A Modified Process For Deprotenization of Green Crab Shells (*Carcinus maenas*) For Extraction of Chitin/Chitosan

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Introduction

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Green Crab Shell – A Potential Source for Chitin/Chitosan

Table 1 – Proximate analysis of Green grab 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

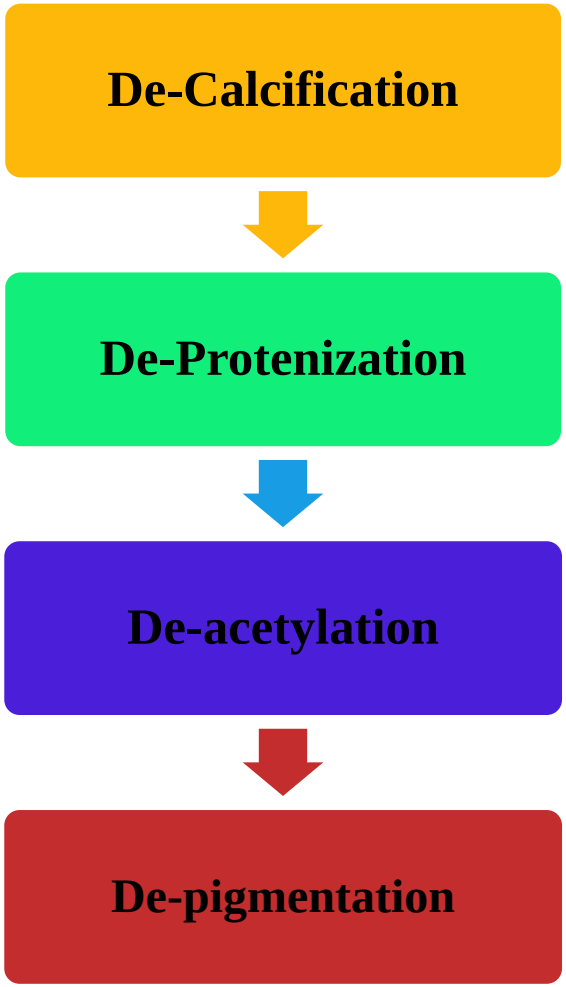
* These value are taken from Beth A. Fulton *et al 2013 "Nutritional Analysis of Whole Green Crab, Carcinus maenas, for Application as a Forage Fish Replacement in Agrifeeds",Sustainable Agriculture Research.*

Chemical Composition of Green Crab Shells

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

1. These values are estimated on the 60 mm carapace width of crab.

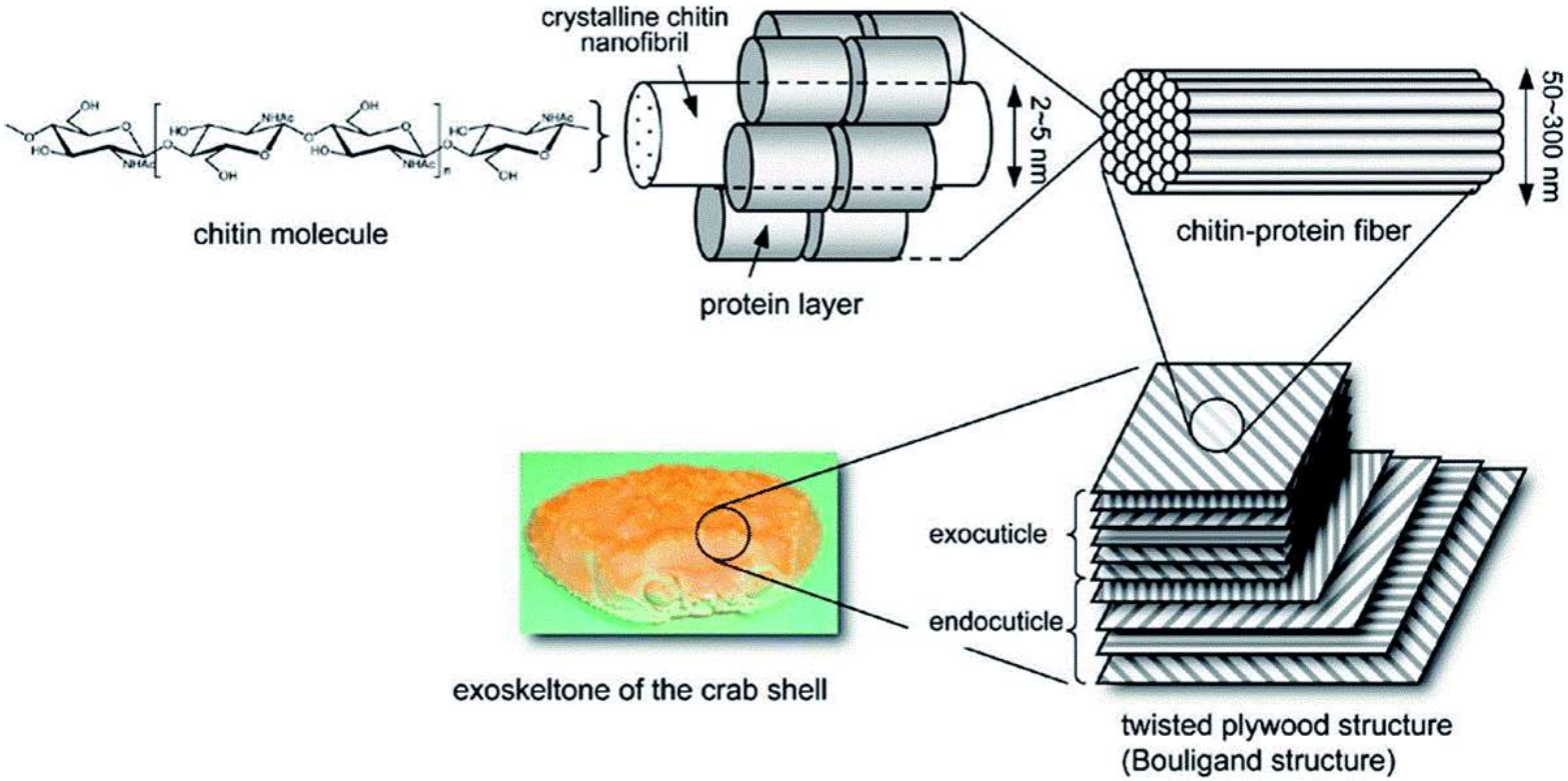
Extraction of Chitin/Chitosan



Limitations in Existing & Previous Methods

- High concentration of Hydrochloric Acid and Sodium Hydroxide (Harsh Chemicals),
- High Temperature.
- It would affect the quality of Chitin/Chitosan Polymer – Molecular Weight and Viscosity,
- Environmental Problems for Treating Waste Water- TDS and Acidity etc.

Structure of Crab Shell



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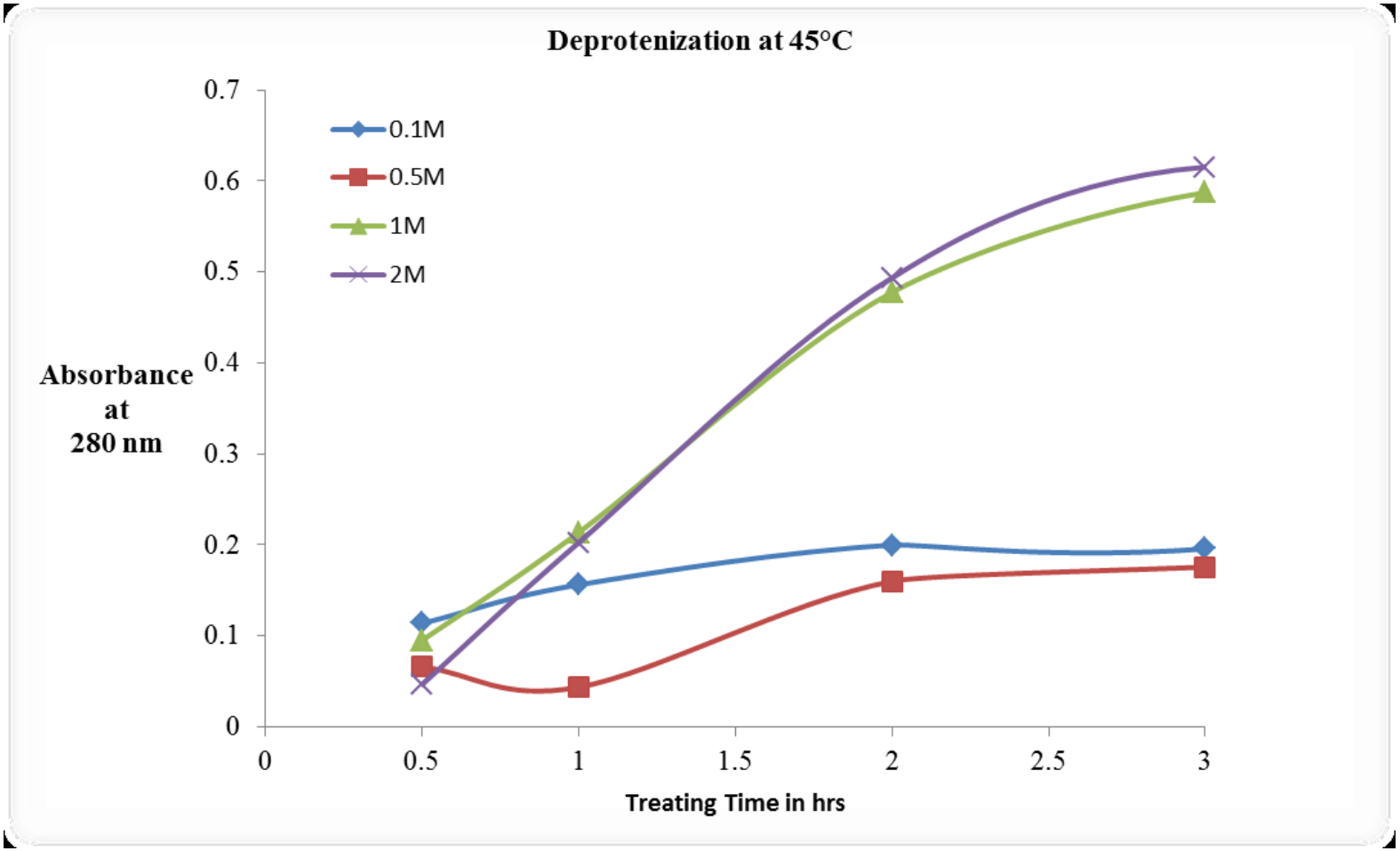
De-mineralization/De-calcification

- 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 is developed and contributed by Dr.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 de-mineralization of crab shells are completed.

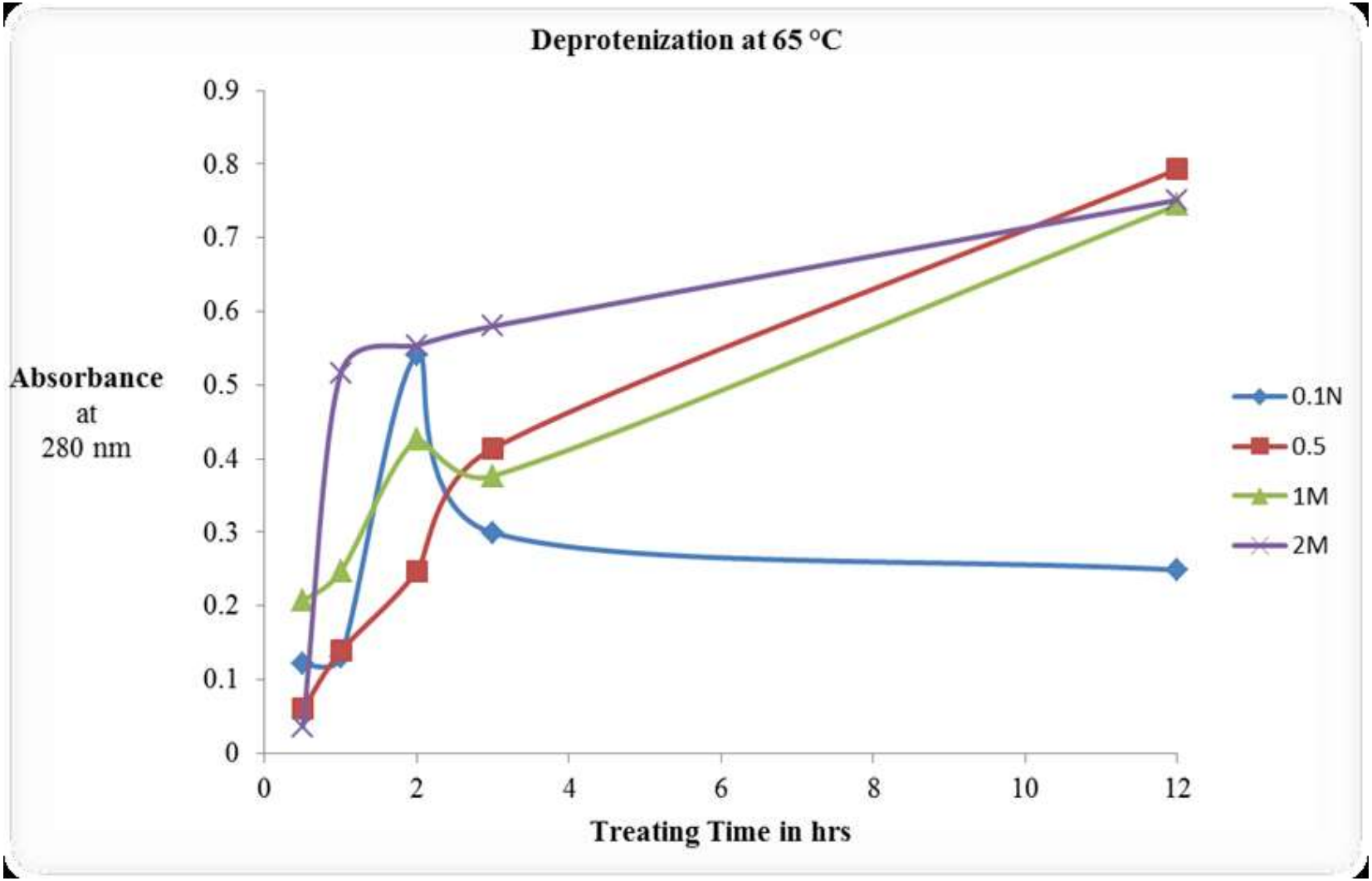
De-protenization

- 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 de-proteinization is a complex process and lack of information of about interaction between proteins and chitin and its chemistry in the literature.
- De-protenization by alkali method such as sodium hydroxide is a common method for removal of proteins from the shrimp shells.

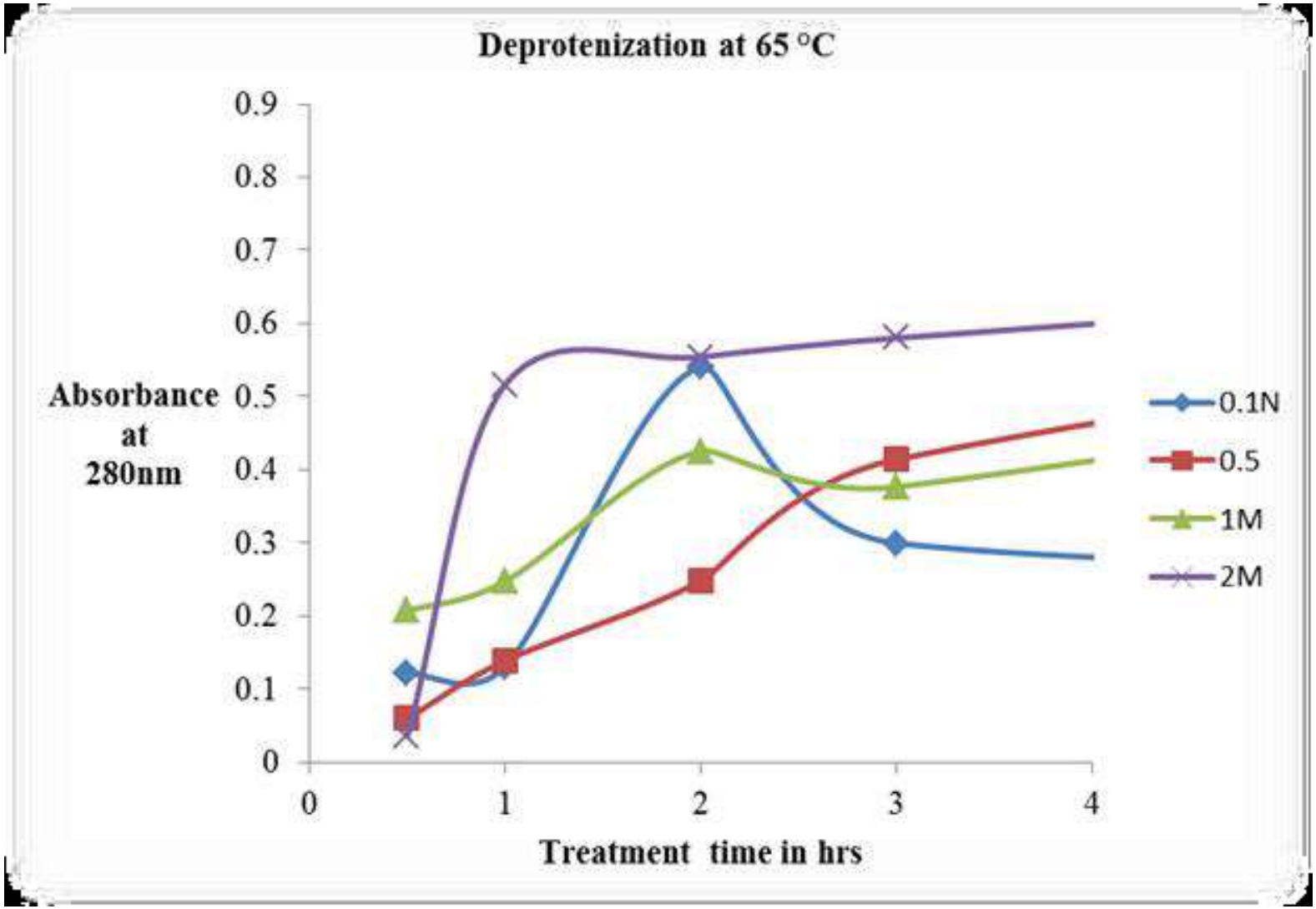
Optimization-Deprotenization at 45 °C



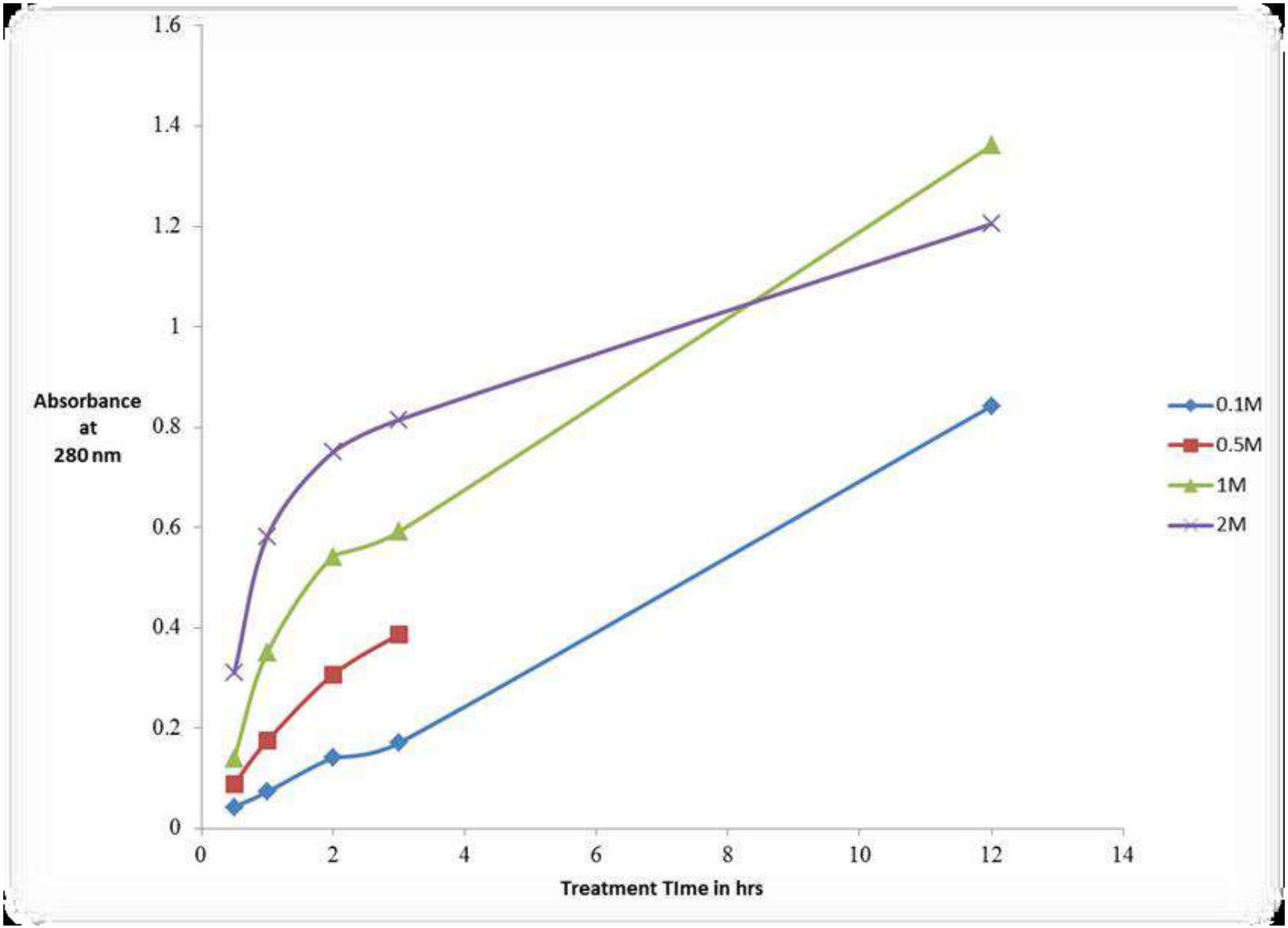
De-Protenization at 65 °C



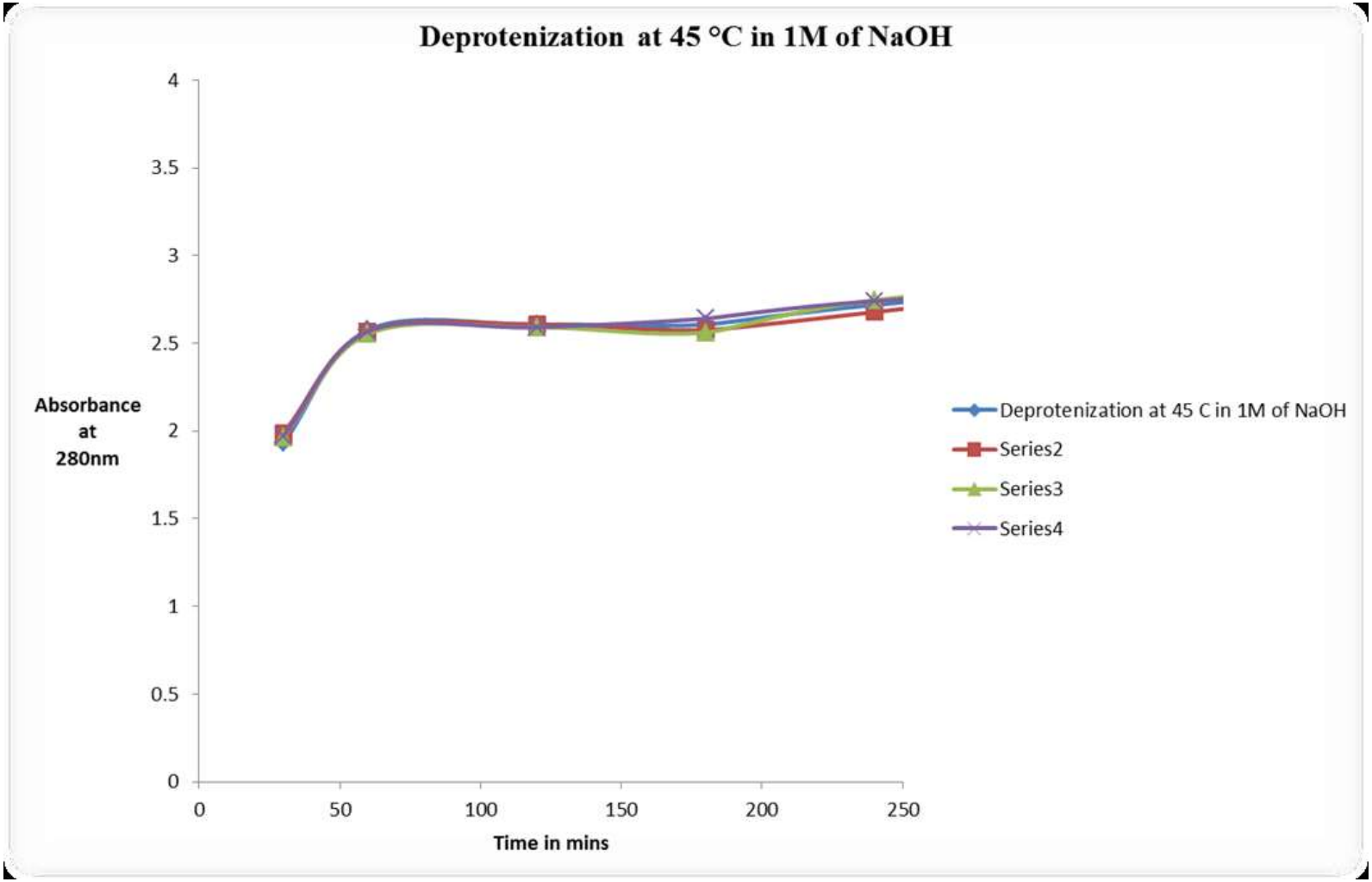
De-Protenization at 65 °C



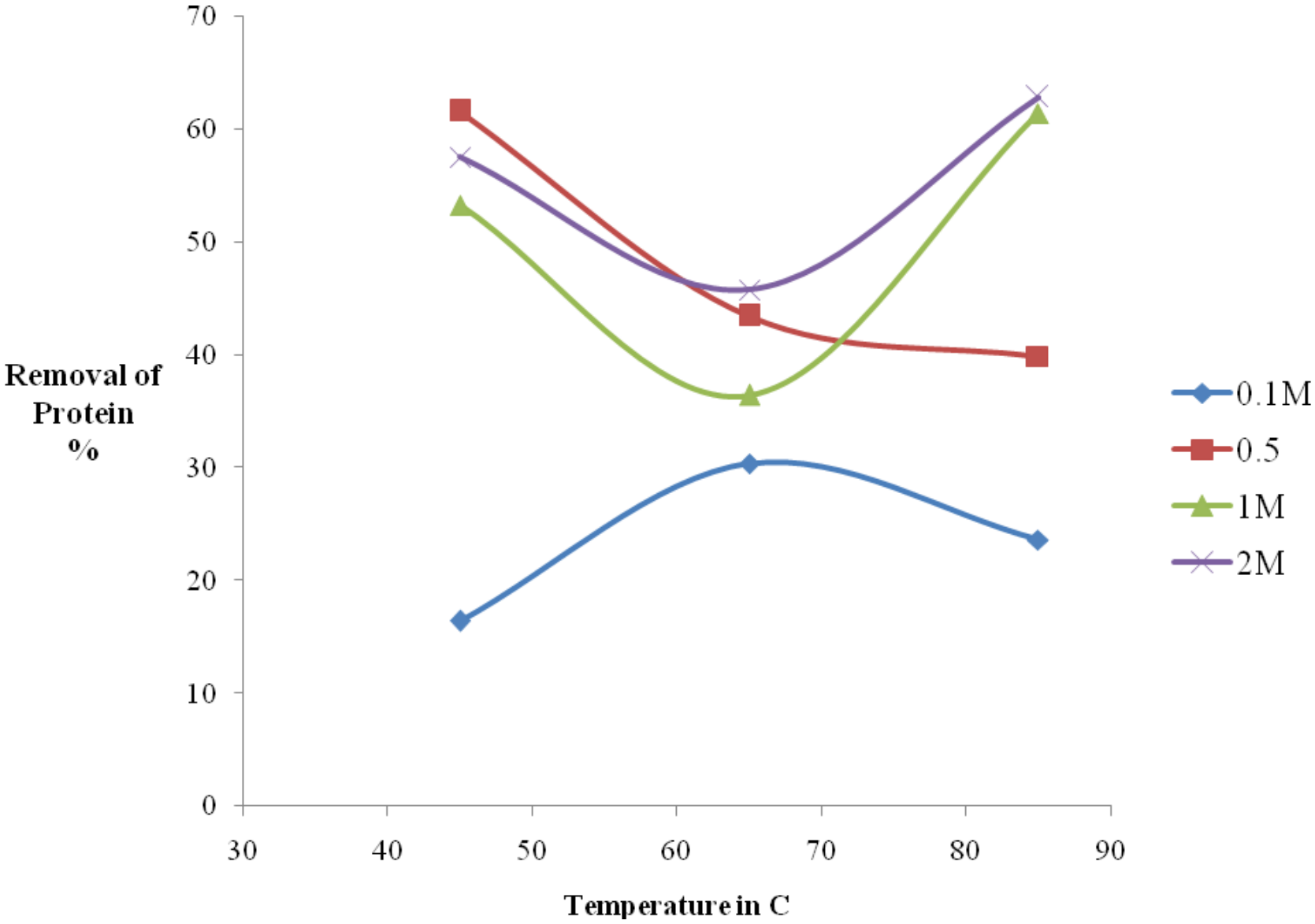
De-protenization at 85 °C



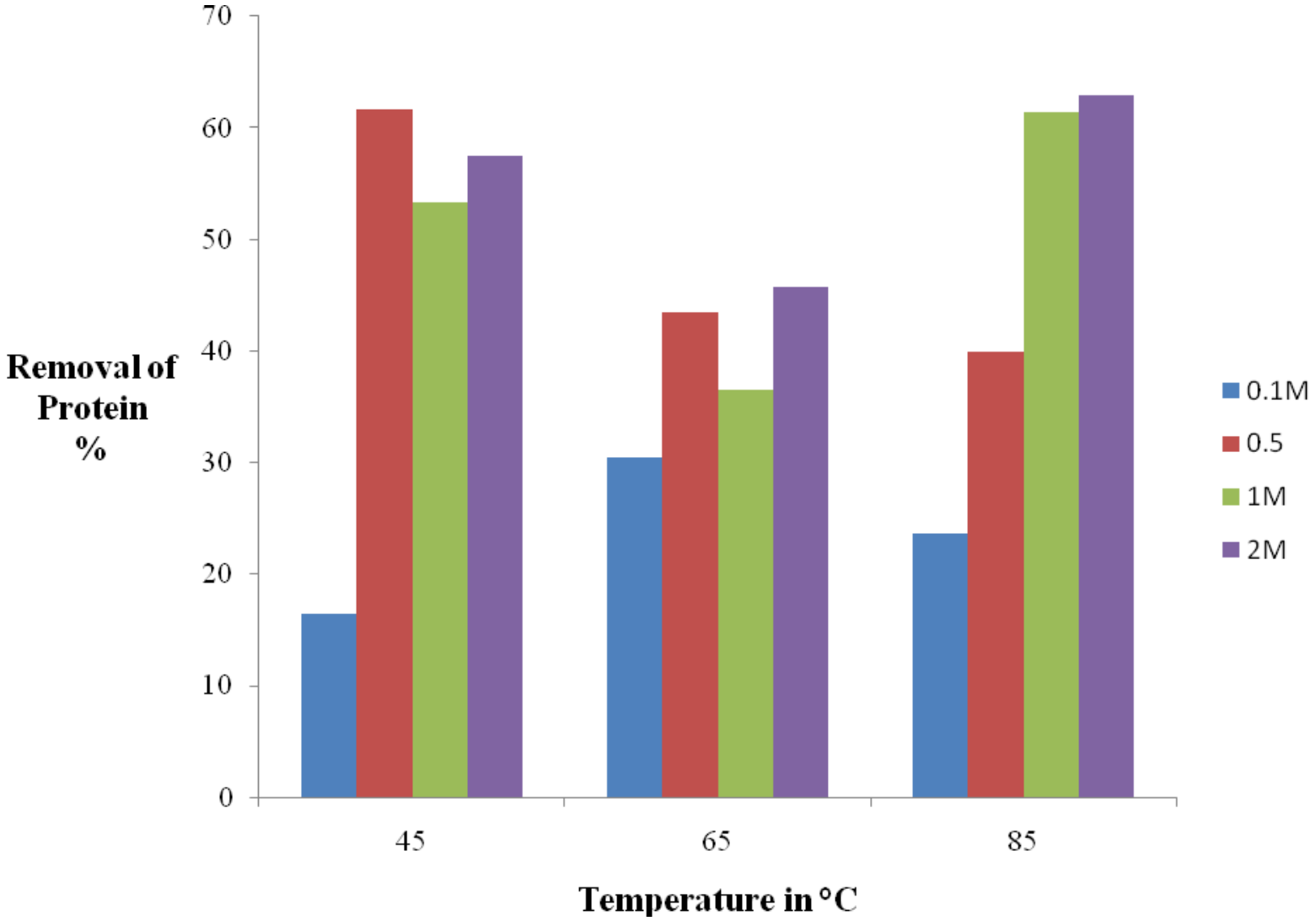
De-protenization at 45 °C



Influence of Temperature



Influence of Temperature



Limitations and Recommendations

- Absorption assay at 280nm is a simple method for finding protein releases from the crab shell. BSA (Bovine Serum Albumin) 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.
- Active Mixing or stirring is provided for de-protenization 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 deprotenized shell such as SEM (Scanning Electron Microscopy), Nitrogen estimating method should be performed

Conclusion

- 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, 1M concentration of NaOH at temperature of 45 °C is suitable for de-protenization of green crab shells for treatment time of 1-2 hrs. However, the thickness of the shell and its protein content (usually 10%) plays major in performance.
- The literature reported that de-protenization of shrimp shell is performed with 1M NaOH for treatment time of 24 hrs. But these experiments are performed in 200ml beakers and effective mixing doesn’t influence on the de-protenization process. Moreover, Shrimp shells are flimsy in nature and thinner than crab shell.
- In our case with thick crab shell, Good contact with NaOH solution is required and it can be provided only by effective mixing

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Thank You