Chitosan Synthesis from Extracted Chitin from Crustacean Shells

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Abstract **- Prawn shell, crab shell, krill shell and craw fish shell deserve special attention for remarkable production of chitin. In this work we have extracted chitin first time from mixture of these shells. An optimization study for extraction of chitin from 1.0 g of this shells mixture of crustaceans using variables like concentration of fixed volume (50 ml) of sulfuric acid and potassium hydroxide was performed and optimal conditions (50 ml 0.5M H2SO4 and 50ml 1M KOH) were resolute with maximum demineralization and deproteination with minimum weight of residue (0.287 g) under secondary treatment gave maximum chitin yield (28.60%). Structures of extracted products were confirmed through structural characterizations like FT-IR and elemental analysis. After then optimization of chitosan synthesis from 1.0 g chitin steeped in 20 ml 30% KOH using variables like time (min) and microwave power was performed and optimal conditions (11 minutes and 560 W microwave power) were resolved with maximum chitosan yield (77.6%).**

Index Terms **- Crustacean shells, Chitin extraction, Microwave illumination, Demineralization, Deproteination, Chitosan synthesis.**

I.INTRODUCTION

Chitin is a naturally copious biopolymer consisting a linear chain of 2-acetoamido-2- deoxy-b-D-glucopyranose units linked through a b (1, 4) linkage functions as structural polysaccharide. According to an estimation of published research, nearly 1010 to 1014 tons of chitin is produced annually from arthropods [1] [2] [3]. 2.8 to 1010 kg chitin from fresh water and 1.3 to 1012 kg chitin from marine ecosystem produced annually from crustaceans [3].

Chitin resides in crustacean shells [4], mollusks [5], insects, fungi and some algae [6], [1]. It can also be carried out from wastes of marine food processing industries of shrimp, krill and crab shells [7] [8] due to its high content and ready availability [9] [10] about 50–60% of the total weight [11]. However, the amount of chitin varies with species prawn shell (20%), crab shell (15%), krill shell (49%) and crawfish shell (30%) [12].

Crustacean shells are consolidated of three fundamental elements: (1) 20 to 30% chitin (serving as a skeleton), (2) 30 to 60% minerals (imparting strength to the shell), (3) 20 to 40% proteins (set the shell as a living tissue) and 0 to 14% lipids [13] [14] [15] [16] [17]. Chitin exists in three types of polymorphs in nature; α-chitin, being the most common structure and corresponding to tightly compacted alternated sheets of antiparallel chains [5] isolated from crustacean exoskeleton [18]; β-chitin, in which the polysaccharide chains are arranged in parallel fashion [19] obtained from squid pens [3]; and γ-chitin, in which arranged two parallel and one antiparallel sheet have been proposed [20] exists in fungi and yeast. In addition c-chitin can be combination of a and b structure suggested by [3].

The comparative study for extraction of chitin by chemical method (demineralization, deproteination and bleaching) [6] [21] [22] and biological method using lactic acid was done [23] from which chemical method is the most common one [24] [22].

Due to insolubility of chitin in majority of solvents its chemical modification [6] [1] [18] to chitosan by partial deacetylation is performed [25] [3] can becomes soluble in acidic aqueous solution behaving as cationic polyelectrolyte [26]. The degree of deacetylation affets its biological [27], physical and chemical characteristics [28].The numbers of method reported for the quantitative determination of degree of deacetylation in chitosan involving potentiometric titration [29], NMR methods [30] [29][31], UV

method [32] [33], Fourier transform infrared (FTIR) [34] [35], and dye adsorption method [36].

The wide range of applications more than 200 [26] of chitin and chitosan such as in medicine, pharmaceutical, personal care products, food, agriculture, environmental sectors [6] [1], cosmetics, paper industry, as absorbent materials for wastewater treatment [37] [38] [39] and as an antimicrobial film on the surface of nonwoven fabrics and plypropylene [40] [41] [42] [25] [43] due to their versatile biological activities having great economical value.

The aim of our present work is to extract chitin with cheaper and benign process from crustacean shells of marine animals obtained from bear garden, Silvassa (Dadra & Nagar Haveli) and synthesis of chitosan from it.

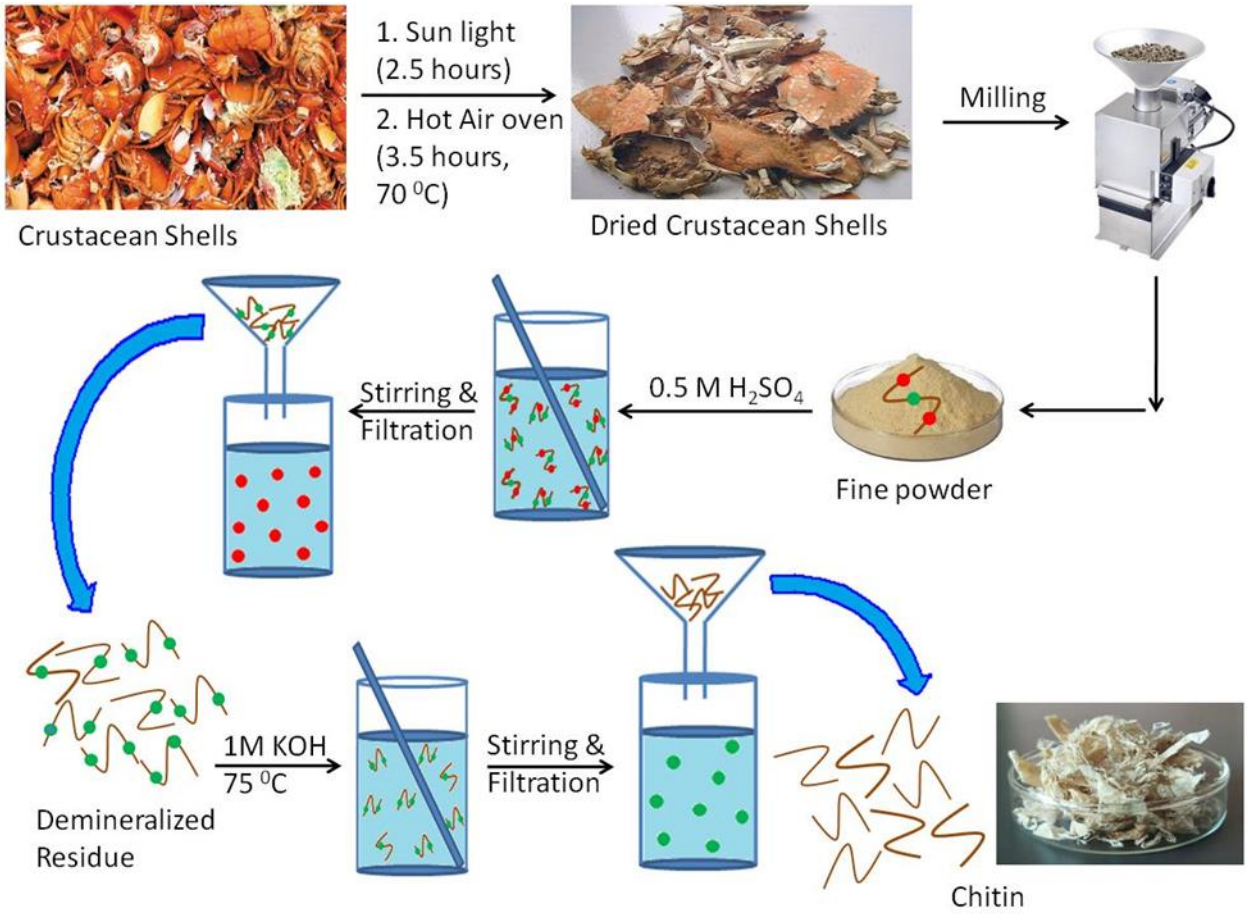
II. EXPERIMENTAL

A. Raw materials

Crustacean shells of marine animals were collected from Bear Garden, Silvassa (Dadra & Nagar Haveli). It were washed with hot distilled water and exposed to sunlight for 2.5 hours in order to expel the surface water and dried in hot air oven (BIOCRAFT, India) at 70 ^{0}C for 3.5 hours. After then milled to get fine powder and subjected to demineralization and deproteinization.

B. Chitin extraction from crustacean shells

Milled fine powder of crustacean shells was subjected to 0.5 M H_2SO_4 (50 mL/g) at room temperature and followed by stirring and then filtration. The resulting solid was washed with double distilled water to neutralize acidity and then demineralized sample was dried. Dried demineralized sample was treated with 1.0 M KOH (50 mL/g) at 75 ^{o}C several times. The removal of proteins was identified by absence of color of medium at last treatment, which was left for a night. The resulting solid residue was washed with distilled water and acetone subsequently to neutrality and to remove impurity respectively. Finally, the purified chitin was dried [12] [44].



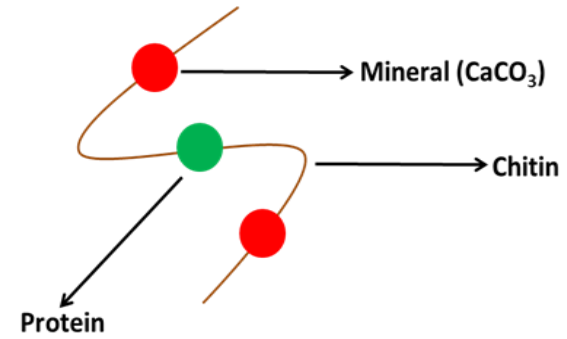


Fig1: Chitin extraction from crustacean shells

C.Chitosan synthesis from extracted chitin

Chitin was steeped in 30% KOH (20 mL/g) at room temperature for 24 hours and placed in 250 mL volumetric flask and covered tightly with cotton, then subjected to microwave irradiation (Catalyst system, CATA-RI) for 7, 9, 11, 13 and 15 min at 210, 420 & 560 W. The mixture was cooled with ice water. The solid residue was then dissolved in 5% acetic acid and reprecipitating it out in 10% KOH solution. Residues were then washed with double distilled water to neutrality and dried [12] [44].

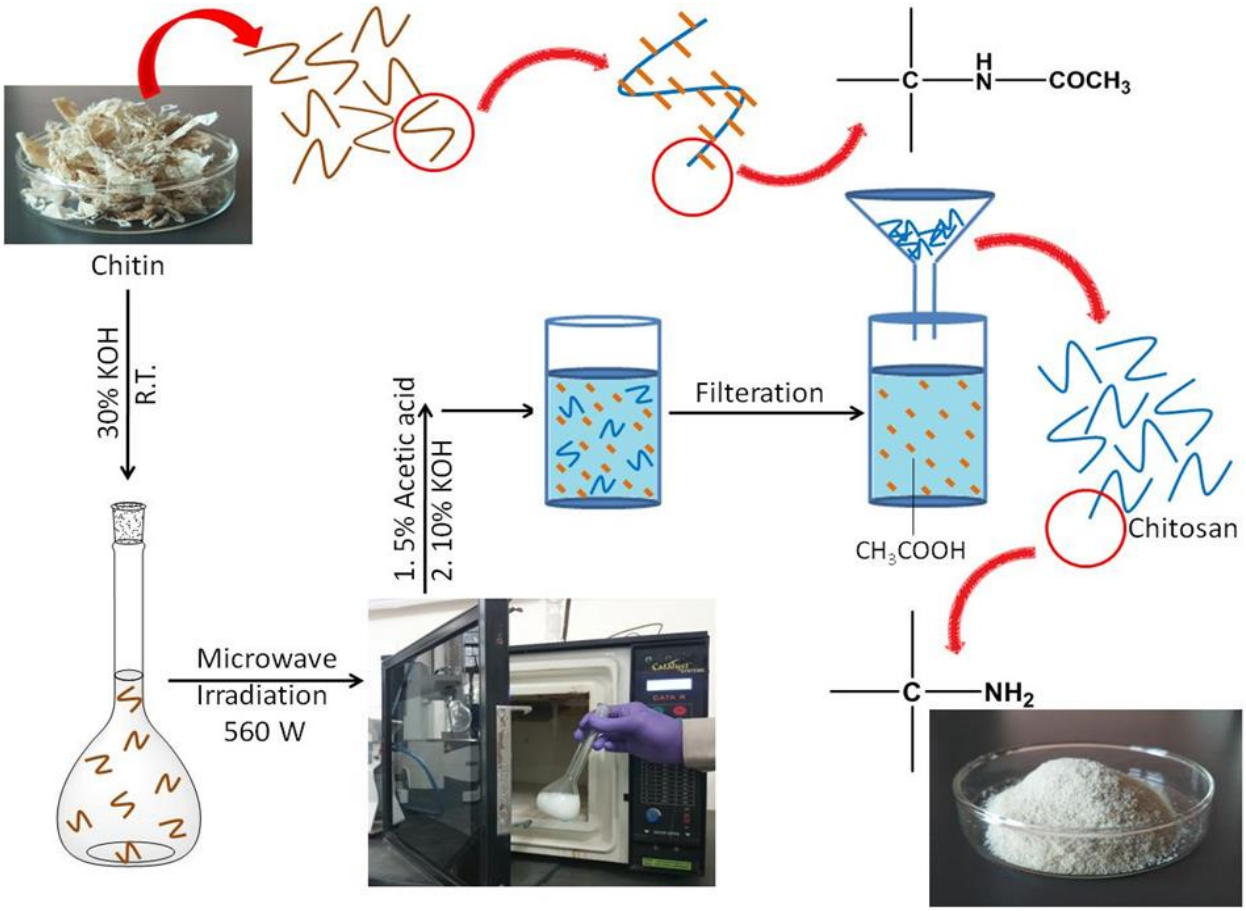


Fig 2: Chitosan synthesis from Chitin

D. Determination of chitin yield and chitosan yield

After extraction of chitin and synthesis of chitosan, the dried chitin was weighed. The chitin yield Y (%, w/w) was calculated from the following equation proposed by Li, Jia, Wei, and Liu (2012) [45].

Y (%, w/w) = (m0/m) x 100

Where, m0 (g) is the weight of dried chitin

m (g) is the weight of crustacean shells fine powder

= (0.285/1.0) x 100

= 28.50%

Y (%, w/w) = (m_0/m) x 100

Where, m_0 (g) is the weight of dried chitosan

m (g) is the weight of chitin

= (0.776/1.0) x 100

= 77.60%

C. Characterizations

SHIMADZU FTIR-8400S was employed for the FTIR spectral analysis of extracted chitin and synthesized chitosan between 400 cm^{-1} and 4000cm^{-1}. The calibration of CHNSO analyzer (Horriba EA3000) with standard sulphanilamide prior to the extracted chitin and synthesizd chitosan samples elemental analysis were done.

III. DISCUSSION & RESULT

A. Optimization of microwave assisted extraction of chitin

The first treatment of crustacean shells powder with 50ml 0.5M H_2SO_4 and 50ml 1M KOH shows that minimum weight of residue was obtained. That means maximum demineralization and deproteination were occurred in this condition than other conditions.

Table 1: Optimization of chitin yield

Sr. No.	Weight of Crustacean shells fine powder m(g)	50ml H_2SO_4 Concentration (M)	50ml KOH Concentration (M)	Weight of residue (g)	Weight of residue after Second treatment with 50ml 0.5M H_2SO_4 followed by 50ml 1M KOH m_0(g)	%Yield Weight of dried Chitin (gm)
1	1.0	0.5	0.5	0.315	0.285	**28.60**
2		1.0		0.295	0.284	
3		1.5		0.401	0.283	
4		2.5		0.321	0.285	
5		3.5		0.455	0.284	
6		**0.5**	**1.0**	**0.287**	0.286	
7		1.0		0.377	0.284	
8		1.5		0.323	0.283	
9		2.5		0.356	0.285	
10		3.5		0.401	0.285	
11		0.5	1.5	0.333	0.285	
12		1.0		0.290	0.285	
13		1.5		0.398	0.285	
14		2.5		0.322	0.285	
15		3.5		0.400	0.284	
16		0.5	2.0	0.404	0.283	
17		1.0		0.285	0.285	
18		1.5		0.291	0.285	
19		2.5		0.387	0.284	
20		3.5		0.326	0.284	
21		0.5	2.5	0.294	0.283	
22		1.0		0.342	0.285	
23		1.5		0.311	0.284	
24		2.5		0.409	0.285	
25		3.5		0.407	0.283	

B.Optimization of microwave assisted synthesis of chitosan from extracted chitin

1.0 g chitin in 20ml 30% KOH upon microwave irradiation at 560W for 11 min yields maximum chitosan product.

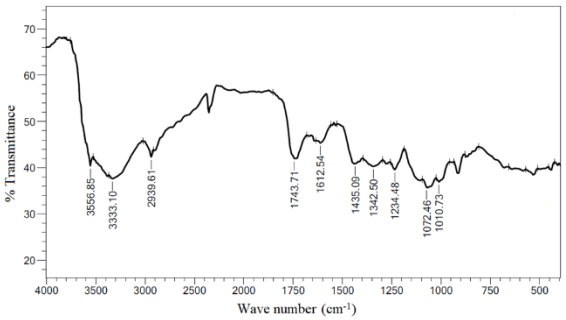
Table 2: Optimization of chitosan yield

Sr. No.	30 %KOH ml/g	Time (Min)	Power (W)	Weight of Chitosan (g)	% Yield
1	20	7	210	0.551	55.1
2			350	0.579	57.9
3			420	0.584	58.4
4			560	0.611	61.1
5		9	210	0.635	63.5
6			350	0.656	65.6
7			420	0.667	66.7
8			560	0.688	68.8
9		**11**	210	0.725	72.5
10			350	0.737	73.7
11			420	0.753	75.3
12			560	0.776	77.6
13		13	210	0.746	74.6
14			350	0.739	73.9

15			420	0.732	73.2
16			560	0.711	71.1
17		15	210	0.694	69.4
18			350	0.657	65.7
19			420	0.590	59.0
20			560	0.572	57.2

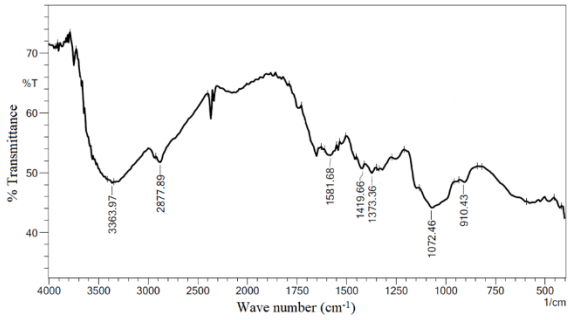
C. Characterizations

I. FT-IR characterization of Chitin



SHIMADZU FTIR-8400S was used to record the IR spectrum of chitin extracted from crustacean shells is shown in Fig.1. The chemical structure of the product was analyzed between 400 cm^{-1} and 4000cm^{-1}. Band in the region 3557 cm^{-1} corresponds to O-H stretching absorption. The band at 3333 cm^{-1} is assigned to -$COCH_3$ stretching absorption. The absorption band at 2940 cm^{-1} corresponds to -C-H stretching of pyranoid ring. The absorption frequencies at 1744 cm^{-1} and 1613 cm^{-1} are assigned to -C=O stretching (Amide-I) and -N-H stretching vibration (Amide-II) of -CONH2 group respectively. The bands 1435, 1343 and 1234 cm^{-1} correspond to -C-H bending of pyranose ring, -O-H bending (in a plane), -C-H bending of -$COCH_3$ respectively. In the finger print region bands at 1072 and 1011 cm^{-1} are assigned to stretching vibrations of glycosidic bond (C-O) and C-C bond of pyranoid ring respectively [46].

II. FT-IR characterization of Chitosan



SHIMADZU FTIR-8400S was used to record the IR spectrum of chitosan obtained by deacetylation of chitin extracted from crustacean shells is shown in Fig.1. The chemical structure of the product was analyzed between 400 cm^{-1} and 4000cm^{-1}. The FT-IR spectra of chitosan obtained by deacetylation of chitin extracted from crustacean shells is shown in Fig.1. The Band in the region 3364 cm^{-1} corresponds to O-H stretching absorption. The absorption band at 2878 cm^{-1} corresponds to -C-H stretching of pyranoid ring. The absorption frequencies at 1582 cm^{-1} is assigned to -N-H stretching of primary amine (-NH_2). The bands 1420 and 1373 cm^{-1} correspond to -C-H bending of pyranose ring and O-H bending (in a plane) respectively. The band in finger print region at 1072 and 910 cm^{-1} are assigned to stretching vibrations of glycosidic bond (C-O) and -C-N- bending of primary amine respectively.

III. Elemental Analysis of chitin

Table 3: Elemental Analysis of extracted chitin

Sr. no	*Type*	*Name*	*C %*	*H %*	*N %*	*S %*	*O %*	*Weight (mg)*
1.	Std	Sulphanilamide	42.11	4.82	18.83	22.01	12.23	0.975
2.	Std	Sulphanilamide	41.04	4.71	16.03	18.62	19.60	1.134
3.	Smp	Chitin	46.97	6.38	7.11	0.01	39.53	1.059

The elemental analysis of extracted chitin from crustacean shells was done using CHNSO analyzer (Horriba EA3000). The Elemental analyzer was calibrated with standard (Sulphanilamide) and after then sample (extracted chitin) was analyzed. Elemental analysis calculated for $(C_8H_{13}NO_5)_n$ (Chitin): C (47.29%), H (6.40%), N (6.90%), O (39.41%); found C (46.97%), H (6.38%), N (7.11%), O (39.53%).

IV. Elemental Analysis of chitosan

Table 4: Elemental Analysis of chitosan obtained by deacetylation of chitin extracted from crustacean shells

Sr. no	*Type*	*Name*	*C %*	*H %*	*N %*	*S %*	*O %*	*Weight (mg)*
1.	Std	Sulphanilamide	42.18	4.61	19.13	21.88	12.20	1.075
2.	Std	Sulphanilamide	41.17	5.01	15.99	18.73	19.10	1.136
3.	Smp	Chitosan	45.11	7.13	8.94	0.00	38.82	1.462

The elemental analysis of chitosan obtained by deacetylation of chitin extracted from crustacean shells was done using CHNSO analyzer (Horriba EA3000). The Elemental analyzer was calibrated with standard (Sulphanilamide) and after then sample (synthesized chitosan) was analyzed. Elemental analysis calculated for ($C_6H_{11}NO_4$) (Chitosan): C (44.72%), H (6.83%), N (8.70%), O (39.75%); found C (45.11%), H (7.13%), N (8.94%), O (38.82%).

IV. CONCLUSION

Two factors such as concentration of KOH and concentration of H_2SO_4 were successfully employed for the optimization of chitin yield. The extraction efficiency of chitin from crustacean shells was influenced significantly by optimizing these chemical process variables. The optimal conditions were as follow: 50ml 0.5M H_2SO_4 and 50ml 1M KOH shown maximum demineralization and deproteination giving minimum weight of residue.

And two factors such as time (min.) and microwave power (W) were successfully employed for optimization of chitosan yield. The synthesis efficiency of chitosan from extracted chitin was significantly influenced by optimizing these process variables of an eco-friendly, time saving and an efficient microwave technique. The optimal conditions were as follows: 560W microwave power and 11 minutes microwave irradiation time to 1.g extracted chitin in 20ml 30% KOH yields maximum chitosan product.

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