# Extraction of Calcium and Protein from Shrimp Shell

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**A Report Submitted in Partial Fulfillment of the Requirements**

**for the Degree of Bachelor of Engineering (Petrochemical Engineering)**

**Department of Chemical Engineering, Faculty of Engineering,**

**King Mongkut's Institute of Technology Ladkrabang**

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# การสกัดแคลเซียมและโปรตีนจากเปลือกกุ้ง

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| **Field of Study** | Petrochemical Engineering |
| **Advisor** | Assoc. Prof. Dr. Prakob Kitchaiya<br>Asst. Prof. Dr. Apinan Namkanisorn |

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Accepted by the Faculty of Engineering, King Mongkut's Institute of Technology Ladkrabang in Partial Fulfillment of the Requirements for the Degree of Bachelor of Engineering (Petrochemical Engineering).

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**Abstract**

In this work, it is focused on demineralization and deproteinization process of shrimp shell by using citric acid and calcium oxide, respectively. The aims of this work are to study extraction of calcium and proteins from shrimp shell. In demineralization process, citric acid with different concentration are used and the solution is mixed between dried shrimp shell and citric acid in ratio 1:5 (w/v) at room temperature and stirred at 100 rpm by different stirring time. In addition, it is solid-liquid adsorption and applying Langmuir equation is used to describe about this demineralization process. Finally, it is found that the value of $k_1$ is equal to 0.05 and $k_2$ is equal to -0.81. In deproteinization process, the kinetics of hydrolysis reaction are studied as a function of temperature between 50-70°C for 48 h. Shrimp shell is incubated in distilled water with a liquid-to-solid ratio of 5 ml/g. The amount of released proteins is measured by biuret protein assay. The results show that the optimal deproteinization condition occurs at 70°C and 48 h which protein is hydrolyzed approximately 50%. Besides, the kinetic study reveals that the reaction was limited by diffusion.

**Keywords:** Shrimp shell, Demineralization and Deproteinization

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| **เรื่อง** | **การสกัดแคลเซียมและโปรตีนจากเปลือกกุ้ง** |
| **โดย** | **นายภัทรภูมิ แจ่มเจริญ** |
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**บทคัดย่อ**

ปริญญานิพนธ์นี้รายงานการศึกษาของกระบวนการกำจัดเกลือแร่ (demineralization) และกระบวนการกำจัดโปรตีน (deproteinization) โดยใช้กรดซิตริกและปูนขาว ตามลำดับ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษากระบวนการสกัดแคลเซียมและโปรตีนจากเปลือกกุ้ง ในกระบวนการกำจัดเกลือแร่ ใช้กรดซิตริกที่มีความเข้มข้นแตกต่างกันและศึกษาโดยใช้เปลือกกุ้งและกรดซิตริกในอัตราส่วน 1:5 โดยน้ำหนักต่อปริมาตร ในสภาวะปกติและอัตราการกวนที่ 100 รอบ/นาที โดยใช้เวลาแตกต่างกัน และกระบวนการนี้ใช้สมการแลงเมียร์ประยุกต์มาอธิบายกระบวนการดูดซับระหว่างของแข็งและของเหลว พบว่า $k_1$ และ $k_2$ ที่ได้มีค่าเท่ากับ 0.05 และ -0.81 ตามลำดับ ในกระบวนการกำจัดโปรตีน ได้ศึกษาจลนพลศาสตร์ของปฏิกิริยาไฮโดรไลซิส ของการสกัดโปรตีนที่อุณหภูมิ 50-70 องศาเซลเซียส เป็นระยะเวลา 2 วันโดยแช่เปลือกกุ้งในน้ำกลั่นในอัตราส่วน 1:5 โดยปริมาตรต่อน้ำหนัก และโดยใช้วิธีไบยูเรต (Biuret method) ในการหาปริมาณโปรตีนในสารละลาย จากการทดลองพบว่าที่อุณหภูมิ 70 องศาเซลเซียส ในเวลา 48 ชั่วโมง สามารถสกัดโปรตีนจากเปลือกกุ้งได้ประมาณ 50% นอกจากนี้จากการศึกษาจลนพลศาสตร์พบว่าปฏิกิริยาถูกจำกัดด้วยการแพร่



คำสำคัญ: เปลือกกุ้ง กระบวนการกำจัดเกลือแร่ และกระบวนการกำจัดโปรตีน

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Phattarapoom Chamcharoen

Sopida Buddawapee

## Table of Contents

Page

**Table of Contents (Cont.)**

Page

**Table of Contents (Cont.)**

Page

## List of Figures

Page

## List of Tables

**Page**

# CHAPTER I

# INTRODUCTION

## 1.1 Background

Nowadays, there is a trend toward to develop biofilm based production in order to use instead of petroleum based films because of environmental problems with plastic waste. Usually, biopolymer based films are produced from protein, polysaccharides, lipids or composite. The main features of biofilm are biodegradability, non-toxicity, non-polluting and edibility. Therefore, chitosan, polysaccharide, is considerably used in biofilm production.

Chitosan is polysaccharide with an amino group derived from chitin by deacetylation in alkaline solution. Chitin and chitosan can be found in crustaceans, insect and related organism. Crustacean shells, especially shrimp shell, can be easily obtained from consuming and freezing industrial waste. Annually, many tonnes of crustacean shells are produced globally which leads to the unavoidable generation of waste. However, the kind of waste consists of three main organic chemical components which are protein 33-40%, chitin 17-20%, and calcium carbonate. Each component can be recovered and extracted by many methods such as chemical or enzyme treating.

In order to obtain chitosan from crustacean shell, the isolation of chitosan involve three main processes including demineralization, deproteinization and deacetylation, respectively. The traditional process, calcium carbonate is extracted by using hydrochloric acid (HCl) in demineralization process but the condition is severe which may damage the nutritional value of protein, chitin and chitosan structures. In addition, the classical deproteinization process has been carried out in strong alkaline media such as 2 M sodium hydroxide (NaOH). However, the cost of sodium hydroxide is expensive and it is a strong alkaline substance causing work up difficulties.

In this work, it is focused on demineralization and deproteinization process. Citric acid, organic acid is used instead of hydrochloric acid, strong acid in demineralization process. Moreover, calcium oxide (CaO), is known as quicklime, is white, less corrosive, alkaline and crystalline substance at room temperature. It is very

cheap and its alkalinity is weaker than sodium hydroxide. Therefore, calcium oxide is considerably used in this deproteinization process.

The objectives of this work are to study kinetic of demineralization process by using citric acid and to study the deproteinization process by using calcium oxide. Moreover, the kinetics of deproteinization were also studied at a function of different temperature, and activation energy were investigated.

## 1.2 Objectives

1.2.1 To extract calcium by using citric acid

1.2.2 To extract protein by using calcium oxide

## 1.3 Scopes of Work

1.3.1 Isolation of calcium carbonate and protein from shrimp shells.

1.3.2 Kinetic study of demineralization process from shrimp shells by using citric acid.

1.3.3 St Kinetic study of deproteinization process from shrimp shells by using 10wt% calcium oxide for 2 days.

## 1.4 Expect outputs

1.4.1 Calcium citrate

1.4.2 Protein solution

# CHAPTER II

# THEORY AND LITERATURE REVIEWS

## 2.1 Shrimp shell components

Shrimp shells consist of three main valuable chemical components which are protein 33-40%, chitin 17-20% and calcium carbonate. Each components can be utilized and extracted through different method such as chemical treating or enzyme treating.[1]

Protein in shrimp shell is closely linked with chitin and calcium carbonate. The difference in a source and species of shrimp result in the difference in protein content. However, protein in shrimp shell can be extracted by hydrolysis reaction in different media. Protein and hydrolysates can be made into animal feed and nutrition additives whereas calcium carbonate is used to produce into dietary supplement and used in application as filler or pigment in various industries.[2]

For the remaining chitin and chitosan, chitin can hydrolyze into N-acetyl, its monomer, and then deacetylate into Glucosamine.[3] Glucosamine is used to produce Glucosamine Hydrochloride or glucosamine sulfate, which is used as dietary supplement in the osteoarthritis treatment.

In addition, chitin is usually deacetylate into chitosan due to chitin because chitosan is more soluble than chitin

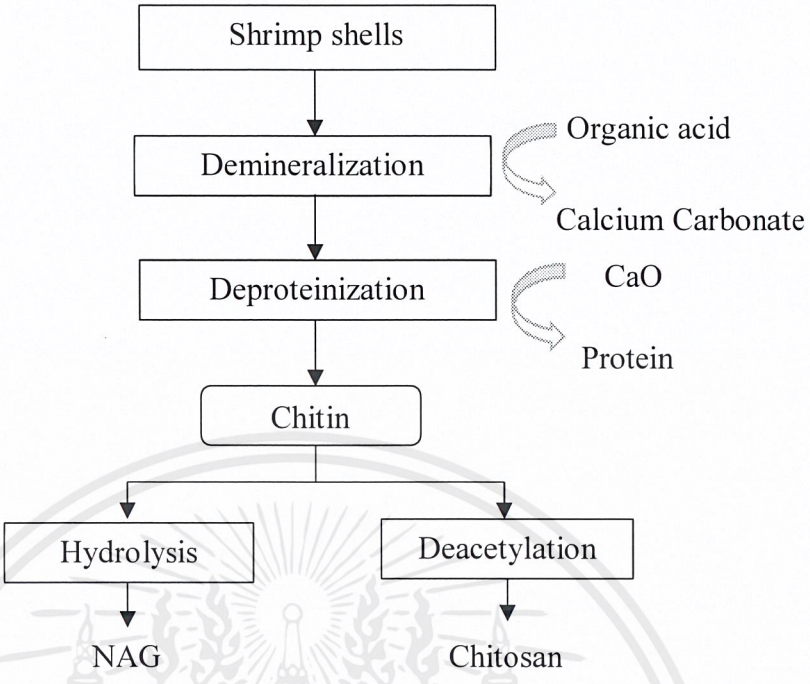


**Figure 2.1** Chemical extraction process [4]

## 2.2 Calcium

Calcium is a chemical element with atomic number which equals to 20 and it is an alkaline earth metal. It is abundant element in Earth's crust as well as calcium is abundant metal which is less than iron and aluminum. The most common calcium compound on Earth is calcium carbonate ($CaCO_3$), considerably found in limestone. In addition, gypsum, anhydrite, and fluorite are likewise the sources of calcium. Calcium is a vital mineral for humans, animals, plants and the environment. It is a major mineral in the human body, which is essential for growing up. Moreover, it is classified as secondary nutrients that are also involved in growing up for the plants.[5]

## 2.3 Calcium carbonate

Calcium carbonate is a chemical compound which has the formula $CaCO_3$. It is a common substance found in rocks as the calcite and it is the main component of crustacean shell, pearls, and eggs. Moreover, in medical applications, it is used as a calcium supplement or as an antacid, but extreme consumption can be dangerous for health.

The amount of calcium carbonate that will dissolve in sea water if thermodynamic equilibrium is reached is directed by the reaction:

$$CaCO_3\ (s) \rightarrow Ca^{2+}\ (aq) + CO_3^{2-}\ (aq)$$

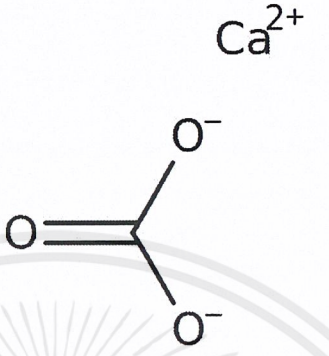


**Figure 2.2** Structure of calcium carbonate [6]

## 2.4 Calcium analyzer

Phenolphthalein was used as indicator in this work. The solution of phenolphthalein has been often applied in titration as a pH visual indicator to point out information of other compounds, including mineral and organic acids. As a phenolphthalein indicator, its color turns into pink in solutions which pH above 10 and changes to colorless in acidic solutions which pH below 8.[7]

$$2(C_6H_8O_7) + 3CaCO_3 \rightarrow Ca_3(C_6H_5O_7)_2 + 3H_2CO_3$$

$$C_6H_8O_7 + 3NaOH \rightarrow Na_3(C_6H_5O_7) + 3H_2O$$

## 2.5 Citric acid

Citric acid is a natural elemental of plants and animals. Citric acid is a tricarboxylic acid with the chemical formula $C_6H_8O_7$. Citric acid exists in a variety of fruits and vegetables, most greatly in citrus fruits. This organic acid has various highly variable properties and it is as a purely biological product, it can be applied this organic acid in the fields of food and pharmaceuticals.[8]

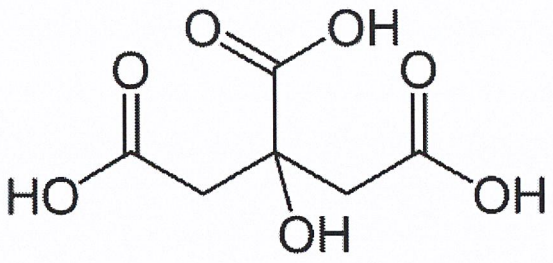


**Figure 2.3** Structure of citric acid [9]

## 2.6 Protein

Protein is an organic compound that consists of a long-chain polymer of amino acids. Proteins are essential nutrients for the human body and animals as well as other species of plants. Protein is the second most abundant component of the body. It is a basic component of cells of all living things, such as enzymes, hormones, which essential for work and living. Proteins are composed of 50% carbon, 20% nitric, 6% nitrogen, and sulfur. The weight of the protein molecules is very low and is synthesized in ribosome cells. The smallest unit of protein is an amino acid. Many amino acids are linked together by peptide bonds in which the carboxyl group of one amino acid is condensed to the amino group of another amino acid with the ejection of water. A linear chain of amino acid residues is called a polypeptide. When an amino acid is formed, it becomes a protein. Each protein provides different benefits.

## 2.7 Protein hydrolysis [10], [11]

Hydrolysis is a decompose reaction involving water that is consumed to break the bond. Make large molecules break down into substances that have smaller molecules. Hydrolysis is opposite the condensation reaction in which two molecules bond together and release a molecule of water. Therefore, water is one of the reactants in hydrolysis reaction, whereas it is a product from condensation reaction.

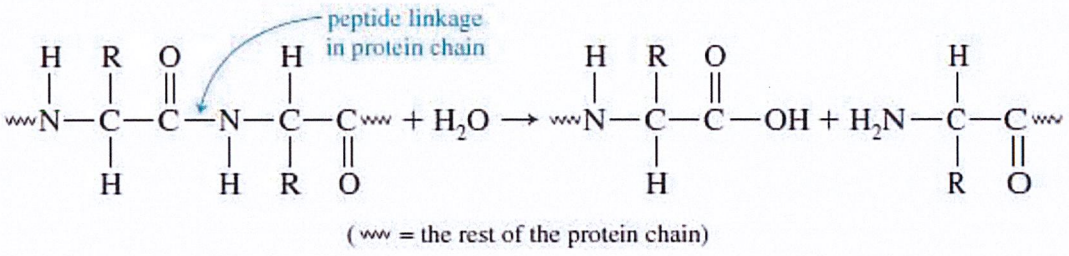


**Figure 2.4** Condensation reaction [12]

Protein hydrolysis is a process that peptide bonds of protein molecules are broken down into smaller peptides or free amino acid by the addition of water. Protein hydrolysis can be catalyzed in several mediums such as acidic, alkaline or enzymatic solutions.

### 2.7.1 Acid hydrolysis

Normally, 6M of hydrochloric acid is used in acid hydrolysis. In this condition, all of the proteins are hydrolyzed to amino acids. However, It is a harass process where some of the amino acids are destroyed.

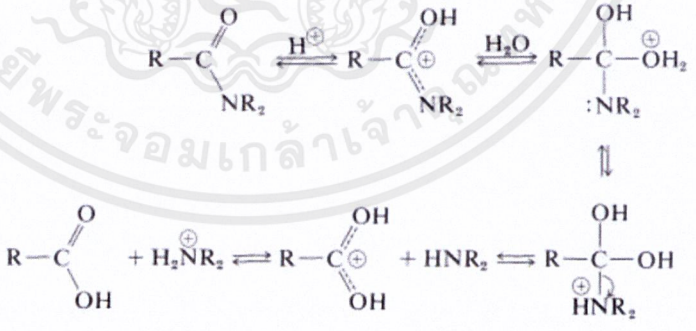

**Figure 2.5** Acid hydrolysis reaction

### 2.7.2 Base hydrolysis

Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are commonly used in alkaline hydrolysis which is in the form of alkali solution of metal hydroxides. In classical deproteiniation process, 2M NaOH is used to extract protein from shrimp shells. However, alkaline hydrolysis destroy some amino acids.

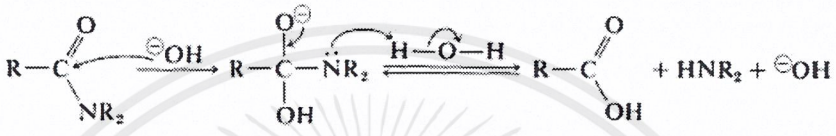

**Figure 2.6** Base hydrolysis reaction.

### 2.7.3 Enzymatic hydrolysis

Enzymatic hydrolysis is the biological process which use proteolytic enzymes, also called protease in hydrolysis reaction. This process is less severe than chemical treatment which lead to less environment burden. It can perform in milder condition and usually target specific peptide bond. However, this process consumes more time and specific to pH and temperature since each enzyme active in different condition.

## 2.8 Protein analyzer

### 2.8.1. Biuret method

The Biuret method is commonly used to analyze protein solutions in concentrations of 5 to 10 mg/ ml. it is a solution of copper sulfate suspension in sodium potassium tartrate. Proteins containing more than 2 peptide bonds will react with $Cu^{2+}$ and form purple complex molecules in alkaline solution. Since the complexes are blue

to purple, therefore it can be measured by absorption spectroscopy at 540 nm. and the concentration of protein can be calculated from the calibration curve.[13]

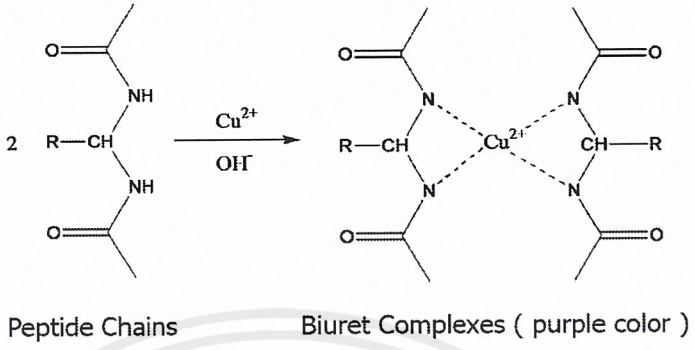


**Figure 2.7** Biuret reaction

### 2.8.2 Ninhydrin [14]

When protein or peptide are reflux with high concentrated hydrochloric (6M) at 110°C for 24 hours, it will be hydrolyzed into amino acid. The free amino group of amino and protein react with ninhydrin will form purple color known as Ruhemann's purple.

### 2.8.3 The Warburg-Christian Assay (UV Absorbance)

This method can be done faster than other methods with a capacity of protein concentrations between 50 – 100 µg/ml. The protein solution can be determined directly by ultraviolet spectroscopy due to the present of tyrosine and tryptophan which absorb at 280 nm. However, this method requires ultraviolet spectroscopy and special UV-quartz cuvette which is has a high price.

### 2.8.4 Kjeldahl method [15]

Kjeldahl is one of protein analysis by analyzing the total amount of nitrogen contained in the sample. This method was developed by Dane Johan Kjeldahl, a Danish citizen during the year 1800. It is widely used as a protein analysis which is recognized as an accurate method. The method consists of 3 steps:

**1. Digestion**

Sample is digested with high concentration sulfuric acid at 360 – 410 °C. The Nitrogen within sample will turn into ammonium sulphate $(NH_4)_2SO_4$. This step is catalyzed by $CuSO_4$, Se, or $HgSO_4$

**2. Distillation**

The sample is distilled with a small amount of sodium hydroxide (NaOH). When $(NH_4)_2SO_4$ reacts with NaOH, ammonium ion ($NH^{4+}$) will turn into ammonia ($NH_3$), which is distilled off. Then, ammonia gas is trapped with standard acid solution.

**3. Titration**

Ammonium ion in the standard acid solution is titrated with a standard solution. Then nitrogen content can be calculated

The amount of proteins can be calculated by multiplying the corrected nitrogen content with 6.25, the theoretical percentage of nitrogen in proteins. The corrected nitrogen content was nitrogen in shrimp shell minus the nitrogen content of chitin (1.18%) as following equation.[16]

$$\%P = (\%\text{Total nitrogen} - 1.18) \times 6.25$$

However, this method also requires special equipment, is time consuming for. This is not used routinely in biochemistry.

### 2.9 Calcium oxide

Calcium oxide (CaO), commonly known as quicklime or burnt lime, is a material obtained from the process of burning limestone a temperature above 825 degrees Celsius to eliminate carbon dioxide. This quicklime can react with carbon dioxide in the air, it will be converted back to calcium carbonate in enough time. Therefore, storage must be careful not to allow air to pass into the storage container. It is an odorless, white, corrosive, alkaline and crystalline substance at room temperature. It is widely applied in many areas such as agriculture, building, and construction.

However, the solubility of calcium oxide is relative low compared to sodium hydroxide. But the price of calcium oxide is less expensive compared to the other chemicals but it can be used as a substance to produce many other chemicals.

$$CaCO_3\ (s) \rightarrow CaO\ (s) + CO_2\ (g)$$

### 2.10 Application of protein

Proteins and hydrolysates extracted from shrimp shell can be potential used in many fields, especially proteins extracted by enzymatic or autolytic hydrolysis since the value of some amino acid is not destroyed. Some proteins and hydrolysates have such interesting biological properties such as antioxidant, anticoagulant, and antihypertensive.

#### 2.10.1 Food

Some proteins obtained from hydrolysis, have been shown anti-oxidant activity. It prevents lipid oxidation in food processing which leads to reduce the formation of undesirable flavors or odor.[17]

#### 2.10.2 Agricultural

Another way to use protein or hydrolysate is to make fertilizer for organic farms. Because proteins contain nitrogen which is the main nutrient for plants Nitrogen nutrients will increase the growth of leaves in plants especially vegetables.

## 2.11 Chemical Kinetics [18]

In order to study kinetic of chemical reaction, concentration and time are collected to analyze and determine the rate law. In particular, order of reaction (n) and rate constant (k) are parameters which are determined.

### 2.11.1 Differential method

For the general reaction,

$$A \rightarrow \text{Product}$$

Then combine the mole balance with rate law given by Equation (1)

$$-\frac{d[A]}{dt} = k_A[A]^n \qquad (1)$$

After taking the natural logarithm of both sides of above Equation

$$\ln\left(-\frac{d[A]}{dt}\right) = \ln k_A + n \ln[A] \qquad (2)$$

From the equation (2), the slop of the plot of $\ln(-dC_A/dt)$ as a function of $(\ln C_A)$ is the rate constant of reaction and the y-intercept is $\ln k_A$.

### 2.11.2 Integral method

Generally, integral method is used to determine specific rate constant when order of reaction is known and it used to determine activation energy when different in temperature.

However, if the order of reaction is unknown, order of reaction will be guess and integrate differential equation to find appropriate order of reaction.

For the general reaction,

$$A \rightarrow \text{Product}$$

Rate of reaction will be as equation (3)

$$-\frac{dC_A}{dt} = r_A \qquad (3)$$

### 1) Zero-order Reaction

For a zero-order reaction, $r_A = -k$, and the combined rate law and mole balance is

$$\frac{d[A]}{dt} = -k \qquad (4)$$

Obviously, concentration of A is not effect to rate of reaction because order of A is zero. When plot relation of rate of reaction versus concentration of A, it will be constant as seen in figure 6.


**Figure 8.** The relation between the concentration of A, [A] and rate of reaction 1

When integrate equation (4) with $[A] = [A]_0$ at $t = 0$, equation (5) will be obtain

$$[A] = [A]_0 - kt \qquad (5)$$

A plot of the concentration of A as a function of time will be linear as shown in figure 7 with slope (-k) for a zero-order reaction.

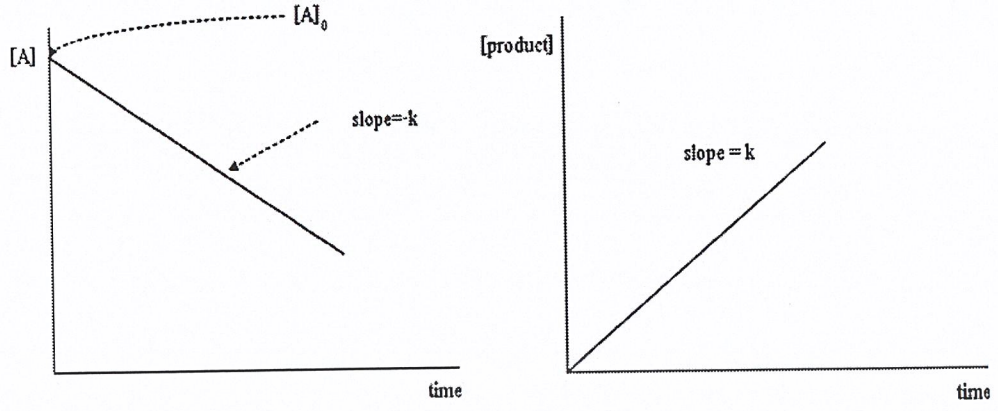


**Figure 2.9** The relation between the concentration of A, [A] and time, t

**2) First-order Reaction**

Rate of reaction of first order will be

$$-\frac{d[A]}{dt}=k[A] \qquad (6)$$

When integrate equation (6) with the limit $[A] = [A]_0$ at $t = 0$ gives

$$\ln\frac{[A]_0}{[A]} = kt \qquad (7)$$

After equation (7) obtained, the plot of $[\ln[A]_0/[A])]$ as a function of time is linear with slope as shown in figure 8.

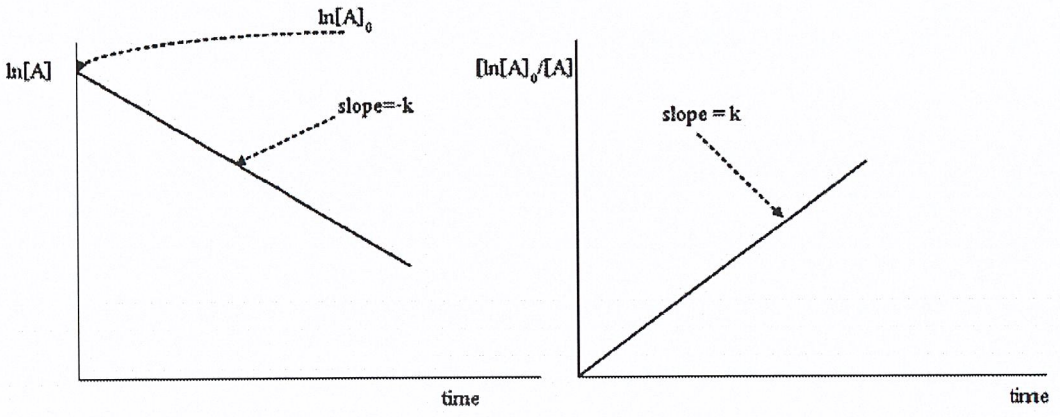


**Figure 2.10** The relation between ln[A], and $\ln[A]_0/A$ with time, t

### 3) Second-order Reaction

Rate of second order reaction is

$$-\frac{dC_A}{dt} = k[A]^2 \qquad (8)$$

Integrating with $[A] = [A]_0$ initially and $t = 0$ gives

$$\frac{1}{[A]} - \frac{1}{[A]_0} = kt \qquad (9)$$

For a second-order reaction, the plot (1/[A]) as a function of time should be linear with slope k and y-intercept is $1/[A]_0$

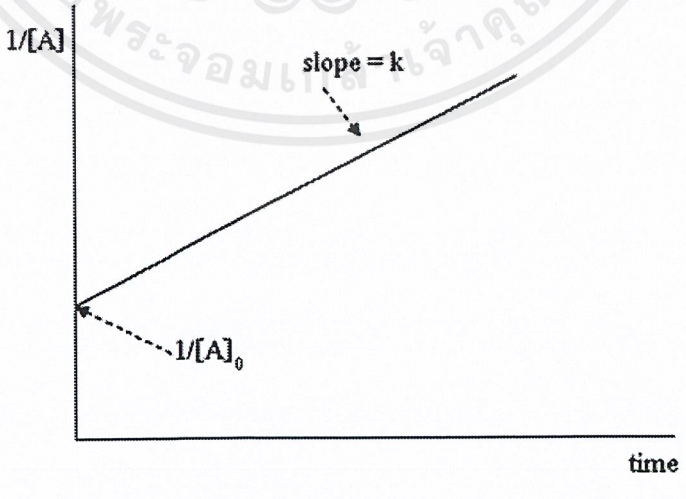


**Figure 2.11** The relation between 1/[A] with time, t

**2.12 Kinetic of deproteinization**

The Deproteinization process can be describe by first-order reaction

Define,

$m_{p_{s0}}$ = Initial protein contained in shrimp shell (g)

$m_{p_s}$ = Remaining protein contained in shrimp shell (g)

$m_{p_l}$ = Mass of protein contained in solution (g)

$C_p$ = Concentration of protein in solution (g/ml)

V = Volume of protein solution (ml)

Therefore, $$m_{p_{s0}} - m_{p_s} = m_{p_l} = C_p V \quad (10)$$

$$-\frac{d(m_{p_{s0}} - m_{p_l})}{dt} = k \times m_{p_s} = k \times (m_{p_{s0}} - m_{p_l}) \quad (11)$$

$$-\frac{d\frac{(m_{PSo}-m_{Pl})}{(m_{PSo}-m_{Pl})}}{dt} = k \quad (12)$$

$$\int \frac{d(m_{PSo}-m_{p_l})}{(m_{p_{s0}}-m_{p_l})} = -\int k\, dt \quad (13)$$

When integrate equation (13) with the limit $m_p = 0$ at $t = 0$ gives

$$ln\frac{m_{p_{s0}}}{m_{p_{s0}} - m_{p_l}} = kt \quad (14)$$

$$ln\,(m_{p_{s0}} - m_{p_l}) = -kt + ln\,(m_{p_{s0}}) \quad (15)$$

Therefore, when ln $(m_{p_{s0}}-m_{p_l})$ is plotted as a function of time, the slope will be the rate constant (k) in the unit of 1/time.

## 2.13 Literature review

There are many researcher works study about demineralization and deproteinization of crustacean shells. Most of researcher works optimize the condition in demoralization and deproteinization processes before deacetylate chitin in order to obtain chitosan.

In 2017, Ahmad Fadli et.al[19] studied about demineralization kinetics of chitin isolation from shrimp shell waste by using sodium hydroxide solution at concentration 2.5%, shrimp shell, and hydrochloric concentration solution ranges 0.2-1.8 N. In addition, the chitin filtered and broke by $H_2SO_4$ and $HNO_3$ to determining calcium carbonate content by titration of complexometry. Finally, they found that the kinetic was following pseudo second order equation and the reaction rate increased by increasing of HCl concentration. Moreover, the effect of stirring rate to reaction rate was not defined by kinetic homogenous reaction. In addition, the results showed that the range of reaction rate was 0.00167-0.394 $min^{-1}$.

In 2015, R. Baron et al.[20] studied the kinetic of solid phase demineralization by weak acids in one-step enzymatic bio-refinery of shrimp cuticles. In this work, demineralization kinetics were followed by many acids including phosphoric, hydrochloric, acetic, formic and citric acids with $pK_a$ ranges from 2.1-4.76. It was found that formic acid met the purpose of 99% of demineralization yield and 95% deproteinization yield at a pH close to 3.5 and a molar ratio of 1.5. Moreover, the offered one-step process is proven to be effective in this work. In addition, they found that demineralization kinetics based on simplified physical considerations could not explicate the kinetics for times that more than 30 min, but the empirical one could perform properly.

In 2003, Aline Percot et.al[21] studied about kinetic of deproteinization of shrimp shell in NaOH solution with different temperature. The condition was performed in solution of NaOH 1 M with solution/solid ratio was 15 mL/g. and

temperature was varied in the range of 16 – 70 °C. In this work, elemental and amino acid analysis were used to analyze the residue amino acid remained in shrimp shell and the amount of peptide released was analyzed by UV absorbance at 280 nm. The resulting characteristic of deproteinization is belong to first order reaction in three stages and rate constants were calculated with different 4 temperatures: 14, 27, 50 and 70°C. Moreover, activation energy and frequency factors of collision were also calculated.

In 1997, R. Chang et.al[16] also studied about the kinetics of removal of protein from pink shrimp shell by using NaOH for 6 h. This work also studied the optimal condition in deproteinization such as solution/solid ratio, concentration and temperatures. Kjeldahl method was used to determine the protein content by using a corrected nitrogen multiply with 6.25. The corrected nitrogen content was minus by 1.18, the nitrogen content of chitin. The result shows that the reaction obey first-order reaction with two stages and the optimal condition occurs at 75 °C, 2.5 N NaOH.

In 2012, Islem Younes et.al[22] studied about the effect of ratio of enzyme to solid, temperature and incubation time of deproteinization process. In this work, *Bacillus mojavensis* A21 is used as protease to hydrolyze protein from shrimp shell. Protein content was calculated from nitrogen content by using Kjeldahl method. The optimum conditions were: an enzyme/substrate ratio of 7.75 U/mg, a temperature of 60 °C and incubation time 6 h. Moreover, this work also remove mineral and deacetylate chitin to obtain chitosan.

# CHAPTER III

# RESEARCH METHODOLOGY

This work focuses on the isolation of calcium carbonate and protein from the shrimp shells by using citric acid and calcium oxide in demineralization step and deproteinization step, respectively. Kinetic of both processes were study.

## 3.1. Chemicals and material

1. Pacific white Shrimp Shell (*Litopenaeus Vannamei*)
2. Citric acid
3. Phenolphthalein
4. Calcium oxide
5. Distilled water
6. Sodium chloride
7. Sodium hydroxide
8. Bovine Serum Albumin
9. Copper (II) sulphate pentahydrate
10. Potassium sodium tartrate tetrahydrate
11. Potassium iodide

## 3.2 Equipment and apparatus

1. Beaker
2. Oven
3. Analytical balance
4. Filter paper
5. Wash Bottle
6. Stirring rod
7. Magnetic bar
8. Magnetic stirrer

9. Stand
10. Clamp
11. Burette
12. Pipette
13. Graduated cylinder
14. PP plastic bag
15. Shaker
16. Grinder
17. Centrifugal
18. Centrifugal tube
19. Filtering funnel
20. Rubber
21. Micropipette
22. Volumetric flask
23. Test tube
24. Plastic cuvette
25. Spectrophotometer

## 3.3 Procedures

### 3.3.1 Study of kinetic of Demineralization of shrimp shells

1. Prepare 0.5, 1, 1.5, and 2 M citric acid solution, by weighting citric acid 48, 96, 144, and 192 g., respectively, then add distilled water until volume of each solution equal to 500 ml.
2. Prepare 0.5 M sodium hydroxide solution, by weighting NaOH 20 g., then add distilled water until volume equal to 1,000 ml.
3. Weight dried shrimp shell 5 g. into beaker, then add 0.5 M citric acid solution 25 ml and add distilled water 25 ml.
4. Stir the solution by magnetic bar on magnetic stirrer for 5 min.
5. After that, filter the solution and take 10 ml. of the liquid sample from step 4 by pipette into Erlenmeyer flask. Repeat this step 2 times by

taking the sample into another Erlenmeyer flask. (In this step, solid sample is taken into the oven for drying)

6. Then add phenolphthalein indicator 2-3 drops to each sample and shake it.
7. Titrate each sample from step 6 by 0.5 M NaOH, and record the volume of NaOH that used.
8. Repeat step 3 to 7 by varying the concentration of citric acid solution (1.0, 1.5, and 2.0 M) and varying the stirring time for 10, 15, 30, 45, 60, 90, 120, 150 and 180 min.
9. Calculate the percent of extracted calcium.

### 3.3.2 Study of kinetic of Deproteinization of shrimp shells

1. Weight dried shrimp shell 30 g. into PP plastic bag for 11 samples.
2. Add CaO 3 g. into each sample.
3. Add distilled water 150 ml into each sample.
4. Strap the plastic bag with a rubber band.
5. Heat samples up to 50°C by placing sample in the oven.
6. Take sample at time 10, 20, 30, 60, 120, 180, 360, 540, 720, 1,440 and 2,880 min.
7. After taking sample, centrifuge sample at 5000 rpm in order to separate solid and liquid phase.
8. Then, analyze amount of proteins released in solution by biuret method.
9. Repeat step 1 to 8 but change the temperature in step 4 to 60 and 70°C.

**1) Protein analysis by biuret method**

**1. Preparation of the biuret reagent**

1.1 Weight $CuSO_4.5H_2O$ 1.5 g. and $NaKC_4H_4O_6.4H_2O$ (Sodium potassium tartrate) 6 g.

1.2 Dissolve chemicals (1.1) in distilled water 500 ml.

1.3 Add 10% (w/v) NaOH (carbonate free) 300 ml.

1.4 Add KI 1 g. into solution and then, add distilled water until volume equal to 1L.

1.5 Store solution in plastic container in the dark.

**2. Calibration curve**

2.1 Preparation Bovine Serum Albumin (BSA) solution in concentration of 10 mg/ml

2.2 Prepare the dilution of BSA as table 1 below

**Table 3.1** Standard dilution for preparation of a calibration curve.

| Final Concentration (mg/ml) | Volume of a 10mg/ml standard (μl) | Volume of water or dilution buffer (μl) |
|---|---|---|
| 0 | 0 | 500 |
| 2 | 100 | 400 |
| 4 | 200 | 300 |
| 6 | 300 | 200 |
| 8 | 400 | 100 |
| 10 | 500 | 0 |

2.3 Transfer the samples into cuvettes and measure the absorbance at 540 nm.

2.4 Plot standard calibration curve of protein concentration and absorbance.

**3. Protein solution analysis**

3.1 Turn on the spectrophotometer and allow it to warm up.

3.2 Prepare samples by mixing 0.05 ml of sample with distilled water 0.45 ml such that the final volume is 0.5 ml.

3.3 Add 2.5 ml. of biuret reagent to each sample, vortex and allow to react 30 min

3.4 Transfer the samples into cuvettes and measure the absorbance at 540 nm.

3.5 Then, calculate protein content from calibration curve.

**2) Kjeldahl method**

The nitrogen content in dried shrimp shell and protein solution at optimum condition were determined by Kjeldahl method.


**Figure 3.1** Kjeldahl apparatus

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 Effect of concentration of citric acid to percentage of extracted calcium

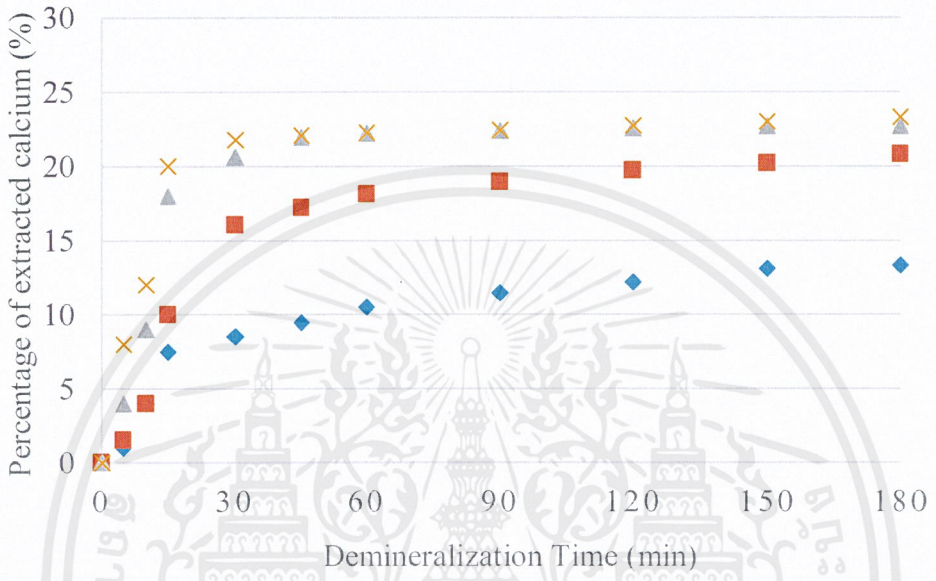


**Figure 4.1** Percentage of different concentration of citric acid at 0.5 M (◇), 1.0 M (□), 1.5 M (Δ) and 2.0 M (x) as a function of time

In this work, it is focused on demineralization process and citric acid, organic acid is used instead of hydrochloric acid, strong acid. Citric acid with concentration of 0.5, 1.0, 1.5 and 2.0 M are used. From Figure 4.1, it is found a great effects of citric acid concentration to percentage of extracted calcium at any time. Higher extracted calcium percentage is obtained by adding 1.5 and 2.0 M of citric acid which increase the percentage of extracted calcium to approximately 23-25%. However, they reach to be stable at 22-23% after 45 minutes due to the amount of citric acid is excess to extract calcium from shrimp shell.

In addition, the percentage of extracted calcium by adding 0.5 M of citric acid reaches to be stable at 13% after 150 minutes because the amount of citric acid is not enough to extract calcium from shrimp shell and citric acid is as known as a limiting reactant in this condition. On the other hand, the percentage of extracted calcium by adding 1.0 M of citric acid shows about 21%, which is higher than expectation and

almost closely to the highest obtained extracted calcium in this work. Therefore, adding 1.0 M of citric acid to extract the calcium from shrimp shell is suitable in term of worthiness and wastefulness of this work.

### 4.2 Concentration of calcium in shrimp shell as a function of time

In addition, Figure 4.2 shows about the remained concentration of calcium in shrimp shell of different concentration of citric acid which are 0.5 and 1.0 M at any time by beginning of the amount of calcium in shrimp shell is 25%.

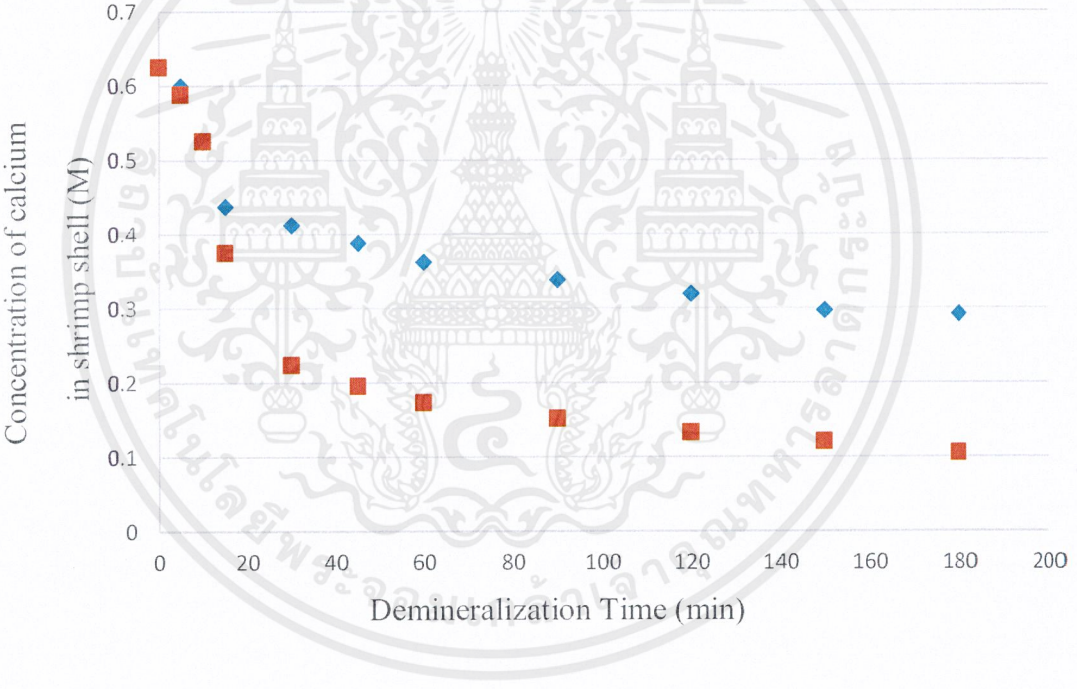


**Figure 4.2** Concentration of calcium in shrimp shell of different concentration of citric acid at 0.5 M (◇) and 1.0 M (□) as a function of time

### 4.3 Isotherm of adsorption

Isotherm of adsorption is important to explain the adsorption characteristics. Langmuir's equation is popularly used to describe about adsorption and this isotherm is the easiest isotherm which is very commonly used for single layer adsorption.

$$-\frac{dC_A}{dt} = \frac{k_1 {C_A}^m {C_B}^n}{1 + k_2 {C_A}^m} \quad (16)$$

Define,

$C_A$ = Remained concentration of calcium in shrimp shell (M)

$C_B$ = Remained concentration of citric acid (M)

$k_1$ = rate constant (1/M.s)

$k_2$ = rate constant (1/M)

In this work, applying Langmuir isotherm (equation 16) is used to describe this demineralization process because it is solid-liquid adsorption. In addition, Table 4.1 shows the value of $k_1$, $k_2$, m and n of applying Langmuir's isotherm by Statistica program.

**Table 4.1** The value of k, m and n of Langmuir's isotherm

| Langmuir | | | |
|---|---|---|---|
| $k_1$ | $k_2$ | m | n |
| 0.05 | -0.81 | 1 | 1 |

When these values are used in Polymath program, concentration of calcium in shrimp shell of different concentration of citric acid at 0.5, 1.0, 1.5 and 2.0 M as a function of time are obtained. Therefore, Figure 4.3, Figure 4.4, Figure 4.5 and Figure

4.6 show concentration of calcium in shrimp shell of different concentration of citric acid at any time between the results from this experiment and the polymath.

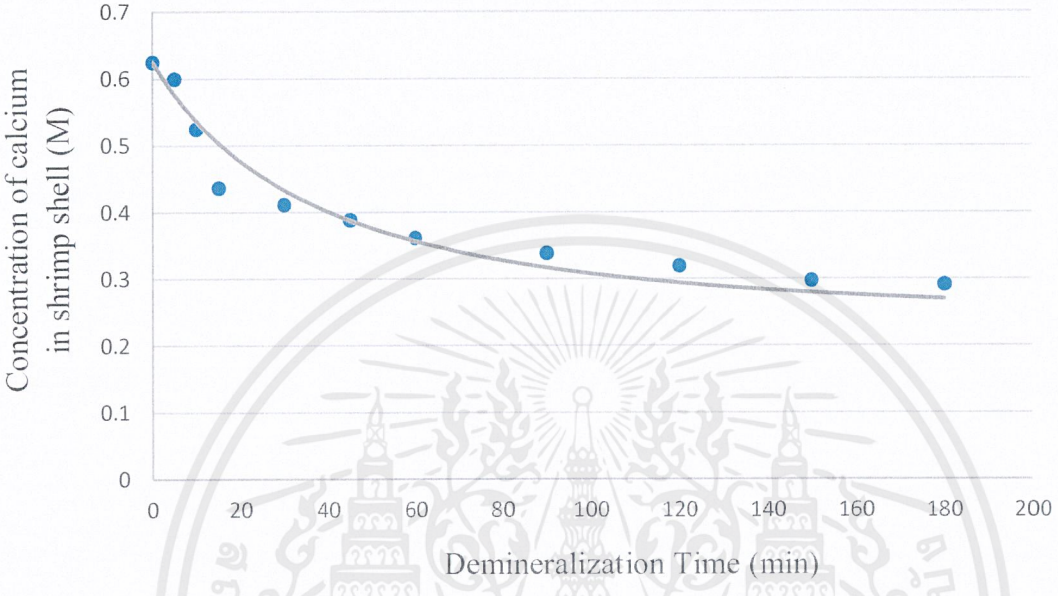


**Figure 4.3** Concentration of calcium in shrimp shell of 0.5 M concentration of citric acid at any time between the results from this experiment (o) and the polymath (-)

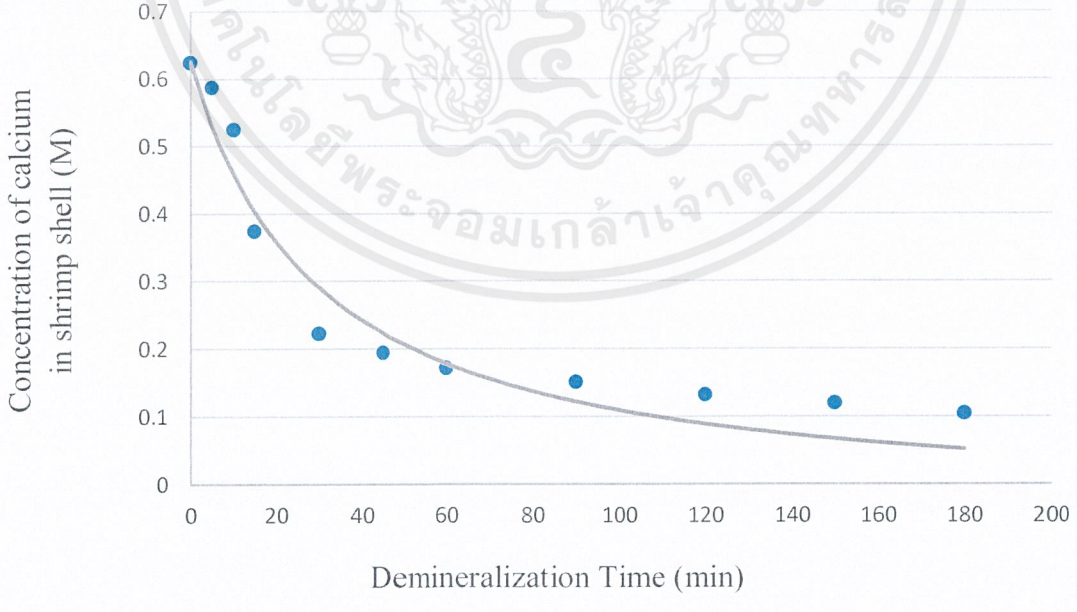


**Figure 4.4** Concentration of calcium in shrimp shell of 1.0 M concentration of citric acid at any time between the results from this experiment (o) and the polymath (-)

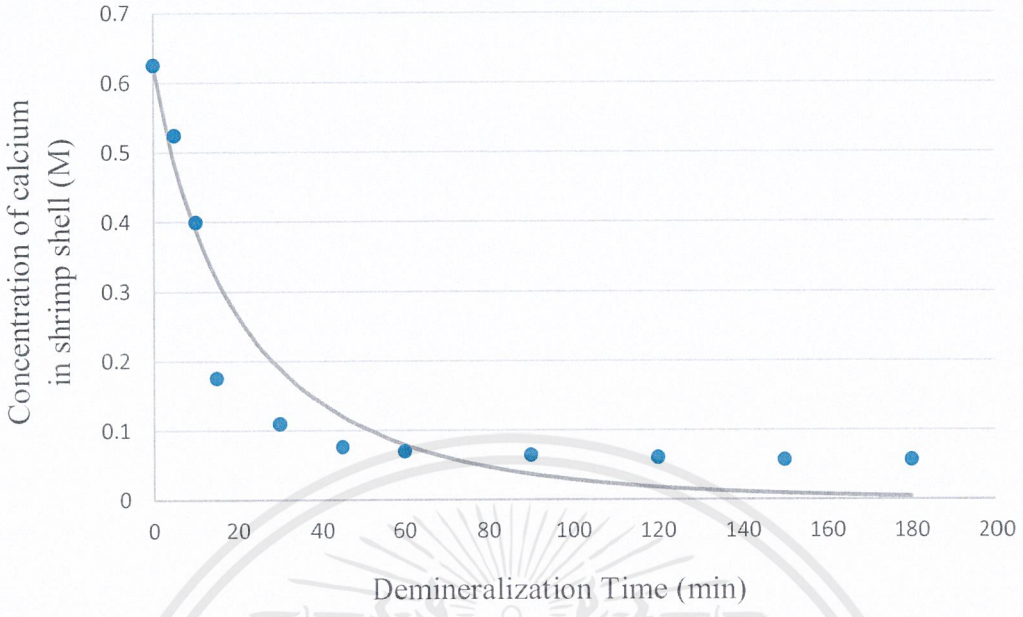


**Figure 4.5** Concentration of calcium in shrimp shell of 1.5 M concentration of citric acid at any time between the results from this experiment (o) and the polymath (-)

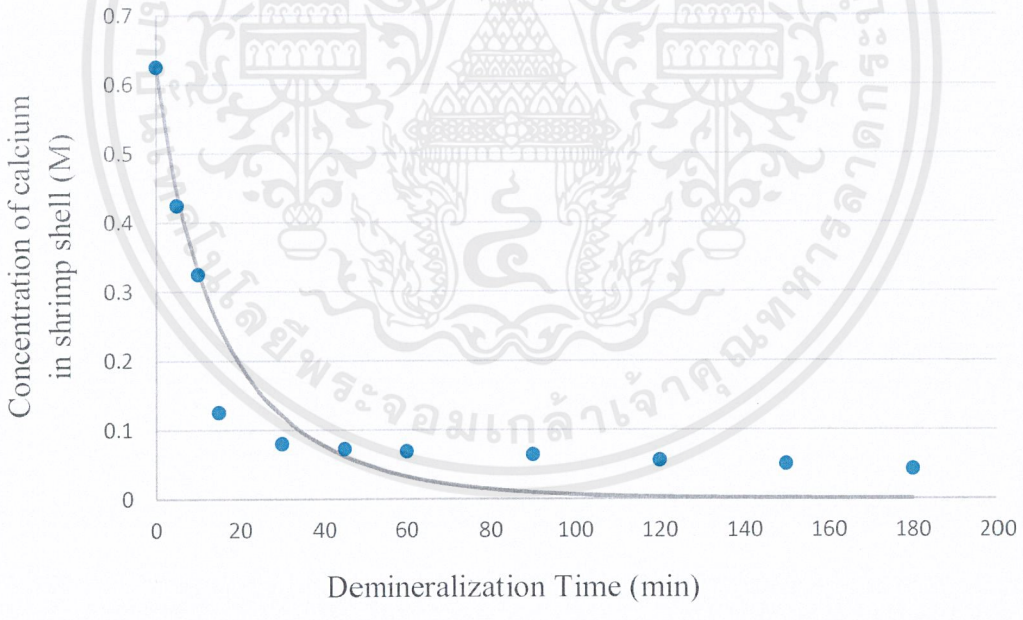


**Figure 4.6** Concentration of calcium in shrimp shell of 2.0 M concentration of citric acid at any time between the results from this experiment (o) and the polymath (-)

### 4.4 Effect of temperature on the deproteinization

In order to study the effect of temperature (between 50-70 °C) on the shrimp shell deproteinization catalyzed by CaO at 3 grams (10wt%), the reaction was performed for 2 days at each temperature and the released protein in the supernatant was deduced by the biuret method. The hydrolyzed protein concentration was plotted versus time at 50, 60, and 70°C as shown in the Figure 4.7. As expected, increasing temperature increases hydrolyzed protein concentration accordingly. At the first 10 minutes, one-third of the hydrolyzed protein concentration was rapidly released into the solution of CaO base, compared to the later one-third which took about after 250 minutes and the last one that took thousands of minute. Results from Kjeldahl method revealed that 50% of protein inside shrimp shell was hydrolyzed in 2 days at 70°C.

Shrimp shell is not a uniform solid which has protein and calcium carbonate distribute unevenly. More protein is found on the inner surface of the shrimp shell and vice versa for calcium carbonate on the outer surface for shrimp shell protection. Hydrolysis takes place on the contact of hydrolysable solid surface and attacked catalytic chemicals in liquids. Therefore, it is not able to explain this hydrolysis by a simple kinetics model.

Initially, proteins that attached to the outer surfaces, mostly the inner one, were quickly hydrolyzed with a small effect of temperature difference. It demonstrates the process is not kinetically controlled. Hydroxide ion has to diffuse from bulk solution to the protein surface in order to catalyze the hydrolysis reaction.

Later on, hydroxide ion diffuses into swell surface of shrimp shell to catalyze hydrolysis of proteins located inside the shell that close to the shell surface. This interval is shown in Figure 14(a) with the much lower slope.

In Figure 4.7(b), proteins that stay far more inside are much more slowly hydrolyzed and being mainly remained, since there is a low concentration of hydroxide ion from lime (pH ~12) in solution and the ion has to penetrate into more complex and dense structures of calcium carbonate and chitin. Thus, temperature has a very small effect on kinetics basis, but it can have some effects on the structure of the remaining shell that enhances hydroxide diffusion.

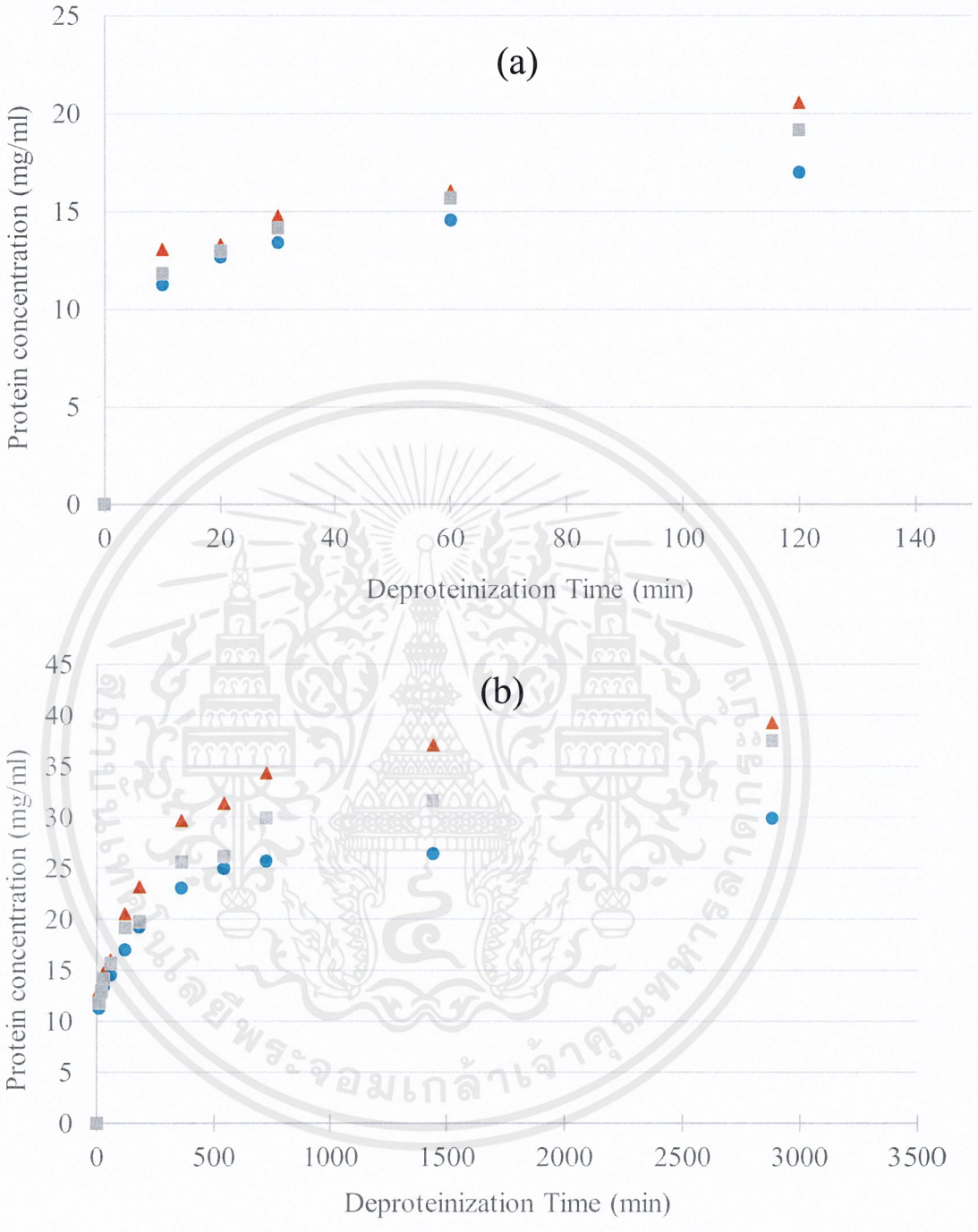


**Figure 4.7** Protein concentration in 10wt% of CaO supernatant as a function of time at 50 (o), 60 (□), and 70°C (Δ).

### 4.5 Falsified kinetics of deproteinization

The deproteinization process can be described by equation 17

$$-\frac{d(m_{p_{s0}} - m_{p_l})}{dt} = k \times m_{p_s} = k \times (m_{p_{s0}} - m_{p_l}) \quad (17)$$

where, $m_{pl}$ is mass of the released protein in supernatant which deduced from biuret method, $m_{ps0}$ is initial mass of protein contained in shrimp shell which is equal to 12.6 g (42 wt% of dry shrimp shell, determined by Kjeldahl method), t = deproteinization time, and k = rate constant.

From equation (17), we obtained

$$-\frac{d\frac{(m_{PSo} - m_{Pl})}{(m_{PSo} - m_{Pl})}}{dt} = k \quad (18)$$

$$\int \frac{d(m_{PSo} - m_{p_l})}{(m_{p_{s0}} - m_{p_l})} = -\int k\, dt \quad (19)$$

When integrate equation (19) with the limit $m_{pl} = 0$ at t = 0 gives

$$ln \frac{m_{p_{s0}}}{m_{p_{s0}} - m_{p_l}} = kt \quad (20)$$

$$ln\,(m_{p_{s0}} - m_{p_l}) = -kt + ln\,(m_{p_{s0}}) \quad (21)$$

Therefore, when ln ($m_{p_{s0}}$-$m_{p_l}$) is plotted versus time, we get a straight line. The slope of this line represents the rate constant.

Data from Figure 4.7(a) in the initial time which is less likely limited by diffusion than the rest were chosen for kinetics calculation and shown in Figure 4.8 The curve is not linear and it has two stages of hydrolysis rate. The first stage which is rapid hydrolysis within 10 minutes has linear pattern (due to drawing a straight line between two points). According to above discussion, this initial less diffusion limit rate constants were determined as shown in Table 4.2. The result shows that there is no significant different rate when increasing temperature. Moreover, the activation energy, obtained from the Arrhenius plot as shown in Figure 4.9, is very low (7.2 kJ/mole) which indicates that the deproteinization is limited by diffusion. It may be noted that the structures of protein inside shrimp shell is complex and their interaction with chitin

such as, hydrogen bonding or covalent bonding, which largely affect the hydrolysis rate.

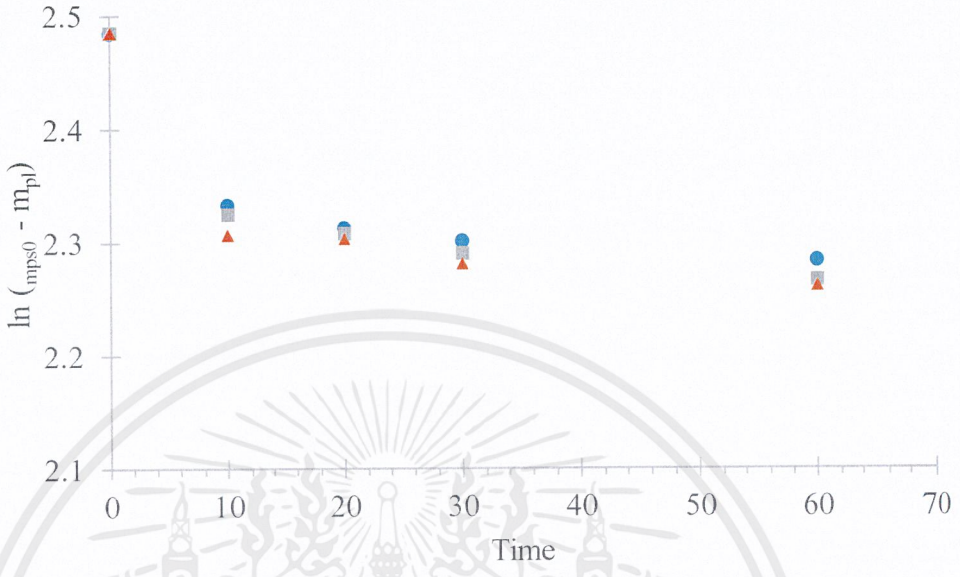


**Figure 4.8** Logarithmic variation of the protein content in shrimp shell as a function of initial deproteinization time at 50 (o), 60 (□), and 70°C (Δ)

**Table 4.2** Rate constants of initial deproteinization

| Temperature (°C) | k ($min^{-1}$) |
|---|---|
| 50 | 0.0152 |
| 60 | 0.0160 |
| 70 | 0.0178 |

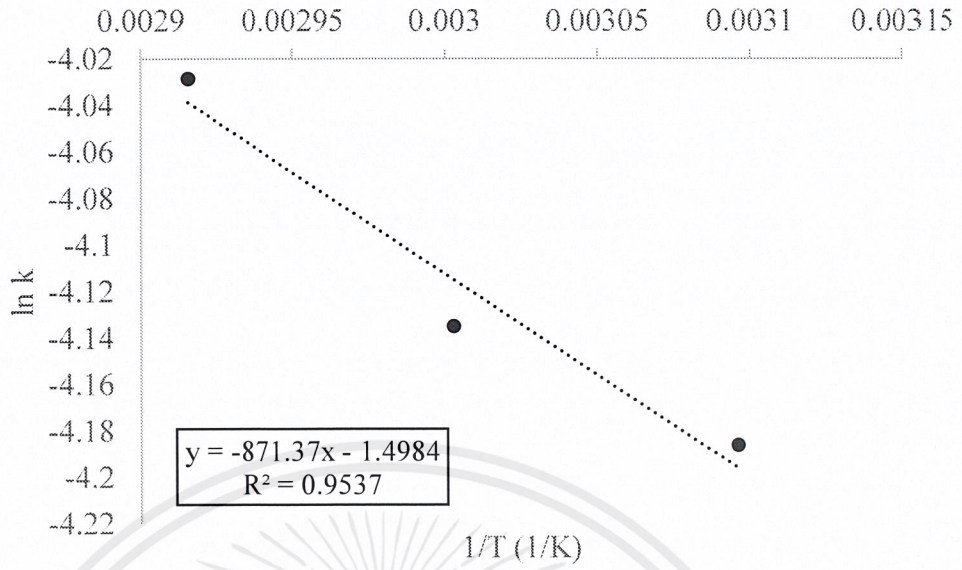


**Figure 4.9** Arrhenius plot

# CHAPTER V

# CONCLUSIONS

## 5.1 Conclusions

In this work, it is focused on demineralization and deproteinization process of shrimp shell by using citric acid and calcium oxide, respectively. The aims of this work are to study extraction of calcium and proteins from shrimp shell.

In the demineralization process, citric acid, organic acid with different concentration (0.5, 1.0, 1.5 and 2.0 M) are used instead of hydrochloric acid, strong acid and the solution is mixed between dried shrimp shell and citric acid in ratio 1:5 (w/v) at room temperature and stirred at 100 rpm by different stirring time. It is found a great effects of citric acid concentration to percentage of extracted calcium at any time. The percentage of extracted calcium by adding 1.0 M of citric acid shows about 21%, which is almost closely to the highest obtained extracted calcium in this work. Therefore, adding 1.0 M of citric acid to extract the calcium from shrimp shell is suitable in term of worthiness and wastefulness of this work. In addition, it is solid-liquid adsorption and applying Langmuir equation is used to describe about this demineralization process. Finally, it is found that the value of $k_1$ is equal to 0.05 and $k_2$ is equal to -0.81.

In the deproteinization process, shrimp shells can be extracted by using 10wt% CaO. In this work, the optimum condition occurs at temperature 70°C for 2 days which can extract proteins from shrimp shell approximately 50wt% of total protein content. The kinetic results show that there is no significant different rate when increasing temperature. Besides, the measured activation energy is low which indicate that the deproteinization is limited by diffusion. It may be noted that the structures of protein inside shrimp shell is complex.

## 5.2 Suggestions

5.2.1 Should study the characteristic of extracted calcium in demineralization process

5.2.2 Should study the condition by vary the size of dried shrimp shell in demineralization and deproteinization process.

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# APPENDIX

# APPENDIX A

# EXPERIMENTAL RESULTS

## A.1 Experimental results of demineralization process

**Table A.1** Percentage of extracted calcium at any time by adding 0.5 M citric acid

| Time (min) | NaOH (ml) | g of Ca | % of Ca |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | 14 | 0.05 | 1 |
| 10 | 11 | 0.2 | 4 |
| 15 | 7.5 | 0.375 | 7.5 |
| 30 | 6.5 | 0.425 | 8.5 |
| 45 | 5.55 | 0.4725 | 9.45 |
| 60 | 4.5 | 0.525 | 10.5 |
| 90 | 3.55 | 0.5725 | 11.45 |
| 120 | 2.8 | 0.61 | 12.2 |
| 150 | 1.9 | 0.655 | 13.1 |
| 180 | 1.7 | 0.665 | 13.3 |

**Table A.2** Percentage of extracted calcium at any time by adding 1.0 M citric acid

| Time (min) | NaOH (ml) | g of Ca | % of Ca |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | 28.5 | 0.075 | 1.5 |
| 10 | 26 | 0.2 | 4 |
| 15 | 20 | 0.5 | 10 |
| 30 | 13.95 | 0.8025 | 16.05 |
| 45 | 12.8 | 0.86 | 17.2 |
| 60 | 11.9 | 0.905 | 18.1 |
| 90 | 11.05 | 0.9475 | 18.95 |
| 120 | 10.3 | 0.985 | 19.7 |
| 150 | 9.8 | 1.01 | 20.2 |
| 180 | 9.2 | 1.04 | 20.8 |

**Table A.3** Percentage of extracted calcium at any time by adding 1.5 M citric acid

| Time (min) | NaOH (ml) | g of Ca | % of Ca |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | 41 | 0.2 | 4 |
| 10 | 36 | 0.45 | 9 |
| 15 | 27 | 0.9 | 18 |
| 30 | 24.4 | 1.03 | 20.6 |
| 45 | 23.05 | 1.0975 | 21.95 |
| 60 | 22.8 | 1.11 | 22.2 |
| 90 | 22.6 | 1.12 | 22.4 |
| 120 | 22.45 | 1.1275 | 22.55 |
| 150 | 22.3 | 1.135 | 22.7 |
| 180 | 22.3 | 1.135 | 22.7 |

**Table A.4** Percentage of extracted calcium at any time by adding 2.0 M citric acid

| Time (min) | NaOH (ml) | g of Ca | % of Ca |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 5 | 52 | 0.4 | 8 |
| 10 | 48 | 0.6 | 12 |
| 15 | 40 | 1 | 20 |
| 30 | 38.2 | 1.09 | 21.8 |
| 45 | 37.9 | 1.105 | 22.1 |
| 60 | 37.75 | 1.1125 | 22.25 |
| 90 | 37.55 | 1.1225 | 22.45 |
| 120 | 37.25 | 1.1375 | 22.75 |
| 150 | 37 | 1.15 | 23 |
| 180 | 36.7 | 1.165 | 23.3 |

**Table A.5** The remained concentration of calcium in shrimp shell of different concentration of citric acid which are 0.5, 1.0, 1.5 and 2.0 M at any time by polymath program

| Time (min) | 0.5 M | 1.0 M | 1.5 M | 2.0 M |
|---|---|---|---|---|
| 0 | 0.625 | 0.625 | 0.625 | 0.625 |
| 4.44046 | 0.580940 | 0.539176 | 0.499716 | 0.4625415 |
| 5.88046 | 0.568400 | 0.515874 | 0.4673316 | 0.4226406 |
| 7.32046 | 0.556592 | 0.494353 | 0.4380238 | 0.3872808 |
| 10.20046 | 0.534934 | 0.455893 | 0.3870658 | 0.3275449 |
| 11.64046 | 0.524980 | 0.438636 | 0.3647921 | 0.3021569 |
| 13.08046 | 0.515550 | 0.422519 | 0.3443220 | 0.2792303 |
| 14.52046 | 0.506604 | 0.407434 | 0.3254526 | 0.2584543 |
| 17.40046 | 0.490027 | 0.379981 | 0.2918483 | 0.2223519 |
| 18.84046 | 0.482335 | 0.367454 | 0.2768357 | 0.2066174 |
| 20.28046 | 0.475004 | 0.355637 | 0.2628613 | 0.1922040 |
| 21.72046 | 0.468011 | 0.344471 | 0.2498273 | 0.1789726 |
| 24.60046 | 0.454951 | 0.323887 | 0.2262474 | 0.1555902 |
| 26.04046 | 0.448845 | 0.314381 | 0.2155586 | 0.1452421 |
| 27.48046 | 0.443000 | 0.305346 | 0.2055221 | 0.1356778 |
| 28.92046 | 0.437399 | 0.296748 | 0.1960847 | 0.1268258 |
| 31.80046 | 0.426875 | 0.280745 | 0.1788222 | 0.1110120 |
| 33.24046 | 0.421926 | 0.273286 | 0.1709160 | 0.1039431 |
| 34.68046 | 0.417170 | 0.266157 | 0.1634457 | 0.0973710 |
| 36.12046 | 0.412597 | 0.259336 | 0.1563799 | 0.0912551 |
| 39.00046 | 0.403961 | 0.246543 | 0.1433507 | 0.0802488 |
| 40.44046 | 0.399881 | 0.240538 | 0.1373377 | 0.0752954 |
| 41.88046 | 0.395948 | 0.234771 | 0.1316297 | 0.0706714 |
| 43.32046 | 0.392156 | 0.229231 | 0.1262070 | 0.0663521 |
| 46.20046 | 0.384964 | 0.218777 | 0.1161463 | 0.0585394 |

| | | | | |
|---|---|---|---|---|
| 47.64046 | 0.381553 | 0.213840 | 0.1114764 | 0.0550066 |
| 49.08046 | 0.378257 | 0.209084 | 0.1070276 | 0.0516992 |
| 50.52046 | 0.375070 | 0.204497 | 0.1027867 | 0.0486016 |
| 53.40046 | 0.369008 | 0.195799 | 0.0948815 | 0.0429783 |
| 54.84046 | 0.366122 | 0.191673 | 0.0911956 | 0.0404270 |
| 56.28046 | 0.363328 | 0.187684 | 0.0876744 | 0.0380336 |
| 57.72046 | 0.360622 | 0.183827 | 0.0843088 | 0.0357876 |
| 60.60046 | 0.355459 | 0.176483 | 0.0780120 | 0.0316999 |
| 62.04046 | 0.352994 | 0.172984 | 0.0750656 | 0.0298407 |
| 63.48046 | 0.350604 | 0.169594 | 0.0722446 | 0.0280940 |
| 64.92046 | 0.348284 | 0.166308 | 0.0695427 | 0.0264527 |
| 67.80046 | 0.343847 | 0.160029 | 0.0644725 | 0.0234597 |
| 69.24046 | 0.341725 | 0.157027 | 0.0620933 | 0.0220961 |
| 70.68046 | 0.339663 | 0.154113 | 0.0598114 | 0.0208135 |
| 72.12046 | 0.337659 | 0.151282 | 0.0576220 | 0.0196071 |
| 75.00046 | 0.333818 | 0.145855 | 0.0535039 | 0.0174042 |
| 76.44046 | 0.331977 | 0.143254 | 0.0515670 | 0.0163992 |
| 77.88046 | 0.330185 | 0.140723 | 0.0497066 | 0.0154533 |
| 79.32046 | 0.328443 | 0.138260 | 0.0479193 | 0.0145628 |
| 82.20046 | 0.325096 | 0.133528 | 0.0445508 | 0.0129352 |
| 83.64046 | 0.323489 | 0.131253 | 0.0429636 | 0.0121919 |
| 85.08046 | 0.321923 | 0.129037 | 0.0414373 | 0.0114918 |
| 86.52046 | 0.320399 | 0.126877 | 0.0399693 | 0.0108325 |
| 89.40046 | 0.317466 | 0.122717 | 0.0371981 | 0.0096265 |
| 90.84046 | 0.316055 | 0.120713 | 0.0358904 | 0.0090753 |
| 92.28046 | 0.314680 | 0.118757 | 0.0346316 | 0.0085560 |
| 93.72046 | 0.313340 | 0.116849 | 0.0334198 | 0.0080668 |
| 96.60046 | 0.310757 | 0.113165 | 0.0311293 | 0.0071712 |

| | | | | |
|---|---|---|---|---|
| 98.04046 | 0.309513 | 0.111388 | 0.0300470 | 0.0067617 |
| 99.48046 | 0.308300 | 0.109651 | 0.0290044 | 0.0063758 |
| 100.9205 | 0.307115 | 0.107954 | 0.0279999 | 0.0060121 |
| 103.8005 | 0.304831 | 0.104673 | 0.0260993 | 0.0053461 |
| 105.2405 | 0.303730 | 0.103087 | 0.0252003 | 0.0050415 |
| 106.6805 | 0.302654 | 0.101535 | 0.0243337 | 0.0047543 |
| 108.1205 | 0.301604 | 0.100017 | 0.0234983 | 0.0044835 |
| 111.0005 | 0.299576 | 0.097078 | 0.0219161 | 0.0039877 |
| 112.4405 | 0.298597 | 0.095655 | 0.0211670 | 0.0037608 |
| 113.8805 | 0.297640 | 0.094262 | 0.0204446 | 0.0035468 |
| 115.3205 | 0.296705 | 0.092898 | 0.0197478 | 0.0033451 |
| 118.2005 | 0.294899 | 0.090252 | 0.0184271 | 0.0029756 |
| 119.6405 | 0.294026 | 0.088969 | 0.0178015 | 0.0028065 |
| 121.0805 | 0.293172 | 0.087712 | 0.0171978 | 0.0026470 |
| 122.5205 | 0.292338 | 0.086480 | 0.0166152 | 0.0024966 |
| 125.4005 | 0.290723 | 0.084088 | 0.0155103 | 0.0022211 |
| 126.8405 | 0.289942 | 0.082927 | 0.0149866 | 0.0020949 |
| 128.2805 | 0.289179 | 0.081788 | 0.0144811 | 0.0019760 |
| 129.7205 | 0.288431 | 0.080671 | 0.0139930 | 0.0018638 |
| 132.6005 | 0.286985 | 0.078499 | 0.0130670 | 0.0016582 |
| 134.0405 | 0.286285 | 0.077444 | 0.0126278 | 0.0015641 |
| 135.4805 | 0.285599 | 0.076408 | 0.0122038 | 0.0014754 |
| 136.9205 | 0.284929 | 0.075391 | 0.0117943 | 0.0013917 |
| 139.8005 | 0.283630 | 0.073412 | 0.0110169 | 0.0012383 |
| 141.2405 | 0.283000 | 0.072450 | 0.0106481 | 0.0011680 |
| 142.6805 | 0.282384 | 0.071504 | 0.0102919 | 0.0011018 |
| 144.1205 | 0.281781 | 0.070575 | 0.0099478 | 0.0010393 |
| 147.0005 | 0.280611 | 0.068767 | 0.0092944 | 0.0009247 |

| | | | | |
|---|---|---|---|---|
| 148.4405 | 0.280044 | 0.067886 | 0.0089842 | 0.0008723 |
| 149.8805 | 0.279489 | 0.067020 | 0.0086846 | 0.0008229 |
| 151.3205 | 0.278945 | 0.066169 | 0.0083952 | 0.0007762 |
| 154.2005 | 0.277891 | 0.064511 | 0.0078453 | 0.0006907 |
| 155.6405 | 0.277379 | 0.063703 | 0.0075843 | 0.0006515 |
| 157.0805 | 0.276878 | 0.062908 | 0.0073320 | 0.0006146 |
| 158.5205 | 0.276387 | 0.062126 | 0.0070883 | 0.0005798 |
| 161.4005 | 0.275434 | 0.060601 | 0.0066252 | 0.0005159 |
| 162.8405 | 0.274971 | 0.059857 | 0.0064052 | 0.0004867 |
| 164.2805 | 0.274518 | 0.059125 | 0.0061927 | 0.0004591 |
| 165.7205 | 0.274074 | 0.058405 | 0.0059873 | 0.0004331 |
| 168.6005 | 0.273211 | 0.057000 | 0.0055969 | 0.0003854 |
| 170.0405 | 0.272793 | 0.056313 | 0.0054115 | 0.0003635 |
| 171.4805 | 0.272382 | 0.055638 | 0.0052322 | 0.0003429 |
| 172.9205 | 0.271980 | 0.054973 | 0.0050590 | 0.0003235 |
| 175.8005 | 0.271198 | 0.053674 | 0.0047297 | 0.0002879 |
| 177.2405 | 0.270819 | 0.053040 | 0.0045733 | 0.0002716 |
| 178.6805 | 0.270447 | 0.052415 | 0.0044221 | 0.0002562 |
| 180 | 0.270112 | 0.051851 | 0.0042879 | 0.0002428 |

## A.2 Experimental results of deproteinization process

**Table A.6** The absorbance of samples analyzed by biuret method

| Temperature (°C) | Time (min) | Round 1 | Round 2 | Average |
|---|---|---|---|---|
| | | Absorbance | Absorbance | |
| **50** | 0 | 0 | 0 | 0 |
| | 10 | 0.146 | 0.146 | 0.146 |
| | 20 | 0.164 | 0.164 | 0.164 |
| | 30 | 0.172 | 0.175 | 0.1735 |
| | 60 | 0.189 | 0.188 | 0.1885 |
| | 120 | 0.245 | 0.195 | 0.22 |
| | 180 | 0.254 | 0.245 | 0.2495 |
| | 360 | 0.31 | 0.287 | 0.2985 |
| | 540 | 0.323 | 0.323 | 0.323 |
| | 720 | 0.333 | 0.279 | 0.306 |
| | 1440 | 0.34 | 0.344 | 0.342 |
| | 2880 | 0.366 | 0.389 | 0.3775 |
| **60** | 0 | 0 | 0 | 0 |
| | 10 | 0.152 | 0.154 | 0.153 |
| | 20 | 0.167 | 0.169 | 0.168 |
| | 30 | 0.186 | 0.18 | 0.183 |
| | 60 | 0.203 | 0.203 | 0.203 |
| | 120 | 0.248 | 0.248 | 0.248 |
| | 180 | 0.256 | 0.256 | 0.256 |
| | 360 | 0.331 | 0.331 | 0.331 |
| | 540 | 0.337 | 0.340 | 0.3385 |
| | 720 | 0.382 | 0.392 | 0.387 |
| | 1440 | 0.363 | 0.454 | 0.40851 |
| | 2880 | 0.461 | 0.508 | 0.4845 |
| **70** | 0 | 0 | 0 | 0 |
| | 10 | 0.170 | 0.168 | 0.169 |
| | 20 | 0.172 | 0.172 | 0.172 |
| | 30 | 0.193 | 0.189 | 0.191 |

| | | | | |
|---|---|---|---|---|
| | 60 | 0.215 | 0.200 | 0.2075 |
| | 120 | 0.293 | 0.239 | 0.266 |
| | 180 | 0.294 | 0.306 | 0.3 |
| | 360 | 0.410 | 0.358 | 0.384 |
| | 540 | 0.406 | 0.406 | 0.406 |
| | 720 | 0.454 | 0.434 | 0.444 |
| | 1440 | 0.465 | 0.495 | 0.48 |
| | 2880 | 0.511 | 0.505 | 0.508 |

The experiment was repeated 2 times, the average values of absorbance were used to deduce the protein concentration.

# APPENDIX B

# CALIBRATION CURVE

## B.1 Calibration Curve of Standard Bovine Serum Solution

**Table B.1** Absorbance of standard bovine serum albumin

| Bovine Serum Albumin Concentrations (mg/ml) | Absorbance |
|---|---|
| 0 | 0 |
| 2 | 0.128 |
| 4 | 0.263 |
| 6 | 0.394 |
| 8 | 0.522 |
| 10 | 0.638 |

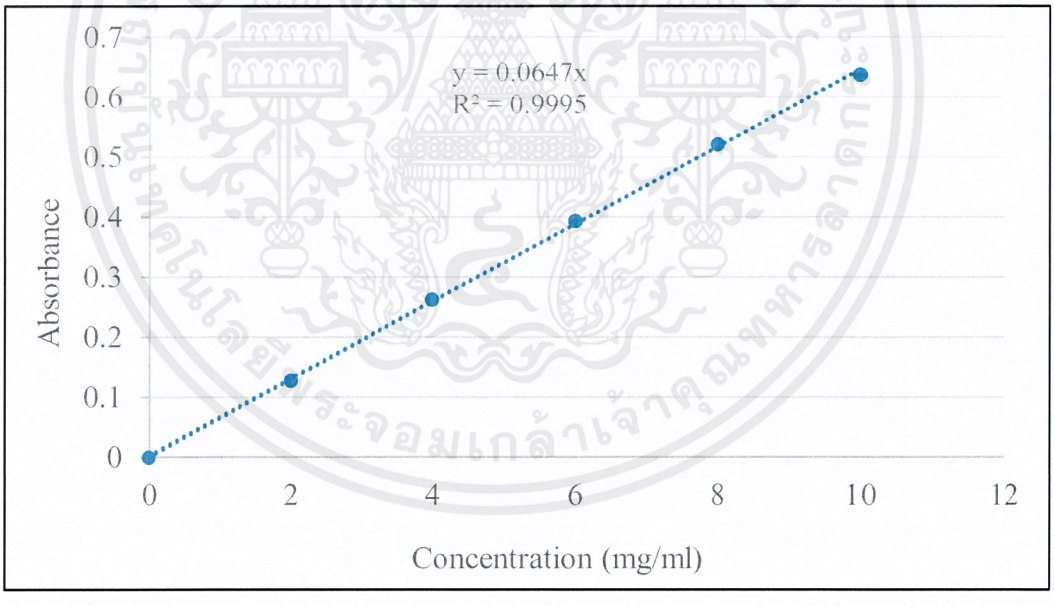


**Figure B.1** Standard Calibration curve

**Example of calculation**

From calibration curve, we obtained

$$Y = 0.0647x$$

Where, Y = Absorbance

X= Protein concentration (mg/ml)

If A = 0.1735

Then, X = 0.1735/ 0.00647

Therefore, protein concentration = 2.68 mg/ml

For a dilution factor of 5, then it multiplied by 5

Final protein concentration is = 2.68 mg/ml × 5 = 13.40 mg/ml.

# APPENDIX C

## Kjeldahl method and calculation

### Calculation of nitrogen percent in the sample

$$\text{Percent of nitrogen (\%N)} = \frac{(A-B)\times N\times 14\times 100}{W\times 1{,}000}$$

Where A = The amount of hydrochloric solution titrated with sample

B = The amount of hydrochloric solution titrated with blank

N = Concentration of hydrochloric (normal)

W = Weight of sample (g)

Then calculate the percent of protein by following equation

$$\text{Percent of protein (\%P)} = \%N \times 6.25$$

Where %N = the percentage of nitrogen

6.25 = Kjeldahl factor

### Calculation of initial protein content

Dried shrimp shell was analyzed to find the amount of initial protein contained in the sample.

Where, the amount of hydrochloric acid titrated with the sample = 57 ml,

the amount of hydrochloric acid titrated with blank = 0.4 ml

the concentration of hydrochloric acid = 0.1 normal

and weight of sample = 1.0380 g

Therefore, $$\text{Percent of nitrogen} = \frac{(50-0.4)\times 0.1\times 14\times 100}{1.0380\times 1{,}000}$$

$$\text{Percent of nitrogen} = 7.633\%$$

However, it is not the corrected percent nitrogen content since in shrimp shells contain nitrogen of chitin 1.18%. Therefore, the corrected percent nitrogen need to minus with 1.18

The corrected percent of nitrogen = 7.63 – 1.18 = 6.45

Therefore, $\text{percent of protein (\%P)} = 6.45 \times 6.25 = 40.33\ \%$

In the initial protein content, there are protein content about 12 g. within shrimp shells of 30 grams.

**Calculation of initial protein content**

The protein solution obtained from the optimal condition, temperature 70°C, 2 days.

Where, the amount of hydrochloric solution titrated with the sample = 45 ml,

The amount of hydrochloric solution titrated with blank = 0.4 ml

The Concentration of hydrochloric = 0.1 normal

And Volume of sample = 10 ml (Density of protein solution = .1.0380 g/ml)

Therefore, Weight of sample = 10.380 g

So, $$\text{Percent of nitrogen} = \frac{(45 - 0.4) \times 0.1 \times 14 \times 100}{10.380 \times 1{,}000}$$

$$\text{Percent of nitrogen} = 0.6244$$

We assume that there is no chitin content in this solution because the solution is not strong alkaline enough to extract chitin.

Therefore,

$$\text{Percent of protein (\%P)} = 0.6244 \times 6.25 = 3.9\ \%$$

The protein solution that we obtained, it has volume = 150 ml or 150 g.

Therefore, total amount proteins are extracted about 3.9 × (150/100) = 5.85 g.

**Calculation of percent protein extraction**

$$\% \text{ protein extraction} = \frac{\text{mass of protein in solution}}{\text{mass of initial protein in shrimp shell}} \times 100$$

Therefore, protein can be extract

$$\% \text{ protein extraction} = \frac{5.85}{12} \times 100 = 48.75\ \%$$

CaO can be used to extract protein from shrimp shell approximately 50%.

# BIOGRAPHY

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