Protein Hydrolysis of Rubber Seed Powder by Using Salts Treatment

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except for the excerpts and summaries that I have clarified the sources.

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Protein Hydrolysis of Rubber Seed Powder by Using Salts Treatment

ABSTRACT

In order to grow economical yet quality poultry feed, poultry requires a high-quality nutrient. For any poultry, protein is one of the essential elements in their feeding meal. Production must need a high feeding quality to produce high-quality poultry, making farmers devote themselves to purchasing the feeding meal. Hence, another solution is replacing the regular soybean meal used in the poultry feed with rubber seed powder. However, this powder is not easily digested. Therefore, this study aims to treat rubber seeds with three different types of salts, including the neutral, acidic, and alkaline salts, at different concentrations and test the protein hydrolysis level of rubber seeds content after the salt's treatment. The results of this study show that rubber seed powder can replace soybean meal as a protein source, and the highest protein decrease is 88 %, which comes from the $KHCO_3$-treated rubber seed powder for 5 hours at 60 °C. The molecular size reduction of protein was confirmed by using the SDS-Page electrophoresis. This study demonstrates the potential of salts to denature and/or hydrolyse protein into smaller molecules to produce a highly digestible protein source.

Keywords: Poultry, Rubber Seed Powder, Salts Treatment, Protein Hydrolysis, SDS-Page electrophoresis

Hidrolisis Protein Serbuk Biji Getah dengan Menggunakan Rawatan Garam

ABSTRAK

Untuk membesarkan ayam yang menjimatkan lagi berkualiti memerlukan nutrien yang berkualiti tinggi. Bagi mana-mana ayam, protein adalah salah satu elemen penting untuk dimasukkan ke dalam makanan penyusuan mereka. Pengeluaran mesti memerlukan kualiti makanan yang tinggi untuk menghasilkan ayam yang berkualiti tinggi, menjadikan penternak menumpukan untuk membeli makanan makanan. Oleh itu, penyelesaian lain ialah menggantikan makanan kacang soya biasa yang digunakan dalam makanan ayam dengan serbuk biji getah. Namun begitu, serbuk ini tidak mudah dihadam. Oleh itu, kajian ini bertujuan untuk merawat benih getah dengan tiga jenis garam yang berbeza, termasuk garam neutral, berasid dan alkali, pada kepekatan yang berbeza dan menguji tahap hidrolisis protein kandungan biji getah selepas rawatan garam-menggunakan rawatan garam sebagai rawatan kimia kepada benih getah untuk menghasilkan makanan penyusuan. Hasil kajian ini menunjukkan serbuk biji getah boleh menggantikan tepung kacang soya sebagai sumber protein, dan penurunan protein tertinggi adalah 88%, yang berasal dari serbuk biji getah yang dirawat $KHCO_3$ selama 5 jam pada suhu 60 ° C dan diteruskan ke SDS. -Elektroforesis halaman, yang menekankan hidrolisis protein. Kajian ini menunjukkan penggunaan serbuk biji getah yang banyak dalam makanan ayam dan potensi garam untuk menyahnaturasi dan/atau menghidrolisis protein kepada molekul yang lebih kecil untuk menghasilkan sumber protein yang sangat mudah dihadam.

Kata kunci: Ayam, Serbuk Biji Getah, Rawatan Garam, Hidrolisis Protein, elektroforesis Halaman SDS

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

APS	Ammonium Persulfate
ANOVA	Analysis of Variance
BSA	Bovine serum albumin
CRD	Customized Randomly Design
EU	European Union
FAO	Food and Agriculture Organization
GFSI	Global Food Security Index
RSP	Rubber Seed Powder
$KHCO_3$	Potassium bicarbonate
KH_2PO_4	Monopotassium phosphate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaH_2PO_4	Monosodium phosphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

LIST OF SYMBOLS

g	Gram
Kg	Kilogram
g kg-1	Gram per kilogram
kcal kg-1	Kilocalories per kilogram
µL	Microliter
mL	Milliliter
L	Litter
mg/mL	Milligram per milliliter
°C	Degree Celsius
M	Molar
hrs	Hours
mins	Minutes
&	And

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Due to the growth of the poultry industry, especially for poultry meat products, irresponsible parties have exploited the cost of raw materials for feed formulation. The protein source is the most expensive feedstuff in feed formulation, but it is essential. This topic had an impact not only on the large-scale feed industry but also on small-scale producers and customers. Hence, poultry development is distinguished by its high economic return due to its limited production time, which lasts 7-8 weeks in poultry production. The capital period can be replicated seven times a year in poultry processing.

In contrast to other species, poultry processing requires a limited number of spaces (N D Wahyono, 2017). This is because poultry is especially important for small farmers and poor rural and urban populations. It is primarily grown in large-scale, intensive operations, making it one of the fastest-growing agricultural sub-sectors (A. MOTTET, 2017).

According to FAO figures from 2000 to 2006, chicken meat demand in developed and developing countries will increase by 2.3 % and 4.0 % per year, respectively, between 2006 and 2016. With a contribution of 43.6 % in 2013, the United States was the leading producer of poultry meat. Asia is in second place with 33.5 %, led by the EU, Africa, and Oceania (N D Wahyono, 2017).

1.2 Problem Statement

Regarding the demand for poultry in this century, the farmers need to increase poultry production to fulfil the consumer's request by controlling feeding and good feeding management to ensure the growth of the poultry. However, poultry feed is well known with expensive, especially with good protein and other nutrients for the development and health of the poultry. Healthy poultry can ensure the high quality of livestock and consumers' satisfaction.

The meal's main ingredient must be local or readily accessible to create cheaper but high-quality poultry feed, such as rubber seed. However, to use rubber seed must go through details about the nutrient content of rubber seed. To ensure that poultry can fit with the nutrient from the rubber seed through feeding and the process for rubber seed can be known from the essential salt's treatment.

1.3 Objectives of the Study

1. To treat rubber seed powder with salts treatment at different temperatures and incubations times.
2. To analyze the percentage of protein hydrolysis in salts-treated rubber seed powder before and after the treatment through Bradford analysis.
3. To determine the relative molecular weight of a protein sample, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

1.4 Hypothesis of the Study

a) H_0: There is no significant value with the percentage of protein hydrolysis in salts-treated rubber seed powder before and after the treatment through Bradford analysis.

b) H_a: There is a significant value with the percentage of protein hydrolysis in salts-rubber seed powder before and after the treatment through Bradford analysis.

a) H_0: There are significant differences that occurred between groups of treatments through statistical analysis by using the Tukey Post Hoc Test in Factorial in Customized Randomly Design (CRD) of Analysis of Variance (ANOVA).

b) H_a: There is no significant difference that occurred between groups of treatments through statistical analysis by using the Tukey Post Hoc Test in Factorial in Customized Randomly Design (CRD) of Analysis of Variance (ANOVA).

1.5 Significant of Study

This study benefits the farmer for a small scale or poultry feed industry manufacturer to formulate broiler chicken feed specifically with the best nutrient recommendation by developing the different inclusion of rubber seed flour in broiler chicken feed to the nearest to their requirement for broiler starter till finisher. Furthermore, this formulation can reduce feed manufacturing costs by substituting protein sources such as soybean, fishmeal, and bone, commonly used in the poultry feed industry.

1.6 Scope of Study

The research aims to improve the degree of hydrolysis of salt-treated rubber seed powder. The percentage of protein hydrolysis in salts-treated rubber seed powder will be determined before and after treatment. Conventional Methodology will be used to treat rubber seed powder with salts. Each temperature will be evaluated at three different temperatures: 30°C, 45°C, and 60°C, and four other incubation times: 0 hours, 1 hour, 3 hours, and 5 hours. Protein hydrolysis of salts-treated rubber seed powder will be determined using the Bradford assay method and an Ultra-Violet Visible Spectrophotometer before and after treatment. The effects of various temperatures and incubation times on the degree of protein hydrolysis will be found, and protein availability data will be evaluated using Factorial in Customized Randomly Design (CRD) Analysis of Variance (ANOVA).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

According to FAO, the global prevalence of malnutrition is 10.8 % and 11.0%, corresponding to 794 and 815 million people in 2015 and 2016, respectively. So, despite all our efforts, the global population of malnourished and hungry people has increased. The World Bank reported in 2017 that 83 million people in 45 countries were famished. In affluent regions, the proportion of undernourished persons does not exceed 5% of the population; in emerging areas, it reaches 13%, African countries 20%, and Asian countries 13%. (Alexander Y. Prosekov, 2018).

In this globalization era, chicken meat has been increasing significantly from time to time. Based on the FAOSTAT current production of chicken meat is around 118 thousand productions and keeps expanding to fulfil the world demand. Poultry meat and eggs are among the most widely-eaten animal source foods worldwide, spanning various cultures, customs, and faiths, making them essential for food protection and nutrition. Poultry appears as the most resource-efficient sub-sector inside the livestock industry, using natural resources and supplying protein to meet rising global demand (A. MOTTET, 2017).

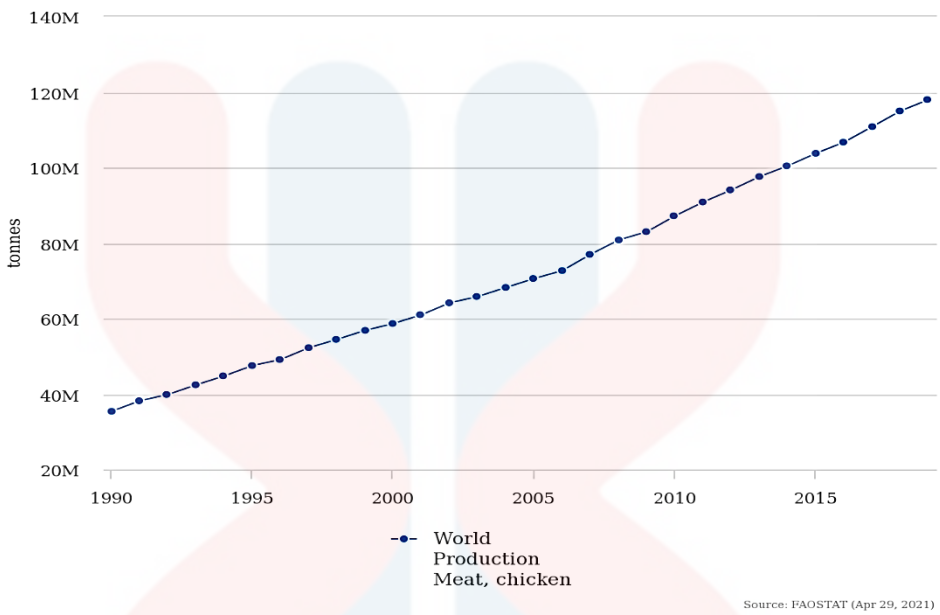


Figure 2.1: World Production of Meat, Chicken

2.2 Food Security Index

The Global Food Security Index (GFSI) is a composite indicator that tracks country-level progress toward food security. The Economist Intelligence Unit (EIU) has produced it every year since 2012, and it covers more than 100 countries. These countries illustrate regional diversity, economic prominence, and population size. The EIU prioritized countries with higher populations to cover as much of the world's population as feasible.

The GFSI seeks to identify which countries are most and least vulnerable to food insecurity. The EIU developed it based on 28 indicators that measure various aspects of food security in over 100 nations. Based on the perspectives of EIU experts and food security professionals, three broad categories were established, each focusing on a distinct component

of food security. (Izraelov, 2019) The main categories are Affordability, Availability, Quality, Safety, Natural Resources, and Resilience. The latest global Ranking are Ireland, Austria, and the United Kingdom as for the regional area of Asia Pacific are Japan, Singapore, and New Zealand, followed by 7th place Malaysia (GFSI, 2021).

2.3 Dependency Imported Feed (Protein)

The feed price of concentrates for livestock rearing is rising due to food-feed rivalry between people and livestock. As a result, scientists and farmers attempt to identify alternate feed supplies from agro-industrial manufacturing firms. To begin with, soybean meal is a popular protein source in livestock feed, but it is more expensive than other protein sources. (Rahman, 2020).

According to FAO statistic data of 2021, the soybean production in Malaysia had been stopping in 1991; meanwhile, the largest producer of soybean, Brazil, had kept growing stronger until now. Hence dependency of soybean for animal feed must import to produce animal feed.

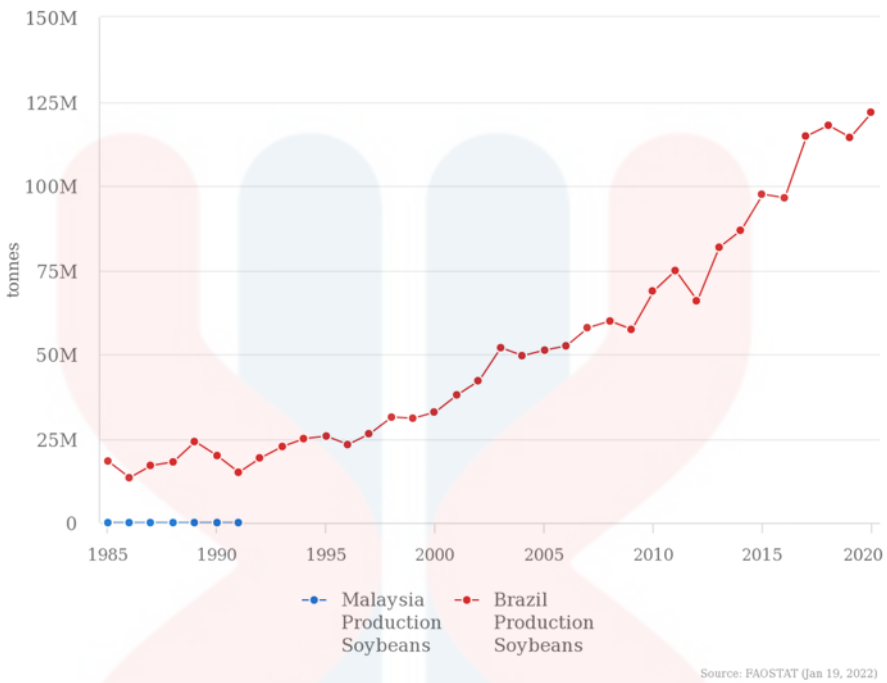


Figure 2.2: Soybean Production in Malaysia and Brazil

2.4 Local Feed Importance and Aspect Need to Be Concerned

One of the essential functions in livestock agriculture is to provide high-quality protein for human consumption, and to do this; animals should be given enough high-quality protein in their diets (Araujo LF, 2007). To maintain uniformity in supplied feed, feed composition is critical in poultry. Later, this would lead to development-level uniformity. In commercial feed formulation, the nutrient requirements and ingredients usually are only matched by the one with the lowest cost.

Since the protocols used to determine protein content are related to amino acid availability, amino acids are essential in animal nutrition. Until recently, poultry feed preparation was based on the principle of crude protein, which often resulted in diets of amino

acid amounts higher than the birds' fundamental requirements (Araujo LF, 2007). The term "ideal protein" was coined recently to describe the ratio of digestible amino acids needed to meet the absolute needs of all amino acids for maintenance and processing, with no excesses or deficiencies. A reference amino acid is selected, and other amino acid requirements are calculated as proportions of this one (Araujo LF, 2007).

2.5 Rubber Seed

Malaysian rubber (Hevea Brasiliense's) plantations, initially planned for latex, are now considered a significant timber source for wood-based industries. It is estimated that around 2 million m^3 of Hevea wood logs are gathered and used to manufacture furniture and furniture components each year. Initially, most rubber plantings occurred in Perak, Selangor, Negeri Sembilan, and Johor – states with the existing developing infrastructure (Organization, 2022).

The rubber tree (Hevea Brasiliense's) yields fruit at four years of age. When the fruit ripens and splits, the seeds fall to the ground, leaving three or four seeds per fruit. Each tree produces approximately 800 seeds (1.3 kilogrammes) twice a year. A rubber plantation is believed to have between 800-1200 kg of rubber seed per ha per year, which is typically regarded as waste. Based on an anticipated average of 1000 kg seeds per ha/year, Malaysia's annual rubber seed production would be 1.2 million metric tonnes (Eka, 2010).

2.5.1 Nutrient content in Rubber Seed

Rubber seed is natural rubber processing from the (Hevea Brasiliense's) plantation. Rubber seeds are a plentiful by-product of rubber plantations, with annual production ranging from 136 to 2000 kg/hectare. However, only 25% of rubber seeds are used for seeding, with the remaining 75% being discarded (Indonesian Directorate General of Plantation 2010) (Miao Yang, 2019). The rubber seed's nutritional composition was found to be 3.99 % moisture, 23 % crude protein, 68.5 % crude fat, and 4.3 grams of ash per 100 grams. Rubber seed had a high Glutamic acid content (16.13%) but a poor Cysteine content (0.78%) (Lukman Abiola Oluodo, 2018).

2.5.2 Protein Hydrolysis of Rubber Seed Powder

The price of main feed ingredients like fishmeal and fish oil has risen significantly due to increased demand for feed ingredients and global economic fluctuations. However, in Indonesia and Malaysia, soya bean meal is not manufactured in adequate amounts domestically. As a result, the countries rely on imports to meet local demand, with the latter placing increased strain on the local aquaculture feed industry due to the higher cost of importing this main ingredient (Muhammad Agus Suprayudi, 2016).

To produce affordable yet high-quality nutrients, especially protein, find other alternative protein sources to replace the international product. Swapping the primary source

of protein from soybean to rubber seed is a good move. Rubber seeds are known as abundant seeds because the rubber only focuses on the latex. Hence the rubber seed itself already contains protein which can replace the soybean.

Chemical and enzymatic processes are used to hydrolyse proteins. Protein hydrolysis is carried out using animal sources such as pepsin and pancreatin. At the right temperature and pH, proteins are hydrolysed by proteolytic enzymes. The various amino acids and peptides are digested because specific peptide cleavage bonds are targeted. Plant enzymes are more complex in their activity than animal enzymes, which are more susceptible to their location of the action.

2.5.3 Utilization and Potential of Rubber Seed Powder as Protein Source in Feed Worldwide

Farm derivative protein, such as corn gluten, soybean, and peanut meals, is typically used as a protein substitute in feeds. On the other hand, these plant-derived protein sources include low levels of lysine, tryptophan, threonine, and methionine.

Even though rubber seeds are still unknown in the poultry industry and yet need to be exposed deeply regarding the excellent potential of protein in the rubber seed. Furthermore, this replacement will help the unfortunate farmers to produce high-quality broiler breeds even though using the by-product from the rubber tree. In some of the articles that had been found, certain animals from overseas had replaced the protein source from regular bean to the rubber seed and got a positive impact from it.

This potential will also help economically for Malaysia and Indonesia because both countries are well known as the most extensive latex production yet exported overseas (Lukman Abiola Oluodo, 2018).

2.6 Salts Treatment

Salts are the ionic compound formed when an acid and a base combine to neutralize. Salts are made up of cations (positively charged ions) and anions (negatively charged ions), and they are electrically neutral in their unsolved, solid-state (without a net charge) (Acid-base properties of salts, 2021)

Chemical hydrolysis is often used to create savoury tastes, while microbial fermentation creates peptides and removes anti-nutritional factors from protein ingredients. Bioactive peptides (usually 2–20 A.A. residues in length) have antimicrobial, antioxidant, antihypertensive, and immunomodulatory properties in addition to their nutritional benefit in supplying A.A.s.

This chemical treatment uses essential salts to neutral salt treatment because chemical hydrolysis mainly uses essential salts treatment to optimum pH 7.0. with this optimum pH value, the protein that had been hydrolysate with combination for the feeding will not be acidic. Meanwhile, the neutral salt is A salt formed when an acid is neutralized by a base and has neither acidic nor basic properties, mainly when dissolved in water (Dictionary, 2016). Hydrolysis of protein using neutral salt will not result because it will neutralize the protein itself.

2.7 SDS-PAGE

Electrophoresis is a technique for separating a complicated protein mixture. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is a technique that uses an electric current to transport charged molecules through a gel matrix. This process evaluates protein component composition, validates protein sample homogeneity, and purifies proteins for usage in other applications. The migration rate of proteins during SDS-PAGE is regulated by the pore size of the gel matrix and the charge, size, and shape. The technique in this section covers gel casting, protein sample preparation, staining and drying of the gels, and protein molecular mass computation based on electrophoretic mobility.

The most frequent method is the Laemmli method, named after U.K. Laemmli, the first to publish a scientific study using SDS-PAGE (Laemmli, 1970). Protein separation by SDS-PAGE can be used to measure the relative molecular mass of proteins, their relative abundance in a sample, their distribution among fractions, and the purity of protein samples. SDS is a negatively charged anionic detergent that may dissolve hydrophobic compounds and denature secondary and non-disulphide-linked tertiary structures.

Polyacrylamide, a polymer of acrylamide monomers, generates a gel matrix that acts as a sieve, limiting the movement of larger molecules more than smaller molecules. In reaction to an electrical field, proteins migrate through the pores in the matrix. The concentration of Acrylamide determines the size of the pores. The smaller the pore size in

the gel matrix, the higher the acrylamide concentration. The migration rate of the protein is regulated by the size of the gel pores and the protein's charge, size, and shape.

CHAPTER 3

MATERIAL AND METHOD

3.1 Protein Hydrolysis

3.1.1 Material

10 gram of rubber seed powder and five types of salt; sodium chloride, sodium bicarbonate, potassium bicarbonate, sodium dihydrogen phosphate, and potassium dihydrogen phosphate, supplied by Miss Athirah, a postgraduate student of UMK Jeli. For protein analysis, the materials needed were Bradford reagent distilled water. For Electrophoresis (SDS-Page), distilled water, Ammonium persulfate (APS) (10%), TEMED, stacking buffer, separating buffer, running buffer, 30% Acrylamide, methanol, Bovine Serum Albumin (BSA), and acetic acid.

3.1.2 Apparatus

Apparatus used in the experiment were zipper bags, spatula, electronic weighing scale, high-speed blender, drying oven, aluminium foil, media bottle, beakers(25ml,50ml,150ml,500ml), measuring cylinder (50ml,1000ml), micropipette (100microlit, 1000microlit), micropipette tips, microcentrifuge tubes(1.5ml), centrifuge rack, distilled water bottle, gloves, plastic dropper, cuvettes(1.5ml), Ultra Visible Spectrophotometer, hot plate, Microcentrifuge machine, pH meter, Incubator shaker, and Vortex machine also water bath, casting plates, spacers, Teflon comb, Vertical electrophoresis, and power supply.

3.1.3 method

a) Experimental Design

Table 3.1 shows the experimental design of salts-treated rubber seed powder includes temperatures of 30°C, 45°C, and 60°C, as well as incubation times of T0 (0 hours), T1 (1 hour), T2 (3 hours), and T3 (5 hours). The weight of rubber seed powder, the volume of distilled water, and the salts were all the same at each temperature: 0.1 g, 30 mL, and 0.9g, respectively. The weight of the untreated rubber seed powder was 1g and 30ml of distilled

water. The experiment was repeated three times to increase the data's precision and obtain a p-value for statistical analysis.

Table 3.1 The experimental design of salts-treated rubber seed

Temperature (°C)	Incubation Time (hrs)
30	0 1 3 5
45	0 1 3 5
60	0 1 3 5

i) Preparation of Phosphate Buffer Solution

Using the formulas below, the number of moles and molarity of Na_2HPO_4, NaH_2PO_4, and NaOH were estimated to make 1000 mL/0.1 M of Phosphate Buffer Solution in pH 8, where the molar mass of Na_2HPO_4 is 14.19 g/mol, NaH_2PO_4 is 11.99 g/mol, and NaOH is 4 g/mol. After combining both solutions to achieve a pH of 8, distilled water was added until the needed volume of solution was attained.

Table 3.2 Equation of Mol

$$Number\ of\ moles\ (mol) = \frac{mass\ (g)}{molar\ mass\ (\frac{g}{mol})}$$

$$Molarity\ (M) = \frac{number\ of\ moles\ (mol)}{volume\ of\ buffer\ solution\ to\ be\ produced\ (L)}$$

ii) Preparation of Protein Standard Curve

Before testing the Bradford assay, a standard curve must be prepared to assess the protein level of the sample. Various Bovine serum albumin (BSA), buffer, and Bradford working buffer volumes were combined in the test tube according to Table 3.3. After 5 minutes, the mixture was transferred to the cuvette, and the absorbance value was measured at 595 nm.

Table 3.3 Amount of Solution Used in Protein Standard Curve

Standard Concentration (µg/mL)	**Bovine Serum Albumin (BSA) Stock Solution 1mg/mL(µL)**	**Phosphate Buffer Solution (PBS)(µL)**	**Bradford reagent (µL)**
0	0	500	1000
25	12.5	487.5	1000
50	25	475	1000
75	37.5	462.5	1000
100	50	450	1000
200	100	400	1000

The sample standard and 1 mL of Bradford assay were combined in the microcentrifuge tube with the cap. The fluid in the microcentrifuge tube was vortexed to ensure thorough mixing. The solution remained steady after vortexing for 5 minutes. The solution was poured into a cuvette five minutes later and placed in the UV-spectrophotometer. Previously, distilled water was added to a cuvette, and the absorbance at 595 nm against PBS was measured with a UV-spectrophotometer. The absorbance reading for each sample was reported three times to obtain a more precise reading. The reported reading was utilized to create a conventional graph with the dependent variable on the x-axis and the independent variable on the y-axis.

3.2 SDS-PAGE

3.2.1 Preparation of Solution

a) APS 10%

Thermo Scientific Pierce Ammonium Persulfate (APS) is an oxidizing agent used in conjunction with TEMED to catalyse the polymerization of Acrylamide and bisacrylamide to generate polyacrylamide gels for electrophoresis. Prepared fresh APS, 0.1g of APS, and 1ml distilled water and kept it cool in the refrigerator until further use.

b) Resolving Gel Buffer 12%

Resolving buffer is purposely for separating the proteins based on their molecular weight. Required 800 µL distilled water, 4000 µL 30% of Acrylamide,5000 µL separating buffer, 100 µL 10% of APS and 10 µL of TEMED.

c) Stacking Gel Buffer 4%

They were stacking gel function to line up all the protein samples loaded on the gel to enter the resolving gel simultaneously. To make the stacking gel 1400 µL of distilled water, 500 µL 30% of Acrylamide, 2000 µL stacking buffer, 40 µL of 10% APS and 4 µL of TEMED.

d) Running buffer

Contained ions that conduct current through the gel. This running buffer can be used for two gels - 6.05 g Tris Buffer, 28.8g Glycine, 2.0g SLS and 2L of distilled water.

e) De-staining buffer

De-staining buffer aims for binds more tightly to the proteins than to the gel matrix. Required 200ml methanol, 100ml Acetic Acid, and 700ml of distilled water.

3.2.2 Method

Assembled the glass plates as directed by the manufacturer. Prepare the resolving buffer, then swirl the mix. Fill up the casting plates using the pipette and remove the bubbles using distilled water. Let it polymerize entirely for 45 until 60 minutes. When it is completed, polymerized drain the extra fluid, add stacking until the top and remove the bubbles. Let the gel polymerize entirely at room temperature for about 45 until 60 minutes. Prepared the protein sample, which was 60°C of 5 hours $KHCO_3$ and 3 hours $NaHCO_3$ with 5 hours Control (Untreated). Pipette the sample into the microcentrifuge, then add on the buffer. Placed the protein sample into the water bath for 90°C and 5 minutes. After completely polymerizing, took out the comb and set the glass gel into the electrophoresis containing running buffer.

Place the protein sample into the well gently and carefully using the pipette. Connect the cell to the power supply and run constant voltage 200V for 120 minutes until the blue dye reaches the bottom. Turn off the power supply and disconnect it. Remove the cassette and discard the buffer solution. Slowly and carefully lift the top glass plate and rinse it into the container filled with distilled water. Discard the distilled water and fill up with blue staining solution until covered the gel completely.

Closed the container tightly and left it overnight on the mini rocking. After overnight, remove the excess dye and de-stain the gel for about 30 minutes. Then place the gel into the zipper filled with a little bit of distilled water and observe the kDa.

3.3 Statistical Analysis

A factorial in Completely Randomized Design (CRD) of Analysis of Variance (ANOVA) was used with IBM SPSS Statistics 26 software to analyse the results of concentration of salts treated rubber seed powder before and after treatment with a significant difference ($p<0.05$). When two or more components are evaluated, factorial analysis is used. In contrast, CRD analysis is used in laboratory trials with homogeneous experimental units where environmental effects may be controlled. The mean and standard deviation were computed and made public. It related to the alphabet, a, b, and c on Tukey HSD in Post Hoc Test Multiple Comparisons of ANOVA. There was a significant difference when any treatment groups did not have the same letter and vice versa. Because the data set is simple, the graph for the experiment data was made using Excel 2016 software.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Protein Analysis

The influence of temperature and incubation time on the protein hydrolysis of salt-treated rubber seed powder is investigated in this study utilizing Factorial in Completely Randomized Design (CRD) Analysis of Variance (ANOVA). Figure 4.1 depicts the linear regression of the protein standard curve, with the correlation coefficient, R^2, equal to 0.9831 and close to 1.00. When R^2 approaches 1.00, an empirical model is more robust and matches the accurate data better, whereas a lower value of R^2 indicates that the dependent variables in the model are less important.

Figure 4.1: Protein Standard Curve

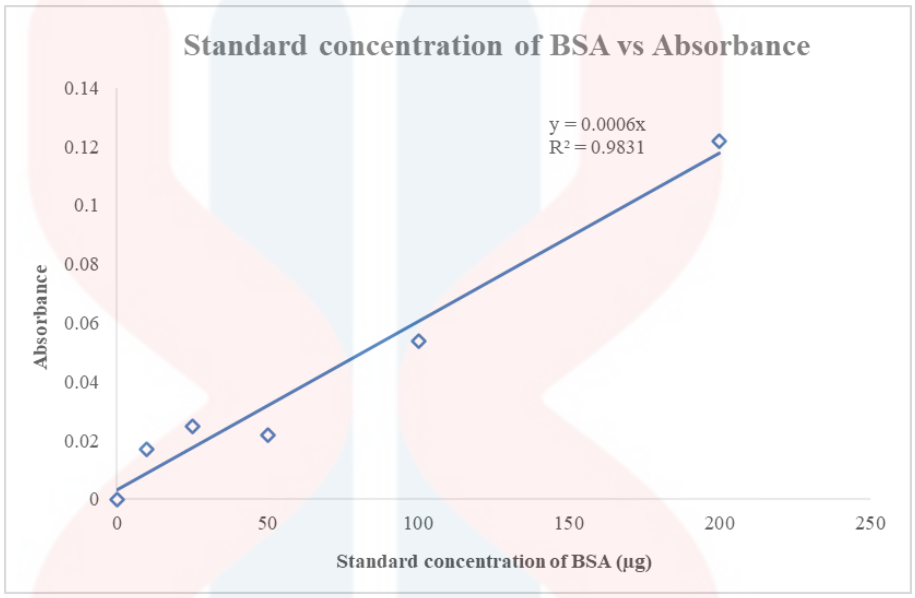


Figure 4.2 shows the percentage of protein hydrolysis versus incubation times for 30 ℃; T0 (0 hours), T1 (1 hour), T2 (3 hours), T3 (5hours) of salts-treated rubber seed powder (RSP).

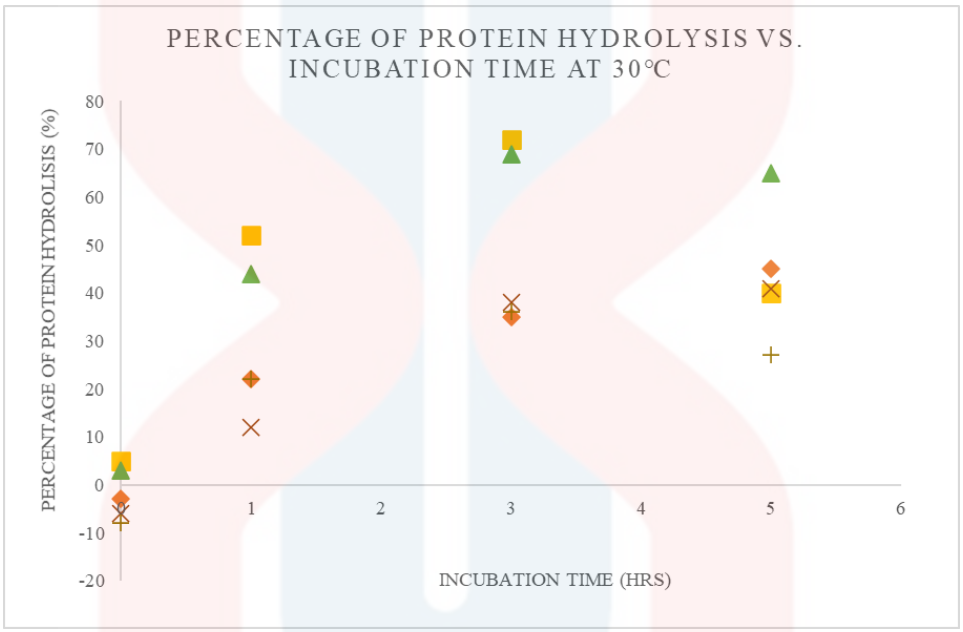


Figure 4.3 shows the percentage of protein hydrolysis versus incubation times for 45 ℃; T0 (0 hours), T1 (1 hour), T2 (3 hours), T3 (5hours) of salts-treated rubber seed powder (RSP).

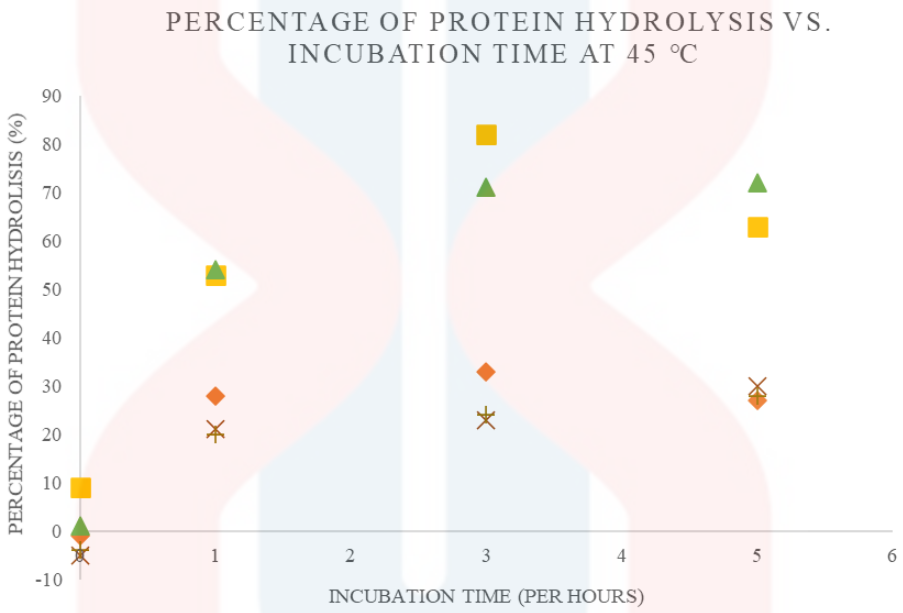


Figure 4.4 shows the percentage of protein hydrolysis versus incubation times for 60 ℃; T0 (0 hours), T1 (1 hour), T2 (3 hours), T3 (5hours) of salts-treated rubber seed powder (RSP).

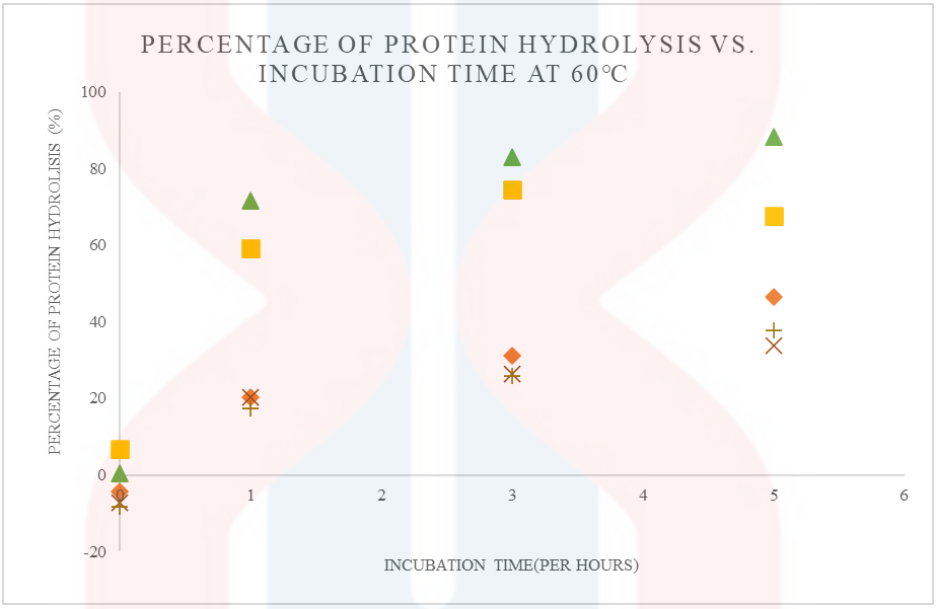


Table 4.1 shows the concentration of untreated rubber seed powder in triplicate and its mean ± standard deviation at three different temperatures: 30°C, 45°C, and 60°C and four other incubation times; T0 (0 hours), T1 (1 hour), T2, (3 hours) and T3 (5 hours). As well as table 4.2,4.3,4.4,4.5, and 4.6 shows the concentration of salts treated rubber seed powder which are NaCl, $NaHCO_3$, $KHCO_3$, NaH_2PO_4, and KH_2PO_4 representative.

Table 4.1: Concentration and mean ± standard deviation of untreated rubber seed powder

Temperature (℃)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for a concentration of Salts-Treated RSP
30	0	1615.00 1581.67 1560.00	1585.55 ± 27.70[a]
	1	2011.67 2081.67 1985.00	2026.11 ± 49.93[ab]
	3	2345.00 2305.00 2333.33	2327.77±20.57[b]
	5	3736.67 3248.33 3948.33	3644.44 ±358.99[c]
45	0	1698.33 1695.00 1675.00	1689.44 ±12.61[a]
	1	2518.33 2248.33 2095.00	2287.22 ±214.32[ab]
	3	1900.00 2126.67 1901.67	1976.11± 130.38[ab]
	5	2913.33 2240.00 2210.00	2454.44± 397.69[b]
60	0	1671.67 1683.33 1693.33	1682.77 ±10.840[a]
	1	2293.33 2143.33 2111.67	2182.77± 97.04[b]
	3	2376.67 2415.00 2236.67	2342.78±93.87[bc]
	5	2463.33 2635.00 2333.33	2477.22± 151.31[c]

Table 4.2: Concentration and mean ± standard deviation of NaCl-treated rubber seed powder

Temperature (℃)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for a concentration of Salts-Treated RSP
30	0	1475.00 1555.00 1566.67	1532.22 ± 49.89[a]
	1	1915.00 1936.67 1968.33	1940.00± 26.82[b]
	3	2161.67 2088.33 2163.33	2137.77± 42.83[bc]
	5	2181.67 2451.67 2255.00	2296.11 ± 139.61[c]
45	0	1653.33 1666.67 1698.33	1672.77 ± 23.11[a]
	1	1888.33 2025.00 2573.33	2162.22 ± 362.53[b]
	3	2196.67 2276.67 2293.33	2255.55 ± 51.67[b]
	5	2126.67 2118.33 2181.67	2142.22 ± 34.41[ab]
60	0	1563.33 1625.00 1643.33	1610.55± 41.91[a]
	1	2061.67 2016.67 2003.33	2027.22 ± 30.56[b]
	3	2230.00 2208.33 2180.00	2206.11 ± 25.07[bc]
	5	2258.33 2345.00 2793.33	2465.55± 287.15[c]

Table 4.3: Concentration and mean ± standard deviation of $NaHCO_3$ –treated rubber seed powder

Temperature (°C)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for a concentration of Salts- Treated RSP
30	0	1740.00 1683.33 1546.67	1656.66 ± 99.38[a]
	1	2458.33 2315.00 2446.67	2406.66 ± 79.59[b]
	3	2700.00 2706.67 2766.67	2724.44 ± 36.71[b]
	5	2041.67 2558.33 2711.67	2437.22 ± 351.03[b]
45	0	1828.33 1888.33 1831.67	1849.44 ± 33.71[a]
	1	2688.33 2615.00 2463.33	2588.88 ± 114.75[b]
	3	3201.67 3001.67 3011.67	3071.67 ± 112.69[c]
	5	3090.00 2843.33 2811.67	2915.00 ± 152.378[c]
60	0	1821.67 1788.33 1781.67	1797.22 ± 21.431[a]
	1	2773.33 2665.00 2600.00	2679.44 ± 87.56[b]
	3	2690.00 2976.67 3150.00	2938.89 ± 232.31[bc]
	5	3018.33 3046.67 3281.67	3115.55 ± 144.55[c]

Table 4.4: Concentration and mean ± standard deviation of $KHCO_3$-treated rubber seed powder

Temperature (℃)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for a concentration of Salts-Treated RSP
30	0	1636.67 1585.00 1661.67	1627.78 ± 39.10[a]
	1	2261.67 2325.00 2250.00	2278.89 ± 40.356[b]
	3	2625.00 2516.67 2896.67	2679.44 ± 195.76[c]
	5	2596.67 2675.00 2575.00	2615.55 ± 52.60[c]
45	0	1673.33 1743.33 1716.67	1711.11 ± 35.32[a]
	1	2625.00 2726.67 2453.33	2601.66 ± 138.15[b]
	3	2975.00 2860.00 2836.67	2890.55 ± 74.054[bc]
	5	3090.00 2843.33 2811.67	2915.00 ± 152.37[c]
60	0	1676.67 1686.67 1706.67	1690.00 ± 15.27[a]
	1	2993.33 2916.67 2750.00	1690.00 ± 15.27[b]
	3	3128.33 3110.00 3000.00	3079.44 ± 69.40[bc]
	5	3275.00 3171.67 3061.67	3169.44 ± 106.68[c]

Table 4.5: Concentration and mean ± standard deviation of NaH_2PO_4-treated rubber seed powder

Temperature (℃)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for a concentration of Salts- Treated RSP
30	0	1533.33 1461.67 1453.33	1482.77 ± 43.97[a]
	1	1761.67 1761.67 1818.33	1780.55 ± 32.71[b]
	3	2206.67 2145.00 2208.33	2186.66 ± 36.09[c]
	5	2083.33 2143.33 2466.67	2231.11± 206.19[c]
45	0	1625.00 1601.67 1598.33	1608.33 ± 14.53[a]
	1	2116.67 2033.33 2008.33	2052.77± 56.72[b]
	3	2040.00 2126.67 2130.00	2098.89 ± 51.02[b]
	5	2300.00 2220.00 2090.00	2203.33 ± 105.98[b]
60	0	1560.00 1558.33 1565.00	1561.11± 3.470[a]
	1	2035.00 2028.33 2018.33	2027.2200 ± 8.39025[b]
	3	2168.33 2110.00 2103.33	2127.22 ± 35.75[bc]
	5	2401.67 2193.33 2155.00	2250.00 ± 132.74[c]

Table 4.6: Concentration and mean ± standard deviation of KH2PO4-treated rubber seed powder

Temperature (℃)	Incubation Time (hrs)	The concentration of Salts-Treated RSP(µg/mL)	Mean ± Standard deviation for the concentration of Salts- Treated RSP
30	0	1446.67 1495.00 1428.33	1456.66 ± 34.44[a]
	1	1835.00 1905.00 2066.67	1935.55 ± 118.81[b]
	3	2161.67 2173.33 2128.33	2154.44± 23.35[bc]
	5	1971.67 1960.00 2106.67	2012.78 ± 81.52[c]
45	0	1620.00 1648.33 1588.33	1618.88 ± 30.01[a]
	1	2033.33 2078.33 1991.67	2034.44± 43.34[b]
	3	1971.67 2040.00 2126.67	2046.11 ± 77.68[Cb]
	5	2221.67 2183.33 2090.00	2165.00± 67.72[b]
60	0	1558.33 1541.67 1535.00	1545.00± 12.01[a]
	1	1943.33 2018.33 1966.67	1976.11 ± 38.38[b]
	3	2178.33 2116.67 2060.00	2118.33± 59.18[b]
	5	2420.00 2286.67 2250.00	2318.89 ± 89.46[c]

At 30°C, 45°C, and 60°C, there is a significant difference between the incubation times at each temperature in which p=0.00 (p<0.05). As for the untreated rubber seed powder, as shown in Table 4.1, there is a significant difference between each temperature, following Table 4.2, which concentration and its mean, standard deviation also indicate a significant difference. As for the Tukey HSD, the highest percentage was 46 % (5 hours) for 60 ℃ showed that which were p=0.211(p>0.05) there is no significant between the percentage of protein availability at hours 3 (31%) and 5 (46%) with mean ± standard deviation 2206.11 ± 25.07bc and 2465.55 ± 287.15c respectively as both incubation times are in the same group of homogeneous subsets which is c.

For $NaHCO_3$, shown in Table 4.3, there is a significant difference between the incubation time at each temperature. The highest percentage of protein availability is at 3 hours (81%) for 45℃, and shown in the Tukey HSD that there is no sign as stated p=1.000 (p>0.05). Next, Table 4.4 for $KHCO_3$ shows a significant difference between the incubation time at each temperature. The highest percentage was at 5 hours (88%) for 60℃ also showed that there is no significant difference that is p=0.110(p>0.05) at 3hours (74%) and 5(88%) with mean ± standard deviation of 3079.4433 ± 69.40771^{bc} and 3169.4467 ± 106.68238^{c} in subsets c.

Table 4.5 also shows a significant difference between the incubation time at each temperature which is NaH_2PO_4. The highest protein availability is for 30℃, 5 hours (40%), and in Tukey HSD shown, there is no significant difference which stated p=1.000(p>0.05). Lastly, Table 4.6 also indicates a significant difference between the incubation time at each temperature. The highest protein availability was shown at 5 hours (33.72%) for 60℃ and shown there is no significant difference shown in Tukey HSD that stated p= 1.000(p>0.05).

4.2 SDS-PAGE

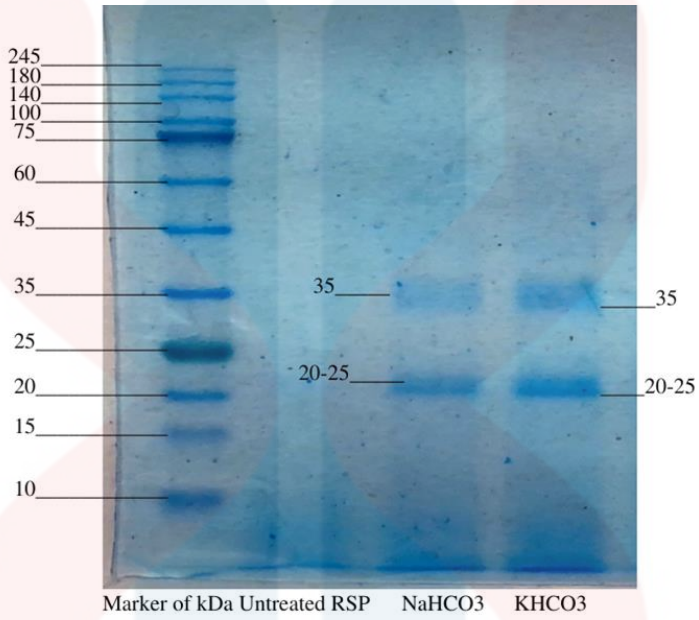
Table 4.7: Percentage of Protein Decrease and Mean ± standard deviation of $NaHCO_3$-treated rubber seed powder

Temperature (℃)	Time (Hrs)	Percentage of Protein Decrease (%)	Mean ± Standard deviation for concentration
30	0	5	1656.66 ± 99.38^{a}
	1	52	2406.66 ± 79.59^{b}
	3	72	2724.44 ± 36.71^{b}
	5	40	2437.22 ± 351.03^{b}
45	0	9	1849.44 ± 33.71^{a}
	1	53	2588.88 ± 114.75^{b}
	3	72	3071.67 ± 112.69^{c}
	5	63	2915.00 ± 152.378^{c}
60	0	7	1797.22 ± 21.431^{a}
	1	59	2679.44 ± 87.56^{b}
	3	75	2938.89 ± 232.31^{bc}
	5	68	3115.55 ± 144.55^{c}

Table 4.8: Percentage of Protein Decrease and Mean ± standard deviation of $KHCO_3$-treated rubber seed powder

Temperature (℃)	Time (Hrs)	Percentage of Protein Decrease (%)	Mean ± Standard deviation for concentration
30	0	3	1627.78 ± 39.10^{a}
	1	44	2278.89 ± 40.356^{b}
	3	69	2679.44 ± 195.76^{c}
	5	65	2615.55 ± 52.60^{c}
45	0	1	1711.11 ± 35.32^{a}
	1	54	2601.66 ± 138.15^{b}
	3	71	2890.55 ± 74.054^{bc}
	5	72	2915.00 ± 152.37^{c}
60	0	0	1690.00 ± 15.27^{a}
	1	71	1690.00 ± 15.27^{b}
	3	83	3079.44 ± 69.40^{bc}
	5	88	3169.44 ± 106.68^{c}

Figure 4.5: SDS-Page untreated rubber seed powder, $KHCO_3$-treated rubber seed powder, and $NaHCO_3$-treated rubber seed powder



As for SDS-Page, to decide which salts treatment should test were determined by the highest percentage of protein decrease. As shown in table 4.7 for $NaHCO_3$- treated rubber seed powder, all temperatures shown at 3 hours of 60℃ has the highest percentage, which was 75%; meanwhile, table 4.8 for $KHCO_3$– treated rubber seed powder shows that at 5 hours of 60℃ also has the highest rate which was 88%.

SDS-Page, known as sodium dodecyl sulphate-polyacrylamide gel electrophoresis, function as an electrophoresis technique that allows for mass separation of proteins also for reduce molecular weight. The blue line shown in Figure 4.5 is the reading for calibration against an unstained protein standard determined the apparent molecular weight (kDa) of each protein; supplemental data should be used for more precise adjustment in different electrophoresis conditions.

To observe, the gel must have the protein ladder on the left side of Figure 4.5, which determines the protein purified. The gel in the figure consists sample of untreated rubber seed powder (5hours) as control, $NaHCO_3$ – treated rubber seed powder (3hours), and $KHCO_3$ – treated rubber seed powder (5hours), both representative 60℃. The sample was decided by the percentage of protein hydrolysis versus incubation times graph.

As for untreated, there is no band shown in the gel. For $NaHCO_3$, the band demonstrated for the first band from the bottom showed the band was between 20-25 kDa. The second band appeared at 35 kDa. Next, for $KHCO_3$, the first band from the bottom is also shown between 20-25 kDa, and the second band is also offered at 35 kDa. Bands that consistently appear after electrophoresis of a specific sample are almost undoubtedly typical of the polypeptides that describe the sample. Bands that show seldom and are very faint, on the other hand, may reflect essential polypeptides or something else (Analysis of protein gels (SDS-PAGE), 2022).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

As a result of the preliminary study, some findings revealed the effects of different temperatures and incubation durations on protein hydrolysis by treating rubber seed powder with salts.

The percentage of protein hydrolysis of salts-treated rubber seed powder before and after treatment was analysed, and it was discovered that at hour 5, 60°C of $KHCO_3$ salts treated rubber seed powder has the highest percentage of protein hydrolysis, which is 88% when compared to the other incubation times and temperatures, and that $KHCO_3$ is better than the other neutral, acidic, and alkaline salts as it hydrolyses protein faster.

Statistical analysis utilizing the Tukey Post Hoc Test in Factorial in CRD of ANOVA was completed satisfactorily, demonstrating the differences between treatment groups. There is a substantial difference between 30°C, 45°C, and 60°C, $p=0.00$ ($p<0.05$). For the

incubation times T0, T1, T2, and T3, there is a significant difference for 30°C, 45°C, and 60°C, with $p=0.00$ ($p<0.05$).

The relative molecular weight of the protein sample using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-Page) was determined and shown $NaHCO_3$, and $KHCO_3$ bands appeared.

5.2 Recommendation

For the future research future areas of research could include a feeding trial for 42 days to record the growth performance, the feed conversion rate, survival rate, and mortality rate of broiler chicken or any poultry that available by developing feed formulation and substituting the protein sources with salts $KHCO_3$-treated rubber seed powder for production purposes.

REFERENCES

A simple guide to the Nutritional Requirements of Poultry. (2021, April 29). Retrieved from The poultry site: https://www.thepoultrysite.com/articles/a-simple-guide-to-the-nutritional-requirements-of-poultry

A. MOTTET, G. T. (2017). Global poultry production: current state. World's Poultry Science Journal, 73. DOI:doi:10.1017/S00439339170000714

Acid-base properties of salts. (2021, April 29). Retrieved from Lumenlearning: https://courses.lumenlearning.com/boundless-chemistry/chapter/acid-base-properties-of-salts/

Alexander Y. Prosekov, S. A. (2018, 5). Geoform. Food security: the challenge of the present, 91, 73-77.

Analysis of protein gels(SDS-PAGE). (2022). Retrieved from Rice.edu: https://www.ruf.rice.edu/~bioslabs/studies/sds-page/gellab3.html#:~:text=Bands%20that%20consistently%20show%20up,they%20may%20represent%20something%20else.

Araujo LF, J. O. (2007). Different Criteria of Feed Formulation for Broilers Aged 43 to 49 Days. Brazilian Journal of Poultry Science, 61-64. Retrieved from https://www.scielo.br/pdf/rbca/v6n1/a08v06n1.pdf

Coon, C. N. (2002). Feeding Broiler Breeders. New York: Springer Science.

Dewi Fortuna, A. R. (2015). Degradation of Acid Cyanide Poison in Rubber Seed (Hevea brasiliensis) after Treatment with Rice Husk Ash. International Journal on Advanced Science Engineering Information Technology. doi:10.18517/ijaseit.5.4.540

Dictionary, L. (2016, April 29). Neutral Salt. Retrieved from Lexico Dictionary: https://www.lexico.com/definition/neutral_salt

GFSI. (2021). Global Food Security Index. Retrieved from Ranking and trends: https://impact.economist.com/sustainability/project/food-security-index/Index

Gous, J. E. (2007). Quantification of reproductive changes and nutrient requirements of broiler breeder pullets at sexual maturity. British Poultry Science, 663-676. doi:10.1080/00071668908417189

HARMS, H. R. (1983). Evaluation of Nutrient Specifications for Broiler Breeders. Poultry Science Department, University of Florida, Gainesville, 1400-1406.

Izraelov, M. S. (2019). An assessment of the global food security index. Food Sec. . Food Security, 1135-1152.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature, 680-685.

LAUW TJIN GI0K, M. S. (1967). Nutritional Value of Rubber-Seed Protein. The American Journal of Clinical Nutrition, 1300-1303.

LEESON, R. S. (1985). Broiler Breeder Performance in Response to Diet Protein and Energy. METABOLISM AND NUTRITION, 683-693.

Lukman Abiola Oluodo, N. H. (2018). Potential Utilization of Rubber Seed Meal as Feed and Food. International Journal of Engineering and Technology, 64-71. doi:http://dx.doi.org/10.14419/ijet.v7i4.43.25821

M. GABRASHANSKA, M. M.-M.-M. (1993). Application of small doses of copper salts (basic and neutral) to Ascaridia galli-initcttd chicks. Journal of Helminthology, 287-290.

M.D. Udo, U. E. (2016). Effect of processing on the nutrient composition of rubber seed meal. Journal of the Saudi Society of Agricultural Sciences.

Miao Yang, W. Z. (2019). Biorefinery of Rubber Seed Kernels for Comprehensive Utilization. Beijing: Research Square.

Muhammad Agus Suprayudi, S. A. (2016). Evaluation of hydrolyzed rubber seed meal as a dietary protein source for Nile tilapia Oreochromis. Aquaculture Research, 1-8. doi:10.1111/are.13206

N D Wahyono, M. M. (2017). A Review of the Poultry Meat Production Industry for Food. The 2nd International Joint Conference on Science and Technology (IJCST) 2017. DOI: DOI:10.1088/1742-6596/953/1/012125

R. L. Dari, A. M. (2005). Use of Digestible Amino Acids and the Concept of Ideal Protein in Feed Formulation for Broilers. Brazil: Poultry Science Association.

Rahman, M. A. (2020). Replacement of soybean meal with levels of inclusion of soya waste in the diet of growing goats. Trop Anim Health Prod 52, 3085-3090.

Sahraei, M. (2012). Feed Restriction in Broiler Chickens Production: A Review. Global Veterinaria, 449-458. Retrieved from http://www.idosi.org/gv/GV8(5)12/4.pdf

WINDHORST, H.-W. (2006). Changes in poultry production and trade worldwide. Germany: World's Poultry Science Association.

Yongqing Hou, Z. W. (2017). Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. China: BioMed Central. DOI:DOI 10.1186/s40104-017-0153-9

APPENDIX A

Descriptive Statistics

Dependent Variable: Concentration Untreated

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1585.5567	27.70523	3
	1	2026.1133	49.92724	3
	3	2327.7767	20.57012	3
	5	3644.4433	358.99766	3
	Total	2395.9725	816.63526	12
Total	0	1585.5567	27.70523	3
	1	2026.1133	49.92724	3
	3	2327.7767	20.57012	3
	5	3644.4433	358.99766	3
	Total	2395.9725	816.63526	12

Figure A.1: Descriptive statistics with mean and standard deviation for incubation time at 30°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration Untreated

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	7070699.16[a]	3	2356899.719	71.118	.000	.964
Intercept	68888210.65	1	68888210.65	2078.660	.000	.996
Temp	.000	0	.	.	.	.000
Time	7070699.158	3	2356899.719	71.118	.000	.964
Temp * Time	.000	0	.	.	.	.000
Error	265125.515	8	33140.689			
Total	76224035.32	12				
Corrected Total	7335824.673	11				

a. R Squared = .964 (Adjusted R Squared = .950)

Figure A.2: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration Untreated

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	1585.5567		
1	3	2026.1133	2026.1133	
3	3		2327.7767	
5	3			3644.4433
Sig.		.070	.254	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 33140.689.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration Untreated

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1689.4433	12.61862	3
	1	2287.2200	214.32778	3
	3	1976.1133	130.38857	3
	5	2454.4433	397.69049	3
	Total	2101.8050	366.33669	12
Total	0	1689.4433	12.61862	3
	1	2287.2200	214.32778	3
	3	1976.1133	130.38857	3
	5	2454.4433	397.69049	3
	Total	2101.8050	366.33669	12

Figure A.4: Descriptive statistics with mean and standard deviation for incubation time at 45℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration Untreated

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1033719.17[a]	3	344573.056	6.229	.017	.700
Intercept	53011011.10	1	53011011.10	958.372	.000	.992
Temp	.000	0	.	.	.	.000
Time	1033719.167	3	344573.056	6.229	.017	.700
Temp * Time	.000	0	.	.	.	.000
Error	442509.070	8	55313.634			
Total	54487239.33	12				
Corrected Total	1476228.237	11				

a. R Squared = .700 (Adjusted R Squared = .588)

Figure A.5: Significant difference between all incubation times at 45°C

Concentration Untreated

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	1689.4433	
3	3	1976.1133	1976.1133
1	3	2287.2200	2287.2200
5	3		2454.4433
Sig.		.057	.136

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 55313.634.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration Untreated

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1682.7767	10.84060	3
	1	2182.7767	97.04184	3
	3	2342.7800	93.87117	3
	5	2477.2200	151.31390	3
	Total	2171.3883	325.83071	12
Total	0	1682.7767	10.84060	3
	1	2182.7767	97.04184	3
	3	2342.7800	93.87117	3
	5	2477.2200	151.31390	3
	Total	2171.3883	325.83071	12

Figure A.7: Descriptive statistics with mean and standard deviation for incubation time at 60℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration Untreated

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1085337.50[a]	3	361779.167	35.088	.000	.929
Intercept	56579127.53	1	56579127.53	5487.481	.000	.999
Temp	.000	0	.	.	.	.000
Time	1085337.500	3	361779.167	35.088	.000	.929
Temp * Time	.000	0	.	.	.	.000
Error	82484.659	8	10310.582			
Total	57746949.69	12				
Corrected Total	1167822.159	11				

a. R Squared = .929 (Adjusted R Squared = .903)

Figure A.8: Significant difference between all incubation times at 60°C

Homogeneous Subsets

Concentration Untreated

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1682.7767		
1	3		2182.7767	
3	3		2342.7800	2342.7800
5	3			2477.2200
Sig.		1.000	.289	.420

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 10310.582.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaCl

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1532.2233	49.89919	3
	1	1940.0000	26.82049	3
	3	2137.7767	42.83011	3
	5	2296.1133	139.61637	3
	Total	1976.5283	305.95653	12
Total	0	1532.2233	49.89919	3
	1	1940.0000	26.82049	3
	3	2137.7767	42.83011	3
	5	2296.1133	139.61637	3
	Total	1976.5283	305.95653	12

Figure A.10: Descriptive statistics with mean and standard deviation for incubation time at 30℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaCl

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	980630.548[a]	3	326876.849	53.288	.000	.952
Intercept	46879971.03	1	46879971.03	7642.513	.000	.999
Temp	.000	0	.	.	.	.000
Time	980630.548	3	326876.849	53.288	.000	.952
Temp * Time	.000	0	.	.	.	.000
Error	49072.833	8	6134.104			
Total	47909674.41	12				
Corrected Total	1029703.382	11				

a. R Squared = .952 (Adjusted R Squared = .934)

Figure A.11: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration of NaCl

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	1532.2233		
1	3		1940.0000	
3	3		2137.7767	2137.7767
5	3			2296.1133
Sig.		1.000	.058	.139

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 6134.104.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.12: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaCl

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1672.7767	23.11317	3
	1	2162.2200	362.53034	3
	3	2255.5567	51.67319	3
	5	2142.2233	34.41538	3
	Total	2058.1942	284.08972	12
Total	0	1672.7767	23.11317	3
	1	2162.2200	362.53034	3
	3	2255.5567	51.67319	3
	5	2142.2233	34.41538	3
	Total	2058.1942	284.08972	12

Figure A.13: Descriptive statistics with mean and standard deviation for incubation time at 45℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaCl

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	616142.642[a]	3	205380.881	6.049	.019	.694
Intercept	50833958.73	1	50833958.73	1497.131	.000	.995
Temp	.000	0	.	.	.	.000
Time	616142.642	3	205380.881	6.049	.019	.694
Temp * Time	.000	0	.	.	.	.000
Error	271634.004	8	33954.250			
Total	51721735.38	12				
Corrected Total	887776.645	11				

a. R Squared = .694 (Adjusted R Squared = .579)

Figure A.14: Significant difference between all incubation times at 45°C

Concentration of NaCl

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	1672.7767	
5	3	2142.2233	2142.2233
1	3		2162.2200
3	3		2255.5567
Sig.		.056	.873

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 33954.250.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.15: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaCl

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1610.5533	41.91097	3
	1	2027.2233	30.56826	3
	3	2206.1100	25.07382	3
	5	2465.5533	287.15167	3
	Total	2077.3600	348.32453	12
Total	0	1610.5533	41.91097	3
	1	2027.2233	30.56826	3
	3	2206.1100	25.07382	3
	5	2465.5533	287.15167	3
	Total	2077.3600	348.32453	12

Figure A.16: Descriptive statistics with mean and standard deviation for incubation time at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaCl

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1163078.33[a]	3	387692.776	18.079	.001	.871
Intercept	51785094.84	1	51785094.84	2414.907	.000	.997
Temp	.000	0	.	.	.	.000
Time	1163078.328	3	387692.776	18.079	.001	.871
Temp * Time	.000	0	.	.	.	.000
Error	171551.448	8	21443.931			
Total	53119724.61	12				
Corrected Total	1334629.776	11				

a. R Squared = .871 (Adjusted R Squared = .823)

Figure A.17: Significant difference between all incubation times at 60°C

Concentration of NaCl

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1610.5533		
1	3		2027.2233	
3	3		2206.1100	2206.1100
5	3			2465.5533
Sig.		1.000	.482	.211

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 21443.931.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.18: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1656.6667	99.38472	3
	1	2406.6667	79.59945	3
	3	2724.4467	36.71825	3
	5	2437.2233	351.03436	3
	Total	2306.2508	442.49570	12
Total	0	1656.6667	99.38472	3
	1	2406.6667	79.59945	3
	3	2724.4467	36.71825	3
	5	2437.2233	351.03436	3
	Total	2306.2508	442.49570	12

Figure A.19: Descriptive statistics with mean and standard deviation for incubation time at 30℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1872253.44[a]	3	624084.480	17.731	.001	.869
Intercept	63825514.88	1	63825514.88	1813.396	.000	.996
Temp	.000	0	.	.	.	.000
Time	1872253.440	3	624084.480	17.731	.001	.869
Temp * Time	.000	0	.	.	.	.000
Error	281573.485	8	35196.686			
Total	65979341.80	12				
Corrected Total	2153826.925	11				

a. R Squared = .869 (Adjusted R Squared = .820)

Figure A.20: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration of NaHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	1656.6667	
1	3		2406.6667
5	3		2437.2233
3	3		2724.4467
Sig.		1.000	.240

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 35196.686.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.21: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1849.4433	33.71822	3
	1	2588.8867	114.75051	3
	3	3071.6700	112.69428	3
	5	2915.0000	152.37893	3
	Total	2606.2500	500.49506	12
Total	0	1849.4433	33.71822	3
	1	2588.8867	114.75051	3
	3	3071.6700	112.69428	3
	5	2915.0000	152.37893	3
	Total	2606.2500	500.49506	12

Figure A.22: Descriptive statistics with mean and standard deviation for incubation time at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2655000.46[a]	3	885000.155	70.484	.000	.964
Intercept	81510468.75	1	81510468.75	6491.763	.000	.999
Temp	.000	0	.	.	.	.000
Time	2655000.465	3	885000.155	70.484	.000	.964
Temp * Time	.000	0	.	.	.	.000
Error	100447.874	8	12555.984			
Total	84265917.09	12				
Corrected Total	2755448.339	11				

a. R Squared = .964 (Adjusted R Squared = .950)

Figure A.23: Significant difference between all incubation times at 45°C

Homogeneous Subsets

Concentration of NaHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	1849.4433		
1	3		2588.8867	
5	3			2915.0000
3	3			3071.6700
Sig.		1.000	1.000	.377

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 12555.984.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.24: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1797.2233	21.43172	3
	1	2679.4433	87.56300	3
	3	2938.8900	232.31551	3
	5	3115.5567	144.55455	3
	Total	2632.7783	543.33259	12
Total	0	1797.2233	21.43172	3
	1	2679.4433	87.56300	3
	3	2938.8900	232.31551	3
	5	3115.5567	144.55455	3
	Total	2632.7783	543.33259	12

Figure A.25: Descriptive statistics with mean and standard deviation for incubation time at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	3081327.16[a]	3	1027109.052	49.503	.000	.949
Intercept	83178261.03	1	83178261.03	4008.924	.000	.998
Temp	.000	0	.	.	.	.000
Time	3081327.156	3	1027109.052	49.503	.000	.949
Temp * Time	.000	0	.	.	.	.000
Error	165986.226	8	20748.278			
Total	86425574.41	12				
Corrected Total	3247313.382	11				

a. R Squared = .949 (Adjusted R Squared = .930)

Figure A.26: Significant difference between all incubation times at 60°C

Homogeneous Subsets

Concentration of NaHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1797.2233		
1	3		2679.4433	
3	3		2938.8900	2938.8900
5	3			3115.5567
Sig.		1.000	.201	.479

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 20748.278.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.27: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1627.7800	39.10046	3
	1	2278.8900	40.35649	3
	3	2679.4467	195.76345	3
	5	2615.5567	52.60732	3
	Total	2300.4183	444.78167	12
Total	0	1627.7800	39.10046	3
	1	2278.8900	40.35649	3
	3	2679.4467	195.76345	3
	5	2615.5567	52.60732	3
	Total	2300.4183	444.78167	12

Figure A.28: Descriptive statistics with mean and standard deviation for incubation time at 30°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2087641.33[a]	3	695880.443	62.907	.000	.959
Intercept	63503094.10	1	63503094.10	5740.607	.000	.999
Temp	.000	0	.	.	.	.000
Time	2087641.330	3	695880.443	62.907	.000	.959
Temp * Time	.000	0	.	.	.	.000
Error	88496.704	8	11062.088			
Total	65679232.13	12				
Corrected Total	2176138.033	11				

a. R Squared = .959 (Adjusted R Squared = .944)

Figure A.29: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration of KHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1627.7800		
1	3		2278.8900	
5	3			2615.5567
3	3			2679.4467
Sig.		1.000	1.000	.877

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 11062.088.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.30: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1711.1100	35.32966	3
	1	2601.6667	138.15579	3
	3	2890.5567	74.05457	3
	5	2915.0000	152.37893	3
	Total	2529.5833	518.73135	12
Total	0	1711.1100	35.32966	3
	1	2601.6667	138.15579	3
	3	2890.5567	74.05457	3
	5	2915.0000	152.37893	3
	Total	2529.5833	518.73135	12

Figure A.31: Descriptive statistics with mean and standard deviation for incubation time at 45℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	2861827.08[a]	3	953942.359	77.812	.000	.967
Intercept	76785502.08	1	76785502.08	6263.267	.000	.999
Temp	.000	0	.	.	.	.000
Time	2861827.076	3	953942.359	77.812	.000	.967
Temp * Time	.000	0	.	.	.	.000
Error	98077.252	8	12259.656			
Total	79745406.41	12				
Corrected Total	2959904.328	11				

a. R Squared = .967 (Adjusted R Squared = .954)

Figure A.32: Significant difference between all incubation times at 45°C

Homogeneous Subsets

Concentration of KHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1711.1100		
1	3		2601.6667	
3	3		2890.5567	2890.5567
5	3			2915.0000
Sig.		1.000	.050	.993

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 12259.656.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.33: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KHCO3

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1690.0033	15.27525	3
	1	2886.6667	124.40869	3
	3	3079.4433	69.40771	3
	5	3169.4467	106.68238	3
	Total	2706.3900	626.76763	12
Total	0	1690.0033	15.27525	3
	1	2886.6667	124.40869	3
	3	3079.4433	69.40771	3
	5	3169.4467	106.68238	3
	Total	2706.3900	626.76763	12

Figure A.34: Descriptive statistics with mean and standard deviation for incubation time at 60℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KHCO3

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	4257395.40[a]	3	1419131.799	177.895	.000	.985
Intercept	87894561.99	1	87894561.99	11018.010	.000	.999
Temp	.000	0	.	.	.	.000
Time	4257395.396	3	1419131.799	177.895	.000	.985
Temp * Time	.000	0	.	.	.	.000
Error	63818.830	8	7977.354			
Total	92215776.21	12				
Corrected Total	4321214.226	11				

a. R Squared = .985 (Adjusted R Squared = .980)

Figure A.35: Significant difference between all incubation times at 60°C

Homogeneous Subsets

Concentration of KHCO3

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	1690.0033		
1	3		2886.6667	
3	3		3079.4433	3079.4433
5	3			3169.4467
Sig.		1.000	.110	.624

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 7977.354.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.36: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1482.7767	43.97861	3
	1	1780.5567	32.71267	3
	3	2186.6667	36.09394	3
	5	2231.1100	206.19502	3
	Total	1920.2775	334.27990	12
Total	0	1482.7767	43.97861	3
	1	1780.5567	32.71267	3
	3	2186.6667	36.09394	3
	5	2231.1100	206.19502	3
	Total	1920.2775	334.27990	12

Figure A.37: Descriptive statistics with mean and standard deviation for incubation time at 30°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1135526.76[a]	3	378508.922	32.335	.000	.924
Intercept	44249588.12	1	44249588.12	3780.126	.000	.998
Temp	.000	0	.	.	.	.000
Time	1135526.765	3	378508.922	32.335	.000	.924
Temp * Time	.000	0	.	.	.	.000
Error	93646.789	8	11705.849			
Total	45478761.68	12				
Corrected Total	1229173.554	11				

a. R Squared = .924 (Adjusted R Squared = .895)

Figure A.38: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration of NaH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1482.7767		
1	3		1780.5567	
3	3			2186.6667
5	3			2231.1100
Sig.		1.000	1.000	.956

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 11705.849.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.39: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1608.3333	14.53005	3
	1	2052.7767	56.72758	3
	3	2098.8900	51.02741	3
	5	2203.3333	105.98742	3
	Total	1990.8333	244.10499	12
Total	0	1608.3333	14.53005	3
	1	2052.7767	56.72758	3
	3	2098.8900	51.02741	3
	5	2203.3333	105.98742	3
	Total	1990.8333	244.10499	12

Figure A.40: Descriptive statistics with mean and standard deviation for incubation time at 45℃

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	620927.159[a]	3	206975.720	47.949	.000	.947
Intercept	47561008.33	1	47561008.33	11018.247	.000	.999
Temp	.000	0	.	.	.	.000
Time	620927.159	3	206975.720	47.949	.000	.947
Temp * Time	.000	0	.	.	.	.000
Error	34532.541	8	4316.568			
Total	48216468.03	12				
Corrected Total	655459.700	11				

a. R Squared = .947 (Adjusted R Squared = .928)

Figure A.41: Significant difference between all incubation times at 45°C

Homogeneous Subsets

Concentration of NaH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	1608.3333	
1	3		2052.7767
3	3		2098.8900
5	3		2203.3333
Sig.		1.000	.087

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4316.568.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.42: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of NaH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1561.1100	3.47078	3
	1	2027.2200	8.39025	3
	3	2127.2200	35.75816	3
	5	2250.0000	132.74087	3
	Total	1991.3875	278.50628	12
Total	0	1561.1100	3.47078	3
	1	2027.2200	8.39025	3
	3	2127.2200	35.75816	3
	5	2250.0000	132.74087	3
	Total	1991.3875	278.50628	12

Figure A.43: Descriptive statistics with mean and standard deviation for incubation time at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of NaH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	815260.765[a]	3	271753.588	57.268	.000	.956
Intercept	47587490.10	1	47587490.10	10028.327	.000	.999
Temp	.000	0	.	.	.	.000
Time	815260.765	3	271753.588	57.268	.000	.956
Temp * Time	.000	0	.	.	.	.000
Error	37962.456	8	4745.307			
Total	48440713.32	12				
Corrected Total	853223.220	11				

a. R Squared = .956 (Adjusted R Squared = .939)

Figure A.44: Significant difference between all incubation times at 60°C

Homogeneous Subsets

Concentration of NaH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1561.1100		
1	3		2027.2200	
3	3		2127.2200	2127.2200
5	3			2250.0000
Sig.		1.000	.349	.207

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 4745.307.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.45: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	1456.6667	34.44085	3
	1	1935.5567	118.81932	3
	3	2154.4433	23.35420	3
	5	2012.7800	81.52022	3
	Total	1889.8617	281.16079	12
Total	0	1456.6667	34.44085	3
	1	1935.5567	118.81932	3
	3	2154.4433	23.35420	3
	5	2012.7800	81.52022	3
	Total	1889.8617	281.16079	12

Figure A.46: Descriptive statistics with mean and standard deviation for incubation time at 30°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	824574.948[a]	3	274858.316	48.874	.000	.948
Intercept	42858925.43	1	42858925.43	7621.002	.000	.999
Temp	.000	0	.	.	.	.000
Time	824574.948	3	274858.316	48.874	.000	.948
Temp * Time	.000	0	.	.	.	.000
Error	44990.333	8	5623.792			
Total	43728490.71	12				
Corrected Total	869565.282	11				

a. R Squared = .948 (Adjusted R Squared = .929)

Figure A.47: Significant difference between all incubation times at 30°C

Homogeneous Subsets

Concentration of KH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	1456.6667		
1	3		1935.5567	
5	3		2012.7800	2012.7800
3	3			2154.4433
Sig.		1.000	.609	.174

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 5623.792.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.48: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
45	0	1618.8867	30.01549	3
	1	2034.4433	43.34073	3
	3	2046.1133	77.68063	3
	5	2165.0000	67.72178	3
	Total	1966.1108	221.64129	12
Total	0	1618.8867	30.01549	3
	1	2034.4433	43.34073	3
	3	2046.1133	77.68063	3
	5	2165.0000	67.72178	3
	Total	1966.1108	221.64129	12

Figure A.49: Descriptive statistics with mean and standard deviation for incubation time at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	513573.759[a]	3	171191.253	51.102	.000	.950
Intercept	46387101.71	1	46387101.71	13847.034	.000	.999
Temp	.000	0	.	.	.	.000
Time	513573.759	3	171191.253	51.102	.000	.950
Temp * Time	.000	0	.	.	.	.000
Error	26799.733	8	3349.967			
Total	46927475.20	12				
Corrected Total	540373.493	11				

a. R Squared = .950 (Adjusted R Squared = .932)

Figure A.50: Significant difference between all incubation times at 45°C

Homogeneous Subsets

Concentration of KH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	1618.8867	
1	3		2034.4433
3	3		2046.1133
5	3		2165.0000
Sig.		1.000	.093

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 3349.967.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.51: Homogeneous subsets by Tukey HSD

Descriptive Statistics

Dependent Variable: Concentration of KH2PO4

Temperature	Incubation Time	Mean	Std. Deviation	N
60	0	1545.0000	12.01619	3
	1	1976.1100	38.38079	3
	3	2118.3333	59.18253	3
	5	2318.8900	89.46282	3
	Total	1989.5833	300.72839	12
Total	0	1545.0000	12.01619	3
	1	1976.1100	38.38079	3
	3	2118.3333	59.18253	3
	5	2318.8900	89.46282	3
	Total	1989.5833	300.72839	12

Figure A.52: Descriptive statistics with mean and standard deviation for incubation time at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of KH2PO4

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	968565.943[a]	3	322855.314	98.404	.000	.974
Intercept	47501302.08	1	47501302.08	14478.085	.000	.999
Temp	.000	0	.	.	.	.000
Time	968565.943	3	322855.314	98.404	.000	.974
Temp * Time	.000	0	.	.	.	.000
Error	26247.285	8	3280.911			
Total	48496115.31	12				
Corrected Total	994813.228	11				

a. R Squared = .974 (Adjusted R Squared = .964)

Figure A.53: Significant difference between all incubation times at 60°C

Homogeneous Subsets

Concentration of KH2PO4

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	1545.0000		
1	3		1976.1100	
3	3		2118.3333	
5	3			2318.8900
Sig.		1.000	.063	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square(Error) = 3280.911.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.54: Homogeneous subsets by Tukey HSD

APPENDIX B

Figure B.1: Rubber seed powder that was kept in the refrigerator

Figure B.2: The sample was centrifuge in the microcentrifuge each hour after incubating

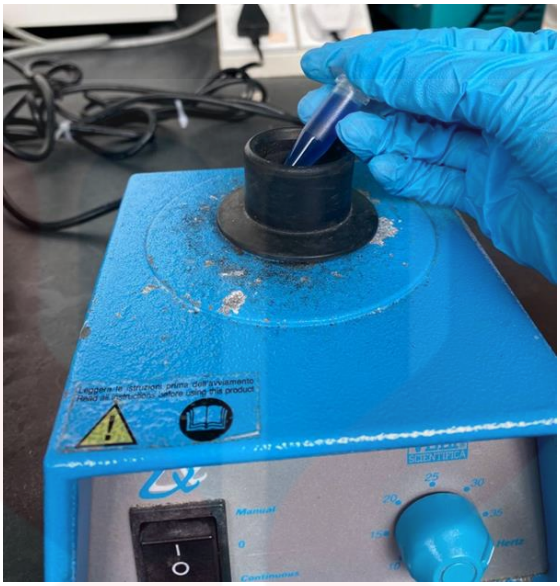


Figure B.3: After centrifuge, put 1000µl of Bradford reagent and vortex it

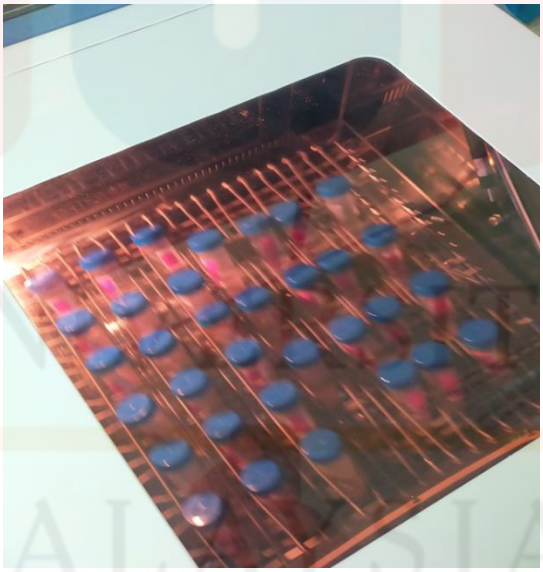


Figure B.4: The sample was in the incubation shaker

Figure B.5: The sample let it cool after incubator before being pipette into the microcentrifuge tube

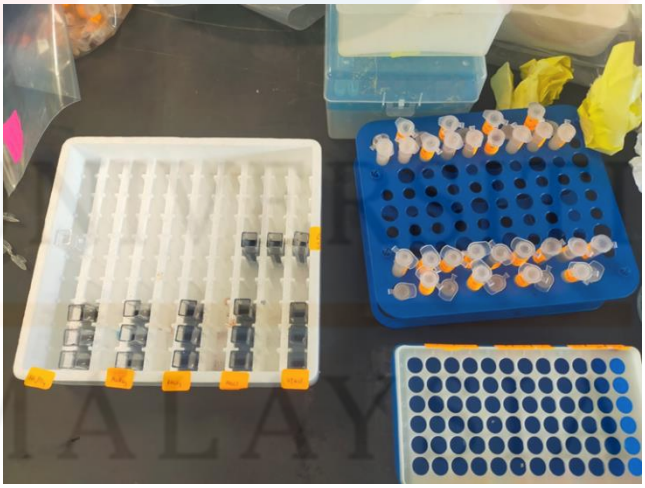


Figure B.6: After vortex, the sample will place in the cuvette (1.5ml) to read the absorbance value

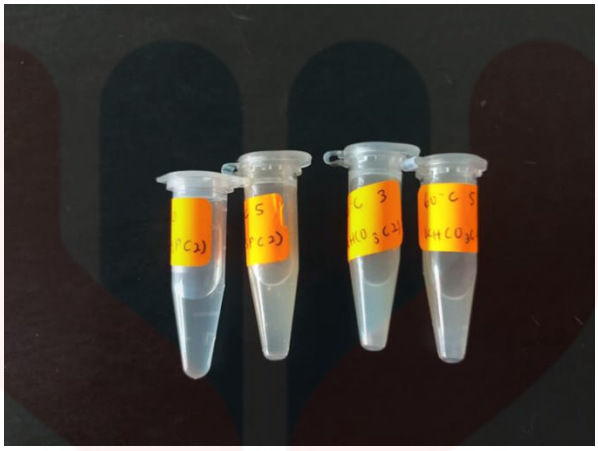


Figure B.7: Choose the best protein sample to do SDS-PAGE

Figure B.8: Using the casting plates to form the gel using the resolving and stacking buffer

Figure B.9: While waiting for the gel to polymerize, prepared the protein sample in the microcentrifuge tube

Figure B.10: The sample will place in the water bath for 5 minutes and 95°C

Figure B.11: Place the casting plates into the electrophoresis and fill up with running buffer for two gels

Figure B.12: The protein sample pipette into the well carefully

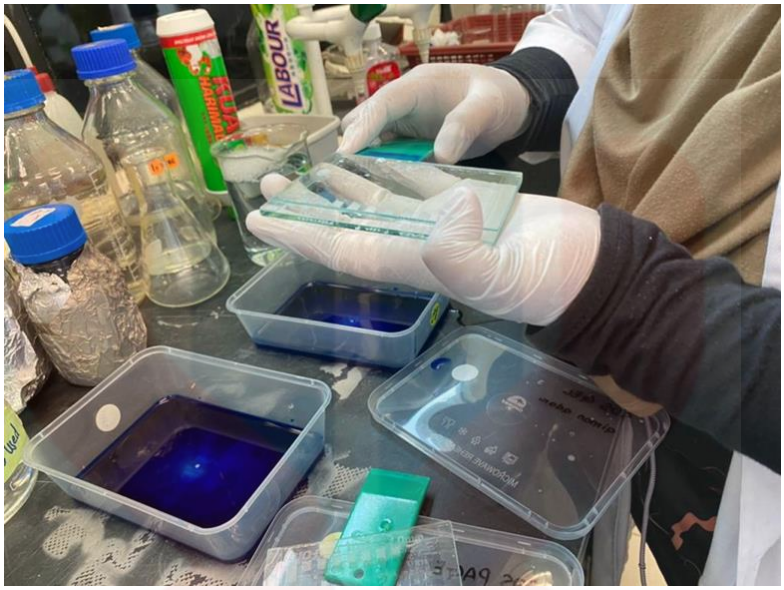


Figure B.13: the casting plates slowly open and place the gel quickly into the blue staining

Figure B.14: Let it overnight on the mini rocking shaker

Figure B.15: The next day, de-stain the gel using de-stain buffer

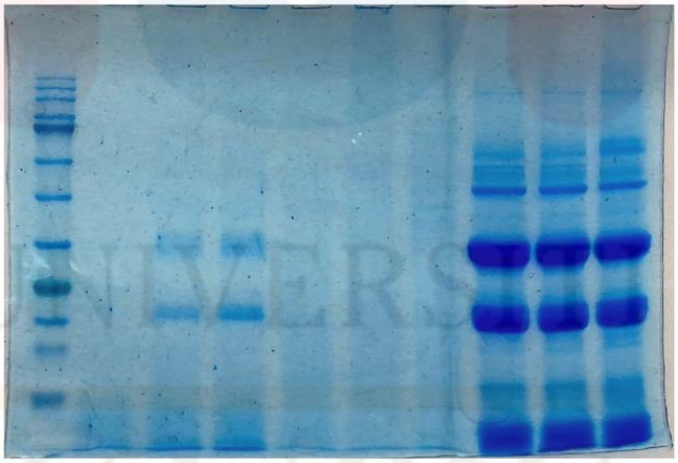


Figure B.16: The gel that had been de-stain shown band from each protein sample

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