3	Potassium, K	0.5
4	Manganese, Mn	0.432
5	Iron, Fe	0.211
6	Bromine, Br	0.158
7	Copper, Cu	0.012
8	Zinc, Zn	-
9	Sulfur, S	-
10	Rubidium, Rb	-

Elemental composition of extracted chitin was determined by EDXRF method at Universities' Research Centre, University of Yangon.

Table (5) Characteristic Absorption Bands in FT-IR Spectra of Extracted Chitin with Different Concentrations of Acetic Acid

Sr. No.	Absorption Bands of Extracted Chitin with Different Concentrations of Acetic Acid, (M)					**Literature Value		
	0.1M	0.3M	0.5M	*0.7M	0.9M	Wave Number, cm^{-1}	Possible Assignment of Absorption Band	Nature of the Peaks
	Experimental Value of Chitin Wavelength in (cm^{-1})							
1	3425.69	3425.69	3419.9	3342.75	3421.83	3448	OH	broad
2				3269.45		3300-3250	N-H stretching	broad
3	-	2885.6	2887.53	2887.53	2891.39	2891	C-H stretching	sharp
4	1637.62	1631.83	1631.83	1662.69	1658.84	1680-1660	C=O stretching	medium
5	1485.24	1483.31	1483.31	1487.17	1483.31	1580-1530	Amide II band	medium
6	1415.8	1415.8	1413.87	1415.8	1417.73	1340	Methyl CH stretch, amide III	weak
7	1151.54	1151.54	1153.47	1151.54	1153.47	1152-1156	Glycosidic linkage, C-H stretch	very weak
8	1070.53	1068.6	1070.53	1070.53	1068.6	1072	C-O-C	weak
9	949.01	873.78	950.94	873.78	952.87	952	Amide III	medium
10	705.97	686.68	696.33	707.9	700.18	750-650	N-H	medium

* suitable condition

** https://www.researchgate.net>publication>Aug2013

Functional Groups of Crude Chitin were determined by FT IR method at Universities' Research Centre, University of Yangon.

Table (6) Characteristic Absorption Bands in FT-IR Spectra of Extracted Chitosan with Different Concentrations of Sodium Hydroxide and Commercial Chitosan

Sr. No.	Absorption Bands of Extracted Chitosan with Different Concentrations of Sodium Hydroxide, (%)			***Commercial Chitosan	****Literature		
	40	45	*50		Wave Number, (cm^{-1})	Possible Assignment of Absorption Band	Nature of the Peaks
	**Experimental Value of Chitosan Wavelength in (cm^{-1})						
1	3363.89	3315.63	3277.06	3298.28	3444.87	H-bonded NH_2 & OH stretching	broad
2	-	-	-	2864.29	2879.72	Aliphatic CH stretching	broad
3	1647.21	1658.78	-	-	1660	C=O stretching	medium
4	-	-	-	1585.49	1558	Amide II band	medium
5	1408.04	1404.18	1408.04	1373.32	1421.54	Amide C=O stretching	sharp
6	1022.27	1024.2	1022.27	1022.27	1029.99	Ring Banding	medium
7	871.82	871.82	871.82	893.04	856.39	N=O=N bending vibration	strong
8	711.73	711.73	711.73	-	713.66	C-O-C stretching	sharp
9	569	557.43	567.07	-	673.16-532.35	Pyranose bending vibration	medium

* suitable condition

**Experimental values of FT-IR spectra for extracted chitosan were measured at Defence Service Sciences and Technology Research Center

*** Tianjin Tianshi Biological Development Co., Ltd. (Address) No.16 Xinyuan Road, Wuqing Development Area, Tianjin, China (Post Code) 301700

****https://www.researchgate.net>publication>Aug2013

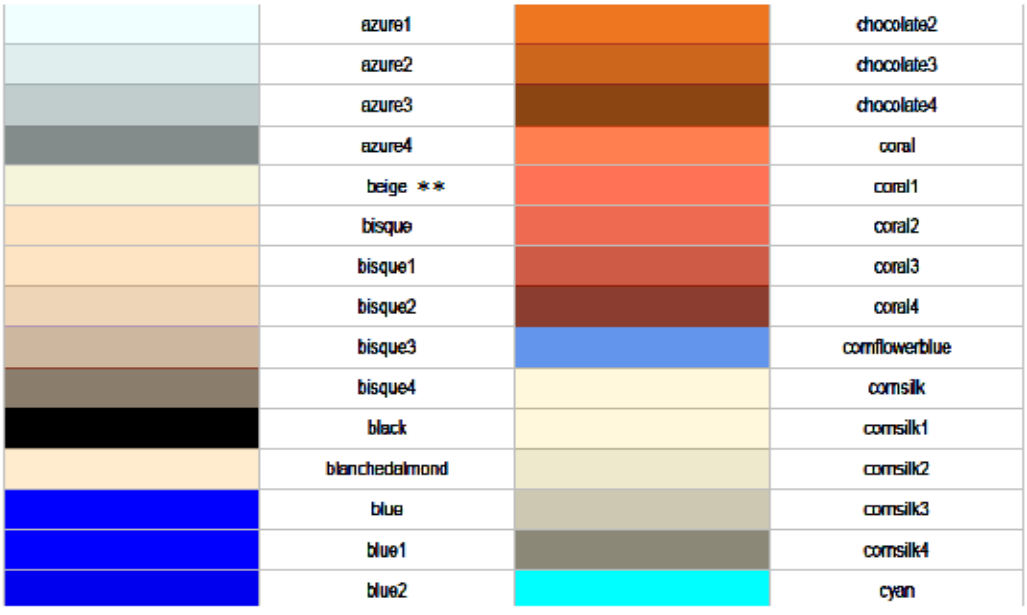

Figure (1) R-colour Chart for Comparison of Colour of Crab Shell Powder (http://www.color in R.com>pdf), **Beige = Colour of Crab Shell Powder

	hotpink1		lightcyan3
	hotpink2		lightcyan4
	hotpink3		lightgoldenrod
	hotpink4		lightgoldenrod1
	indianred		lightgoldenrod2
	indianred1		lightgoldenrod3
	indianred2		lightgoldenrod4
	indianred3		lightgoldenrodyellow
	indianred4		lightgray
	ivory ***		lightgreen
	ivory1		lightgrey
	ivory2		lightpink
	ivory3		lightpink1
	ivory4		lightpink2
	khaki		lightpink3

Figure (2) R-colour Chart for Comparison of Colour of Extracted Chitin and Chitosan

(http://www.color in R.com>pdf)

***Ivory = Colour of Extracted Chitin and Chitosan

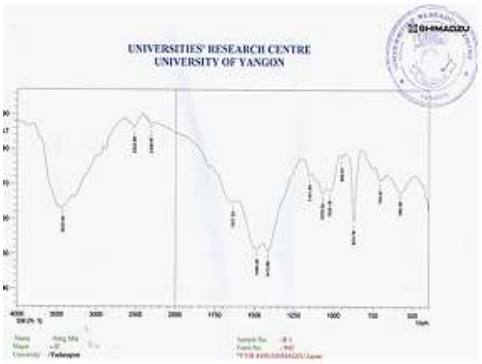

Figure (3) FT-IR Spectra of Chitin Isolated with Acetic Acid (0.1M)

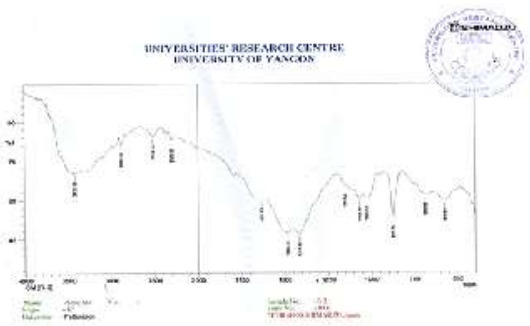

Figure (4) FT-IR Spectra of Chitin Isolated with Acetic Acid (0.3M)

Figure (5) FT-IR Spectra of Chitin Isolated with Acetic Acid (0.5M)

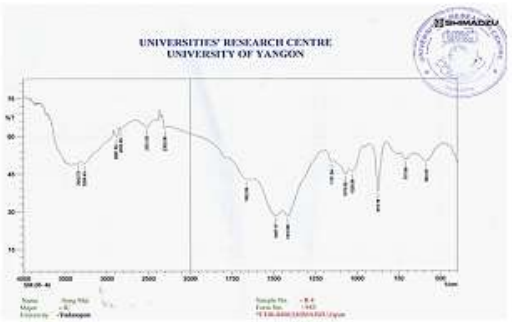

Figure (6) FT-IR Spectra of Chitin Isolated with Acetic Acid (0.7M)

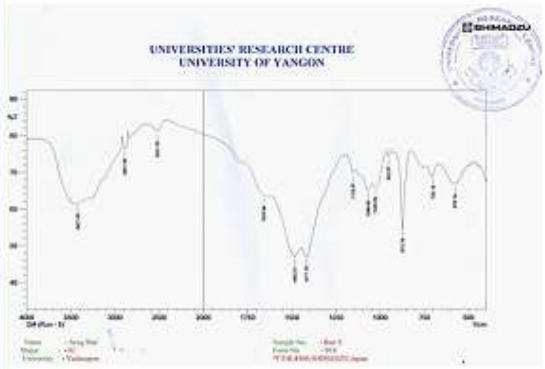

Figure (7) FT-IR Spectra of Chitin Isolated with Acetic Acid (0.9M)

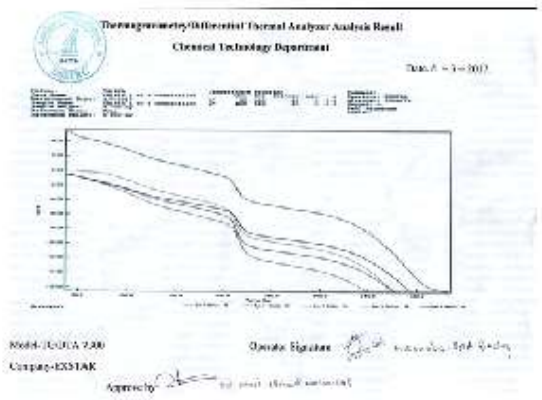

Figure (8) TG Plots of Treated Chitin with Acetic Acid (0.1M, 0.3M, 0.5M,0.7M and 0.9M)

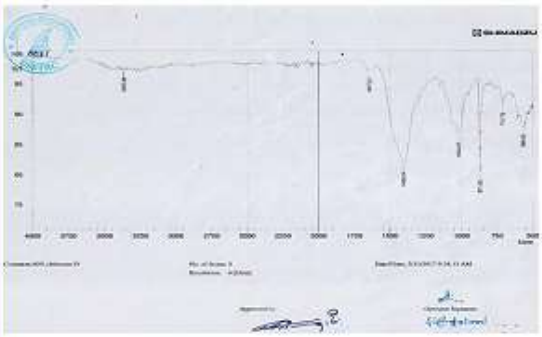

Figure (9) FT-IR Spectra of Extracted Chitosan after Deacetylation of Chitin Using 40% Sodium Hydroxide

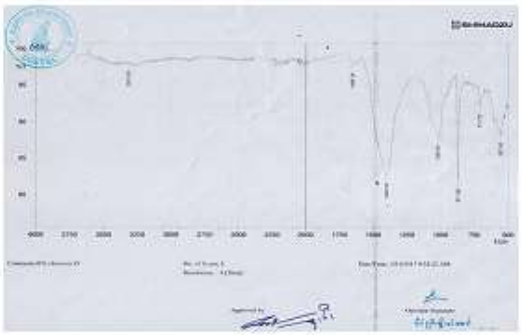

Figure (10) FT-IR Spectra of Extracted Chitosan after Deacetylation of Chitin Using 45% Sodium Hydroxide

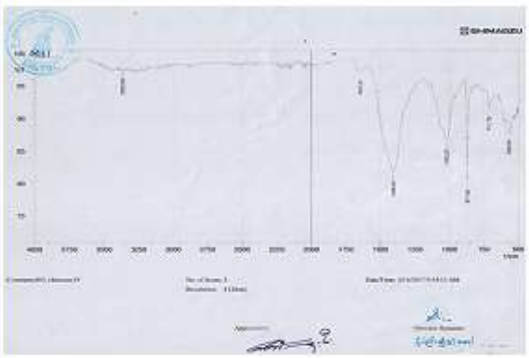

Figure (11) FT-IR Spectra of Extracted Chitosan after Deacetylation of Chitin Using 50% NaOH

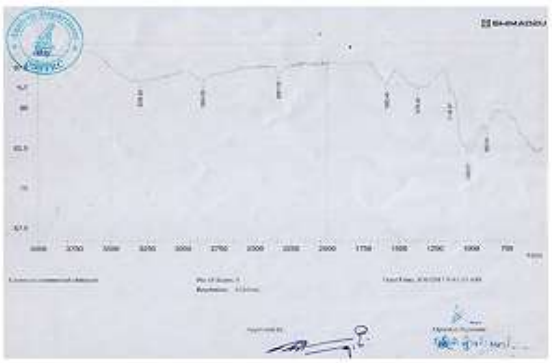

Figure (12) FT-IR Spectra of Commercial Chitosan

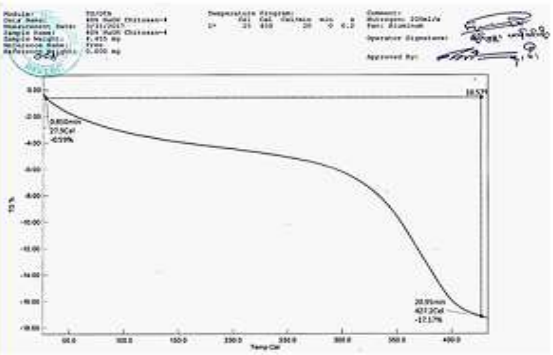

Figure (13) TG Plots of Extracted Chitosan Deacetylated with 40% NaOH in Nitrogen Atmosphere

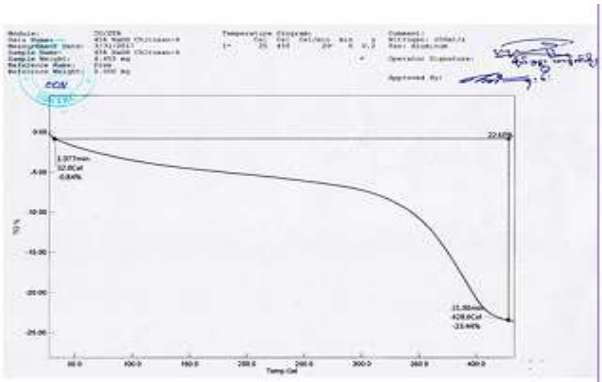

Figure (14) TG Plots of Extracted Chitosan Deacetylated with 45% NaOH in Nitrogen Atmosphere

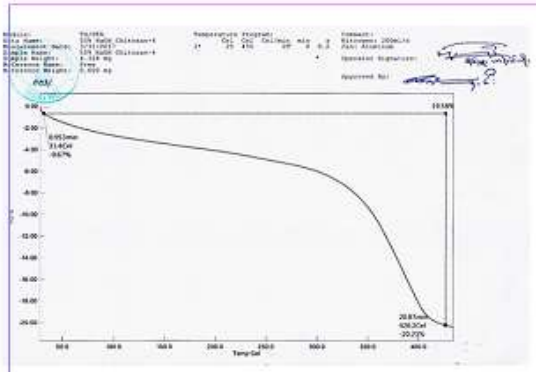

Figure (15) TG Plots of Extracted Chitosan Deacetylated with 50% NaOH in Nitrogen Atmosphere

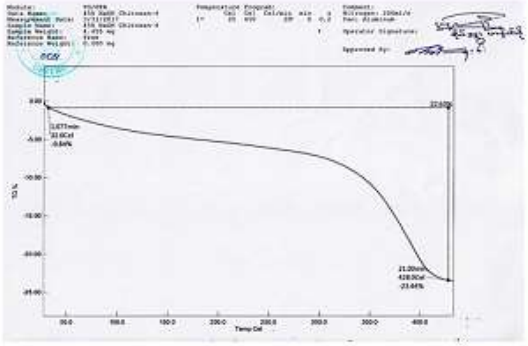

Figure (16) TG Plots of Commercial Chitosan in Nitrogen Atmosphere

Conclusion

The fishery waste from shrimp, crab and squid containing chitinous material can be processed in chitin/chitosan with high value added. Chitin/chitosan can be used in various applications from textile, cosmetics, medicine, composites and nanomaterials, and food and neutriceutical products. To promote the development of chitin-chitosan industry, extensive research activities will be carried out on the applications of chitin-chitosan .

From FT-IR analysis, the peaks indicated that crab shell was a rich source of chitosan and the chemical constituents were very much effective on textile wastewater treatment. From thermogravimetric analysis, the results indicated that the observed temperature of weight loss phenomena for commercial chitosan sample was considerably less than that of the extracted chitosan. This might be due to introduction of weak linkage into the polymer chain depending upon process condition and impurities.

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