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A Preliminary Investigation on Deprotenization of Green Crab Shells *(Carcinus Maenas)*

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Abstract : The green crab *(Carcinus maenas)* shell is the potential source for numerous bio-products that are to be utilized in a variety of biomedical and biotechnology applications. Chitin and Chitosan are the most important materials from crab shells and its application in the design of various medical devices such as drug delivery vehicle and tissue engineering construct. Even though the well established extraction protocol for chitin/Chitosan from crab shell is available, however, there are large usage of harsh chemicals with high concentration such as 2M Sodium Hydroxide solution and 2M hydrochloric acid for chitin extraction. As a consequence, These harsh chemicals affect not only affect the quality of chitin/chitosan polymer also produce numerous environmental problems such as increasing acidity and total dissolved solids in waste water by neutralizing acid with an alkali or vice versa. The most important focus of this investigation is to contribute the interpretation of the deprotenization of the grab shells using various concentration of sodium hydroxide solution and contact time at different temperatures. The protein leached from the grab shell is measured by reading absorbance at 280 nm in UV-Visible spectrophotometer. Furthermore, the percentage removal of protein from the crab shell at various concentration of Sodium hydroxide is carried out and evaluated based on the dry weight of the crab shell. The optimized temperature of deprotenization is found and commented. In addition to that, the kinetic limitation of de-proteinization is mentioned using experimental data. To conclude, the effective de-protenization of green crab shell is carried out in 1M NaOH at 45°C for extraction of chitosan with optimum quantity from green crab shells.

Keywords: Chemical based extraction of Chitosan, Green Crab Shells, De-proteinization and Temperature.

Introduction:

Carcinus maenas (L.), Green crab, is an invasive and globally dispersed species present on both the east and west coasts of Atlantic Ocean of North America, with severe negative ecological impacts on native species (2,4,5,12). Green crabs represent a plentiful, easily-harvested, and underutilized nutrient-rich biomass, although their biomass is largely unquantified and unutilized as their use in human diets is very limited. A fishery for *C. maenas* as a delicacy for humans and as a scent for seafood-based products (8) occurs in Portugal, where the majority of the crabs is exported live to Spain for consumption or re-exports (6). The green crab has flourished in North America because it is able to tolerate a wide range of sea water temperatures and salinities and live in many types of marine habitats. This species of crab is a voracious predator that feeds on a variety of prey in-cluding soft shell clams, quahogs, mussels, and oysters. In addition, the green crab competes with other crustaceans for nutrient resources and habitat, and damages the ecosystem by digging in sediment around grass beds and disturbing the root systems.

Green Crab shells are to be utilized as a potential raw material for the production of chitin and chitosan as a biomaterial for development of medical devices and scaffolds. Chitin is the most important carbohydrate based polymer in the field of natural polymers. The main sources for chitin/Chitosan extraction are two marine crustaceans, shrimp and crabs. The de-acetylated chitin called as Chitosan is soluble in acidic aqueous media and then is used in many formulations and applications such as food, cosmetics, biomedical and pharmaceutical applications. Chitosan is a biodegradable, biocompatible and non-toxic alternative to synthetic polymers that you would find in a whole bunch of different industries. The natural compound chitin/chitosan could be used as an alternative for synthetic polymers in various developments of bioproducts such as cosmetics, toiletries and pharmaceuticals.

The chitosan is produced by chemical based extraction from crab shells which has various sequence steps namely demineralization, de-protenization, deacetylation and de-coluration/de-pigmentation. Deprotenization is the crucial step in extraction process. Because it remains obscure about binding of proteins with chitin in the crab shells. As a consequence, the deproteinization is a complex process and lack of information of about interaction between protein and chitin and its chemistry in the literature. Deprotenization by alkali method such as sodium hydroxide is a common method for removal of proteins from the shrimp shells. Based on the literature review, Sodium hydroxide is chosen as a deprotenization agent for this process. This report explains the optimized concentration of sodium hydroxide, contact time and temperature of the deproteinization process of crab shells and its limitations such process problems.

Figure. 1. European Green Crab from Sea of Atlantic Canada – Halifax, NS,Canada. (Source – The Chronicleherald.ca/business/1182507)

Composition of Green Crab Shells:

These values are taken from (1) Beth A. Fulton et al 2013 "Nutritional Analysis of Whole Green Crab, Carcinus maenas, for Application as a Forage Fish Replacement in Agrifeeds", Sustainable Agriculture Research.

Table.1. Proximate Analysis of Green Crab Shells

Proximate Component	Green Crab mince (%) Wet Basis
Moisture	67.96±0.46
Ash	16.55±0.29
Protein	12.27±0.25
Fiber	02.87±0.15
Fat	00.21±0.07

In the above table, the calcium content in the green crab shell is around 16.55% and in the de-calcification process, it should be neutralized with hydrochloric acid/ any other mineral acid. The protein content is 12.3 % and it is removed by deprotenization process. The above values can vary with source and maturity of green crab and its thickness of shell.

Table -2 –Chemical composition of Green Crab Shell

Chemical Contents	(%)
Ash	38.00
Lipids	3.23
Nitrogen	5.24
Protein	14.08
Chitin	43.9

Structure of Crab shells

The exoskeleton of the crab shells contains three distinct layers namely epicutile, exocuticle and endo cuticle. Generally, the exoskeleton has a high degree of mineralization, typically calcium carbonate as main constituent, in some case calcium phosphate. In exoskeleton, chitin fibrils are wrapped with proteins forms a form of fibers which is assembled further into a bundle of fibers in the exoskeleton. In addition to that, the calcium carbonate in the form of calcite deposited in the chitin–protein matrix. (3)

Current status of extraction process of chitosan

Most of the chemical based extraction processes for chitosan from grabs shells are involved with harsh chemicals with high concentration and temperature. For example, 2M concentration of Hydrochloric acid used for demineralization process and 2M sodium hydroxide solution for de-protenization at 100°C and exposed to 24 hrs., and these prolonged exposure of chitin and chitosan with harsh chemicals affect the quality of the polymer in terms of molecular weight and degree of acetylation. Therefore, The eco-friendly and diluted concentration of Hydrochloric acid HCl and NaOH chemical based extraction is developed for the isolation of chitosan from green crab shells.

Chemical Based Extraction processes

The procedure for the production of chitin from shells of crabs includes demineralization and de-protenization process and then chitosan is produced by deacetylation of chitin. Based structure of crab shell, the calcite/calcium carbonate should be removed the crab shells to weaken the structure of crab shells. Therefore, the demineralization is carried out first step in the extraction process.

Extraction of Chitin/Chitosan from Green Crab Shells:

Demineralization/De-calcification of crab shells is completed in 6 – 7 hrs., and treated with 0.1 M of Hydrochloric acid. It is verified by crab shells lost its brittleness. The de-mineralized Crab shells washed with deionized water to remove the residual acid content in the shell which might reduce the strength of Sodium Hydroxide solution. As a consequence, deprotenization process will be affected. De-proteinization is carried out in 1M of Sodium hydroxide solution at a temperature of 45°C for 2 hrs.

The de-proteinized crab shell washed with deionized water and it is raw chitin material. The de-acetylation process is carried out in 50% wt. Sodium Hydroxide solution at a temperature of 95 C for 2- 3 hrs under nitrogen atmosphere. The de-acetylated crab shells are washed with hot deionized water to remove the sodium hydroxide solution. The washed de-acetylated crab shell are treated with 95% Ethyl alcohol to remove the pigments present in the shell and then washed with deionized water.

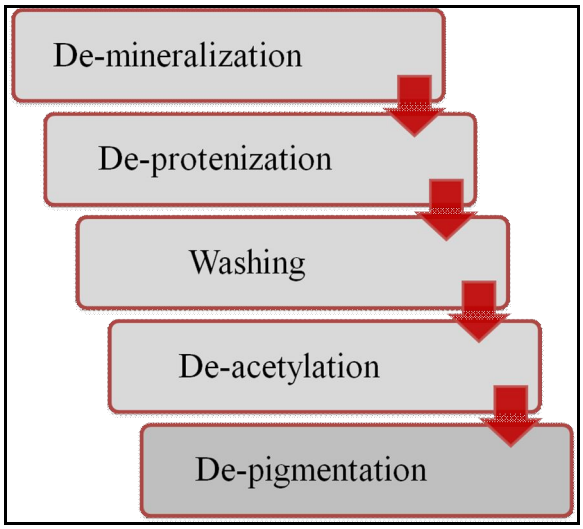

Figure. 2. Sequential Steps in Extraction of Chitin/Chitosan from Green Crab Shells

2. Process Development of Extraction of Chitin/Chitosan from Green Crab Shells

Demineralization

The demineralization process is carried out in 0.1 M hydrochloric acid and it has taken 6-7 hrs., to neutralize the calcium carbonate or calcite in the crab shells. This process has been developed and contributed by Dr. John Young, Professor Emeritus, Advanced Inorganic Chemistry, SMU, Halifax, NS, Canada. In my point of view, after completion of demineralization of green crab shells, the crab shells are filmy and lost its brittleness. Therefore, it is confirmed that the demineralization of crab shells are completed.

Washing

The presence of residual acid content in the demineralized shell should be removed and it will decrease the strength of sodium hydroxide solution and performance of deprotenization could be affected. The demineralized crab shells are washed with de-ionized water to remove the residual acid content and then crab shell are trimmed and crab trimmings are also collected as secondary sources of chitosan.

De-protenization

Deprotenization is the crucial step in extraction process. Because it remains obscure about binding of proteins with chitin in the crab shells. As a consequence, the deproteinization is a complex process and lack of information of about interaction between proteins and chitin and its chemistry in the literature. Deprotenization by alkali method such as sodium hydroxide is a common method for removal of proteins from the shrimp shells. Based on the literature review, Sodium hydroxide is chosen as a de-protenization agent for this process. This step explains the optimized concentration of sodium hydroxide, contact time and temperature of the deproteinization process of crab shells and its limitations such process problems.

De-proteinization Process

The demineralized crab shell is washed with deionized water to remove the acidity present in the crab shell. The residual acidity in crab shell is removed by washing with water and otherwise it reacts and neutralize with sodium hydroxide solution which lowers the strength of NaOH solution. The demineralized shells are treated with various concentrations of sodium hydroxide solution, contact times and temperature. The 2 ml of sample from the de-protenized solution is taken every hour and measured the absorbance at 280nm in UV-Visible spectrophotometer.

Experimental Methods:

Measurement of Protein content in the sample:

Absorption Assay:

The protein in the solution absorb at 280 nm due to the presence of aromatic amino acids in the proteins. Therefore, quantification of the amount of protein in a solution is possible in a simple UV-Visible spectrometer. Absorption of radiation in the near UV by proteins depends on the Tyr and Trp content (and to a very small extent on the amount of Phe and disulfide bonds). Therefore, Absorbance at 280 nm varies greatly between different proteins. Total protein content in the sample is determined by standard curve of BSA proteins.(9,10)

Preparation of Stock Solution of BSA as standard:

Bovine Serum Albumin used as standard protein and dissolved in deionized water at a concentration of 25 mg/ml for preparation of working standard solutions. The working standard is prepared at concentration of 1mg/ml, 0.8mg/ml,0.6mg, 0.4mg/ml and 0.2mg/ml. The absorbance of a protein solution is measured at 280 nm. The experiments have been performed in duplicate in order to get consistent data.

Deproteinization Process:

The demineralized crab shell is washed with deionized water to remove the acidity present in the crab shell. The residual acidity in crab shell is removed by washing with water and otherwise it reacts and neutralize with sodium hydroxide solution which lowers the strength of NaOH solution.The de-mineralized shells are treated with various concentrations of sodium hydroxide solution, contact times and temperature. The 2 ml of sample from the de-protenized solution is taken every hour and measured the absorbance at 280nm.

Result and Discussion:

Standard Curve of BSA:

Using the above chart, the concentration of protein present in the sample has been evaluated. The standard graph is used to estimate protein released from the crab shell.

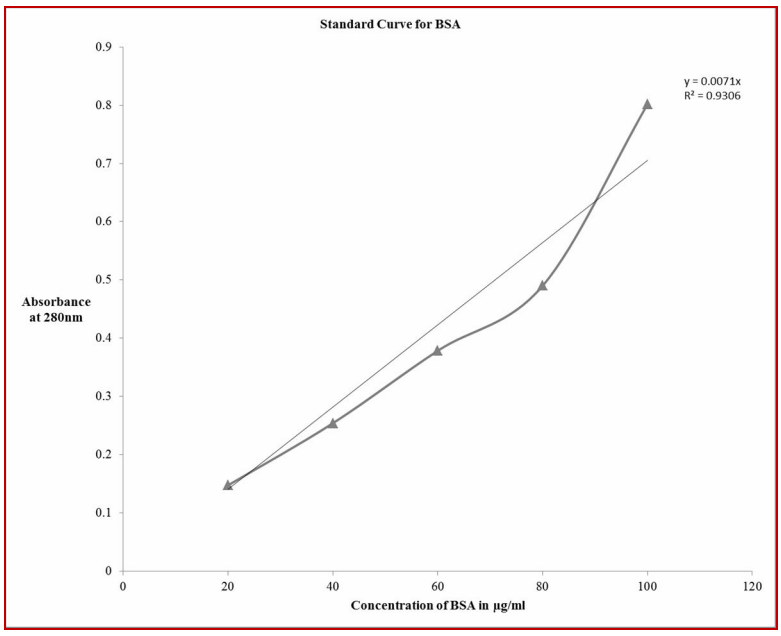

Figure.3. Standard Curve for BSA. In the graph, R^2 = 0.9306. The data is valid for investigation

Deprotenization at 45 °C:

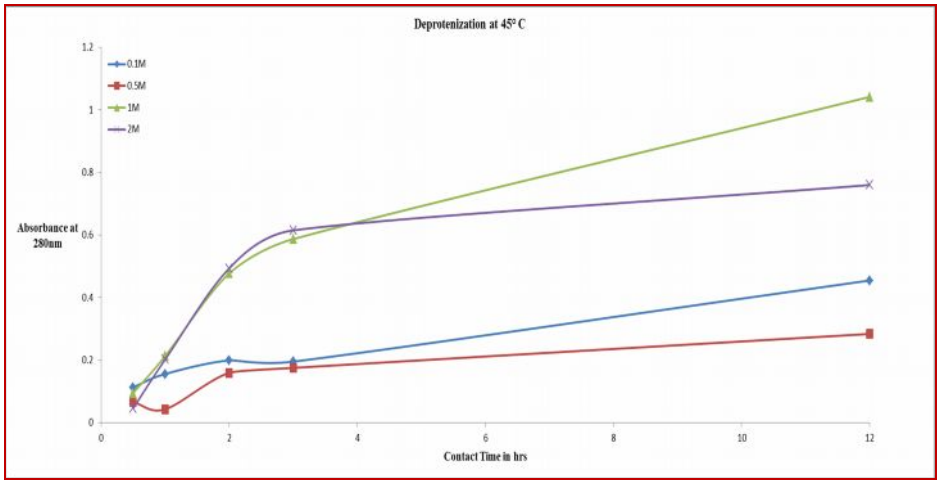

Figure. 4. Protein release in the sample expressed in the absorbance at 280nm vs treating time.

In the figure 3, the amount of protein released is higher in the contact time 3 hr. and it is same for 1M and 2M concentration of NaOH. However, in this experiment, there are some limitations. The demineralized shell is treated with various concentration of sodium hydroxide solution at 45 c in beaker and kept in hot air oven set at 45° C -50 °C. The deprotenization is a mass transfer limited reaction and therefore active mixing is required for better leaching of proteins from the shell. In addition, the prolonged treatment with sodium hydroxide solution enables removal of some pigments from the crab shell and colorizes the solution that gets chance to increase the absorbance.

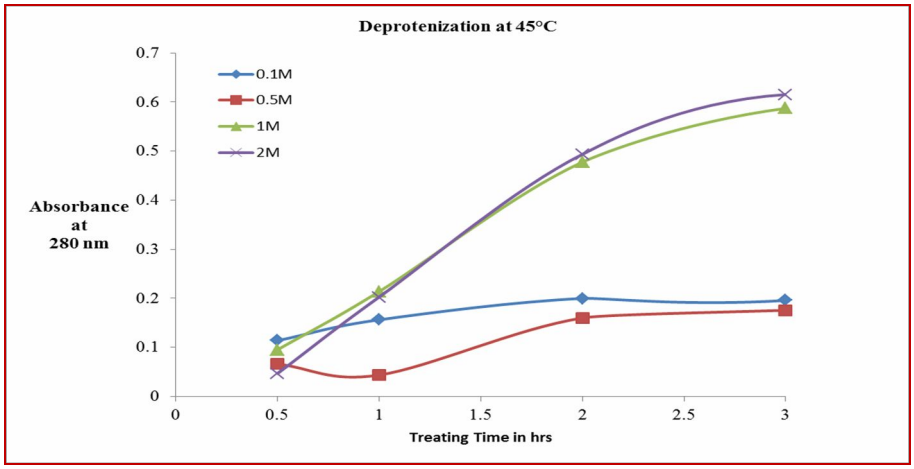

Figure.5. Protein release in the sample expressed in absorbance at 280nm Vs Treating Time in hrs.

In the above graph, the optimized treating time is 3 hrs for 1M and 2M NaOH. It can be reduced by effective mixing in the vessel.

Table 3. De-protenization at Temperature of 45°C -Batch -1:

Notes	2M -NaOH	1M-NaOH	0.5M-NaOH	0.1M-NaOH
Initial moist Weight (g)	1.27198	1.3528	1.2387	1.3565
Initial Dry Weight (g) Before Deprotenization	0.9044	0.788	0.8028	1.0137
Moisture Content (%)	28.884	41.75	35.19	25.27
Moist wt. after 30 mins(g)	0.9044	0.788	8028	1.0137
Moist wt. after 60 mins(g)	0.6437	0.5492	0.5329	0.7466
Moist wt. after 90 mins (g)	0.4594	0.4064	0.3742	0.5467
Moist wt. after 120 mins (g)	0.3577	0.3371	0.3098	0.4285

Moist wt. after 180 mins (g)	0.3073	0.307	0.28385	0.3561
Dry weight before deprotenization (g)	0.3421	0.3036	0.28385	0.3427
Dry Weight after Deproteinization (g)	0.3003	0.2991	0.2813	0.2887
Percentage Loss in weight	12.24	1.48	0.898	15.75
Percentage Removal of Protein from Crab shell	12.24	1.48	0.898	15.75

Table 4. De-protenization at Temperature of 45°C -Batch -2:

Notes	**2M -NaOH**	**1M-NaOH**	**0.5M-NaOH**	**0.1M-NaOH**
Initial moist Weight (g)	1.9393	1.7069	1.4384	1.8608
Initial Dry Weight (g) Before Deprotenization	1.7352	1.4709	1.1633	1.5941
Moisture Content (%)				
Moist wt. after 30 mins(g)	1.7352	1.4709	1.1633	1.5941
Moist wt. after 60 mins(g)	1.5184	1.2328	0.8772	1.3091
Moist wt. after 90 mins (g)	1.3328	1.0579	0.6766	1.0827
Moist wt. after 120 mins (g)	1.1883	0.9027	0.5407	0.913
Moist wt. after 180 mins (g)	0.8835	0.50775	0.3777	0.6669
Dry weight before deprotenization (g)	0.8083	0.3857	0.3281	0.6185
Dry Weight after Deproteinization (g)	0.7469	0.2991	0.2499	0.5507
Percentage Loss in weight	7.59	22.24	23.83	10.96

Deprotenization at 65 °C±5:

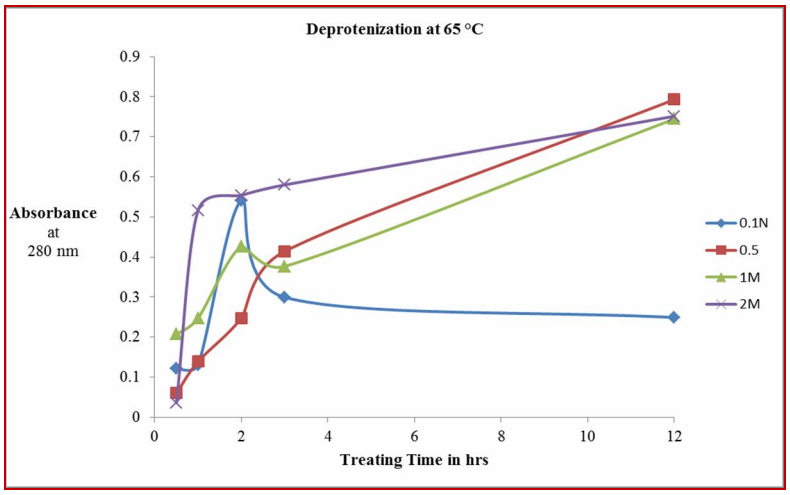

Figure. 6. Protein release in the sample expressed in absorbance at 280nm Vs Treating Time in hrs.

In this case, the amount of protein leached is high and same at 12 hr for 0.5 M,1M and 2M NaOH concentration. However, the absorbance at 3 hrs is not much high and acceptable.

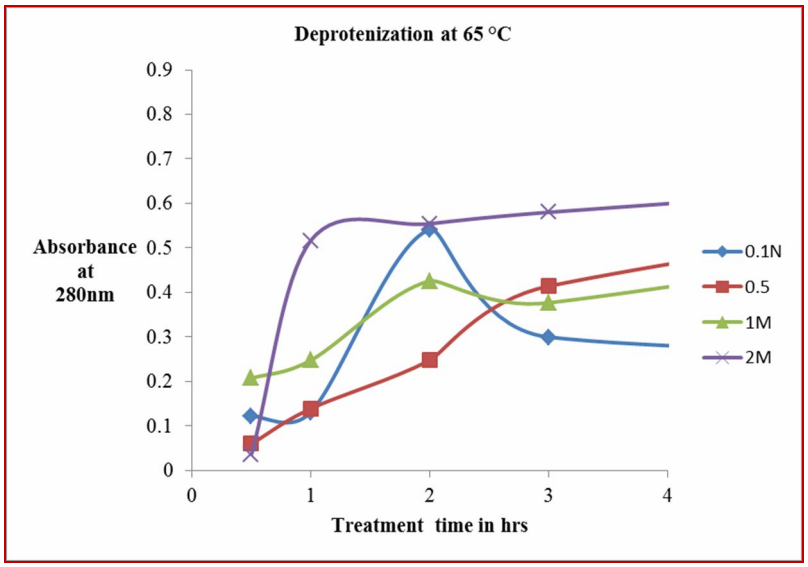

Figure. 7. Protein release in the sample expressed in absorbance at 280nm Vs Treating Time in hrs.

In the above graph, the optimized treating time is 3 hrs for 2M and 2M NaOH. It can be reduced by effective mixing in the vessel.

Table.5. – De-proteinization at 65 °C

Notes	2M -NaOH	1M-NaOH	0.5M-NaOH	0.1M-NaOH
Initial moist Weight (g)	1.4926	1.3416	1.4341	1.3107
Initial Dry Weight (g) Before Deprotenization	1.0114	0.9838	1.1041	0.9675
Moisture Content (%)				
Moist wt. after 30 mins(g)	1.0114	0.9838	1.1041	0.9675
Moist wt. after 60 mins(g)	0.476	0.5915	0.5623	0.4666
Moist wt. after 90 mins (g)	0.3599	0.3175	0.331	0.2766
Moist wt. after 120 mins (g)	0.3569	0.2922	0.3268	0.2732
Moist wt. after 180 mins (g)	0.353	0.288	0.3212	0.2695
Dry weight before deprotenization (g)	0.353	0.288	0.3212	0.2695
Dry Weight after Deproteinization (g)	0.3327	0.258	0.2555	0.2478
Percentage Loss in weight	5.75	10.41	20.45	

Deprotenization at 85 °C:

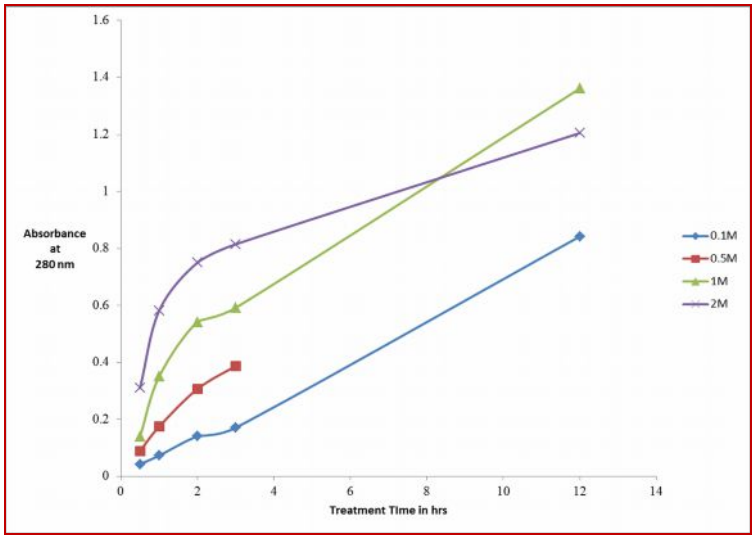

Figure. 8. Protein release in the sample expressed in absorbance at 280nm Vs Treating Time in hrs.

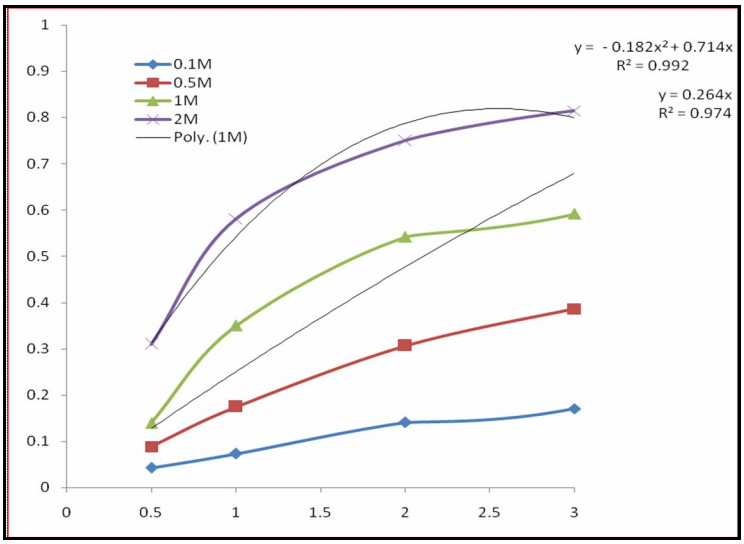

Figure. 9. Protein release in the sample expressed in absorbance at 280nm Vs Treating Time in hrs.

In this case, the absorbance at 2 hrs is almost equal to absorbance at 3 hrs. 1 M is the optimized concentration. Due to high temperature, the decolration of the crab shell is seen and increase the absorbance at 280nm. But generally, the protein can be denatured at higher temperature.

Table.6. - Deprotenization at 85 C:

Notes	2M -NaOH	1M-NaOH	0.5M-NaOH	0.1M-NaOH
Initial moist Weight (g)	1.6339	1.2528	1.2739	0.9654
Initial Dry Weight (g) Before Deprotenization	0.8122	0.5362	0.5078	0.2828
Moisture Content (%)	50.29	57.2	60.138	70.1
Moist wt. after 30 mins(g)	0.8122	0.5362	0.5078	0.2828
Moist wt. after 60 mins(g)	0.3609	0.2701	0.2686	0.2067
Moist wt. after 90 mins (g)	0.3679	0.2739	0.2739	0.2066
Moist wt. after 120 mins (g)	0.3672	0.2736	0.2736	0.2061
Moist wt. after 180 mins (g)	0.3649	0.2714	0.2713	0.2034

Dry weight before deprotenization (g)	0.3649	0.2714	0.2713	0.2034
Dry Weight after Deproteinization (g)	0.3268	0.2675	0.2472	0.1933
Percentage Loss in weight	10.44	1.43	8.88	4.96

In the above experiments, there is no active mixing in the beaker and the experiment is carried out in hot air oven to maintain the temperature. As a result, treating time is extended.

Deprotenization in 1M NaOH at 45 -50°C:

The 37 g of demineralized crab shell is treated with 1 liters of 1M NaOH solution in the beaker and the mixing is provided by magnetic stirrer. The temperature is maintained 45 -50 C. The sample is collected every hour and the absorbance is measured at 280 nm.

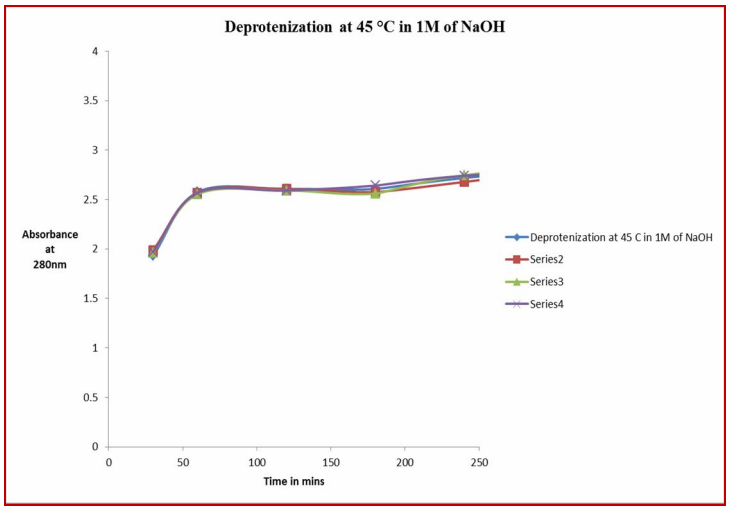

Figure.10. Deprotenization of Crab Shells at 45°C at Lab Scale Batch stirred tank reactor.

In this experiment, the absorbance is almost constant after 60 mins. So mixing is decreased the treatment time.

Influence of Temperature on Process of Deprotenization:

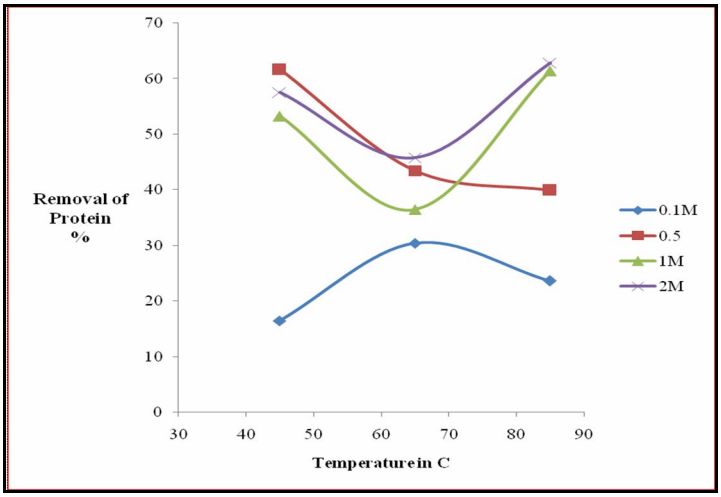

Figure. 11. Temperature Vs Removal of Proteins in % for 3 hrs treatment with various concentration of NaOH.

In above figure, Temperature 45 °C favors high de-protenization at concentration of 0.5 -2 M of NaOH. In the case of 1M and 2M concentration of NaOH, De-protenization is better at a temperature of 85°C, However, high temperature de-protenization may cause the affecting of quality of chitin polymer.

Further Discussion:

The performance of de-protenization by chemical method such as sodium hydroxide depends the crab shell thickness, concentration of NaOH, treatment time, temperature and effective mixing. Based on these investigations, 1 M concentration of NaOH at temperature of 45 C is suitable for deprotenization of green crab shells for treatment time of 3 hrs. However, the thickness of the shell and its protein content (usually 10%) plays major in performance. The literature reported that deprotenization of shrimp shell is performed with 1M NaOH for treatment time of 24 hrs. But these experiments are performed in 200 ml beakers and effective mixing doesn't influence on the deprotenization process. Moreover, Shrimp shells are flimsy in nature and thinner than crab shell. In our case with crab shell which is thick shells, Good contact with NaOH solution is required and it can be provided by effective mixing.

Limitations and Recommendations:

- Absorption assay at 280nm is a simple method for finding protein releases from the crab shell. BSA is not a suitable marker for evaluation of crab shell proteins in the solution. But it used to find the total protein content in the solutions. Further, micro syringe is used for preparation of working standard solution of BSA. I am not sure about how precise is it? Micro pipette is highly recommended for protein analysis.
- Active Mixing or stirring is provided for deprotenization process to minimize the treatment time. In addition, sometime there is a fluctuation in temperature in Hot air oven.
- The complete chemical analysis of grab shell is highly recommended for various analytical purposes.
- Various scientific approach on de-protenized shell such as SEM, Nitrogen estimating method should be performed

De-acetylation:

The deacetylation process is followed as per previous protocol which is reported earlier for isolation of chitosan from crab shells. It is carried out in 50% wt. sodium hydroxide solution with inert atmosphere usually nitrogen blanketing at temperature of 95±2°C for 2–3 hrs. The crab shells are washed with hot deionized water in every hour of de-acetylation process. For the case of de-protenated green crab shells, it is in the planning to find the extent of deacetylation process and to optimize the temperature of deacetylation process.

Washing

The deprotinated crab shells are treated with 50% wt. NaOH solution for deacetylation process. After completion of deacetylation, the crab shells are soapy in nature and washed with hot deionized water to remove the residual sodium hydroxide solution.

Depigmentation

The pigments present in the washed deacetylated chitosan are treated with 95% ethanol in the ratio of 1:5 (crab shell: ethanol) for 30-60 mins. After completion of depigmentation process, the depigmented – deacetylated chitosan (crab shells) are thoroughly washed with deionized water to remove the residual ethanol.

Drying:

The deacetylated chitosan from green crab shells are dried in hot air oven at a temperature of 50-60°C to remove the residual moisture. The dried deacetylated crab shells are stored in desiccator for characterization studies.

Yield

In demineralization, More than 40% loss is observed due to decalcification with 0.1 M hydrochloric acid. However, Dr. John Young has technical data about these processes.

Deprotenization Yield

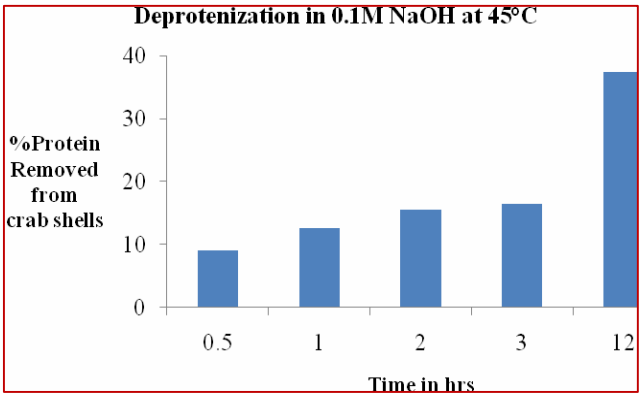

Figure. 12. Percentage of Protein removed in 0.1 M of NaOH in crab shell at 45 °C

In the above figure, Deprotenization is carried out in 0.1M concentration of NaOH solution at temperature of 45 C. The protein removed from the crab shells is calculated by the difference between the amount of protein in the 0.1M solution and the amount of protein retained in the crab shell divided by total protein present in the crab shell which to be considered as 10% wt. of crab shell on dry basis. In the above case, 35 percent of protein only removed from the crab shell in 12th hour.

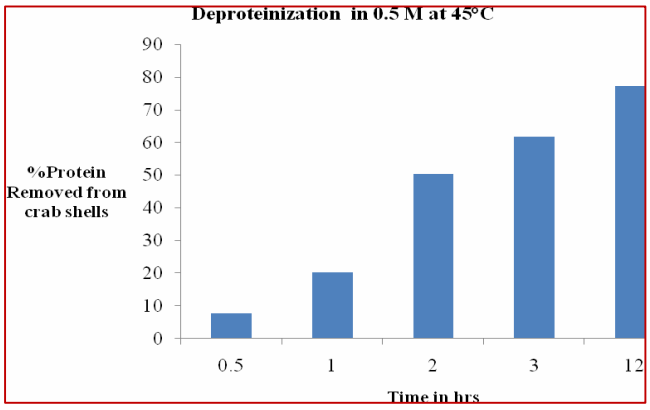

Figure. 13. Percentage of Protein removed in 0.5 M NaOH solution crab shell at 45 °C

In the case with 0.5 M NaOH deprotenization,75 % of proteins from the crab shells are leached into the solution. Therefore, Deproteinization is carried out in 1 M solution of NaOH.

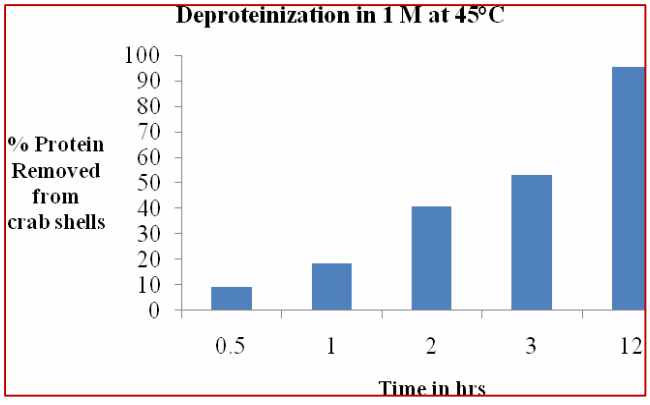

Figure. 14. Percentage of Protein removed in 1 M NaOH solution crab shell at 45 °C

In the case with 1M Solution of NaOH de-protenization, 95 % of protein is removed in 12th hour.

Further, the effective mixing is needed to minimize the time consumption for deprotinization process.

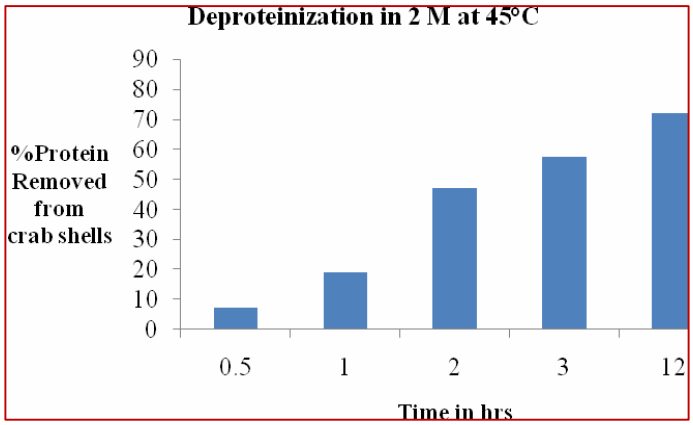

Figure. 15. Percentage of Protein removed in 2 M NaOH solution crab shell at 45 °C

In the case with 2M solution, the protein removal percentage is lesser than 1M concentration.

Influence of Temperature on Deprotenization:

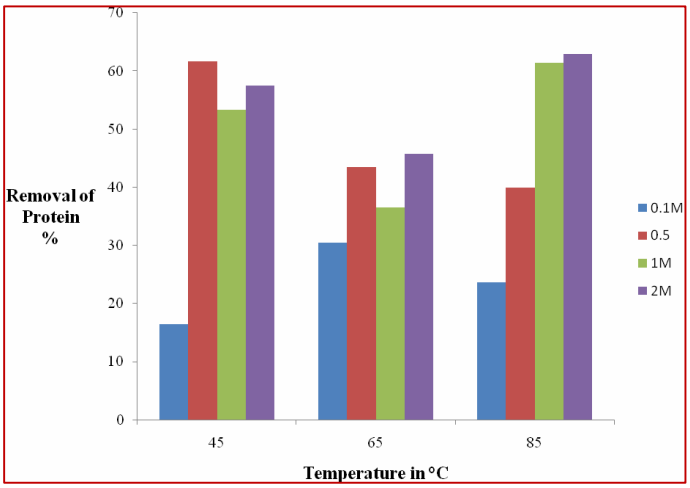

Figure. 16. Effect of Temperature on Percentage of Protein removed from crab shell

As temperature influence the extraction process, Low temperature favors more efficient deproteinization process with 0.5 and 1 M concentration of sodium hydroxide solution. Further, high temperature causes decrease the quality of chitosan and denature the protein that means loss of its quaternary structure.

Deacetylation:

In this process, there is no change in weight loss and is an intramolecular reaction and release of acetyl group from the chitin molecule to become chitosan. At present, the reported deacetylation protocol is used for extraction of chitosan from green crab shells. The extent of deacetylation process is in the planning stage and to find the marker for deacetylation process.

Depigmentation:

It is reported that the amount of pigments present in the crab shells are 43 mg of pigments per 100g of crab shells. Therefore, there is no much weight loss in the depigmented -deacetylated crab shells.

Drying

The depigmented and deacetyalted chitosan contains 70-80%wt. moisture due to wet processing of chitosan. Once it undergoes drying process to retain 10-20% wt. of chitosan on dry basis.

Yield

100 g of raw green crabs processed for recovery of chitosan is retained 5 gram of chitosan excluding crab trimmings. In addition to that, there is no much weight loss in deproteinization, deacetylation and depigmentation. But drying of final product allows 70 -80% weight loss due to removal of moisture.

Limitations and Recommendations

Demineralization:

- The molecular weight and viscosity of demineralized crab shells (chitin) should be analyzed. Then FTIR analysis of chitin is recommended to find the presence of acetyl group in the molecule.

Deprotenization:

- Absorption assay at 280nm is a simple method for finding protein releases from the crab shell. BSA is not a suitable marker for evaluation of crab shell proteins in the solution. But it used to find the total protein content in the solutions. Kjedahl method should be used to find the residual protein present in the deprotinated crab shells.
- Active Mixing or stirring is provided for deprotenization process to minimize the treatment time. Crab shells are flimsy in nature after demineralization. Therefore, it is easily settled in bottom of vessel. So that bottom driven mixing is recommended to avoid settling than top driven.

Deacetylation:

- The extent of deacetylation process is to be performed and then the concentration of sodium hydroxide solution and operating temperature are to be optimized for the case of green crab shells.
- The analytical method for release of acetyl group from the crab shells are utilized for optimization of deacetylation process.

Depigmentation and Discoloration:

- At present, 95% Ethanol is used as solvent for leaching of pigments from the crab shells. The main constituent of pigments in the crab shells is astaxanthin. It is non polar compound and has medicinal value which used as anti-oxidant. It is necessary to recover the pigments from solvent.
- Depigmentation by 95% Ethanol is cost expensive process. To reduce the cost, the solvent is to be formulated for depigmentation process.

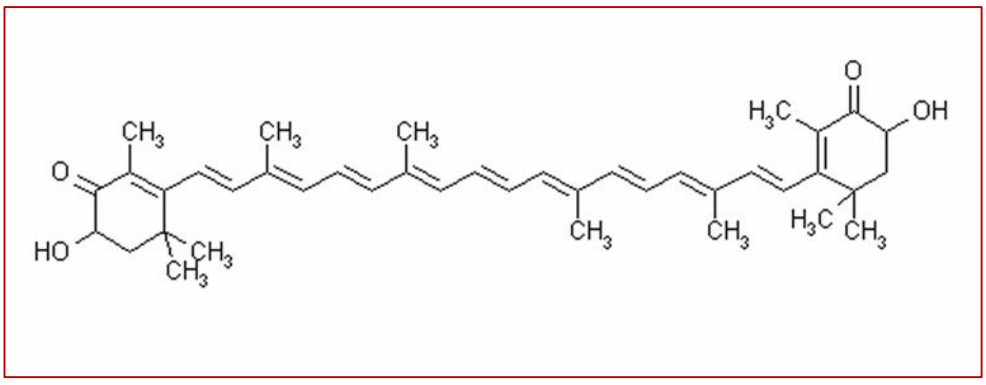

Fig 17**: Structure of astaxanthin**

Suggested Solvents for Depigmentation:

- Non Polar solvents are recommended and easy to recycle in the process. Acetone, Diethyl ether, petroleum ethers or eco-friendly solvents.
- To improve the appearance of chitosan, the chitosan should be bleached with hydrogen peroxide, Sodium hypo chloride solutions and potassium permanganate followed by oxalic acid treatment.

Drying

- The low temperature drying is strongly recommended to retain the quality of chitosan. Vacuum drying is suggested for drying at lower temperature and meantime more efficient removal of moisture from crab shell.

Grinding

- In this process, the carapace of the crab shells during processing is maintained and then should be ground for better storage. As it is flimsy, it needs better grinding methods.

Other factors

- There are various factors affecting the extraction of chitosan from green crab shells such as thickness of the shell, size of carapace and its maturation in terms of calcification in the shell and its contents.

5. Conclusion

The chemical based extractions of chitosan from green crab shells are developed. The demineralization and deproteinization are optimized and controlled for efficient removal of calcium and proteins from the shells. In NaOH 1 M as reactive, the deproteinization process is satisfactory and 1-2 hours are necessary to perform a satisfying de-proteinization process. Moreover, to improve the extraction process, various recommendations are given.

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