Protein Hydrolysis of Pumpkin Seed Powder by Using Enzyme and Fruit Waste 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 made clear of the sources.

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Protein Hydrolysis of Pumpkin Seed Powder by Using Enzyme and Fruit Waste Treatment

ABSTRACT

Pumpkin seed (*Cucurbita maxima*) is an efficient-cost, plentiful agricultural waste that is rich in protein and fibers. Pumpkin seeds' protein content and amino acid profile are like various high-protein feedstuffs, such as fish meals and soybean meals. Nevertheless, only half of the protein content is being absorbed by the chicken gut. Thus, protein hydrolysis could be done by enzymatic methods to improve the digestibility and growth performance of broiler chickens. In this study, the enzyme of commercial protease, pineapple peel powder, bromelain, and date seed powder was used for pre-treatment to hydrolyze protein in pumpkin seed. The pumpkin seed was treated by these four enzymes to evaluate the percentage of protein hydrolysis with increasing temperature and time incubation. There were three tested temperatures; 30°C, 45°C, and 60°C, and four tested time incubations; T_o (0 hours), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours) using these four types of enzyme. The results show a similar percentage of protein decreased when date seed and bromelain were employed (42%), followed by pineapple peel (33%), and commercial protease (28%). To corroborate this hydrolysis, the selected enzyme-treated pumpkin powder was analyzed by using SDS-PAGE. The result from SDS-PAGE confirmed the reduction of molecular size of pumpkin protein. This finding unlocks the potential of treated pumpkins to become a cost-efficient yet highly-digestible protein source for animal feed in the future.

Keywords: Pumpkin seed, protein hydrolysis, SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE), broiler feed

Hidrolisis Protein pada Serbuk Biji Labu Menggunakan Rawatan Enzim dan Sisa Buah

ABSTRAK

Biji labu (*Cucurbita maxima*) ialah sisa pertanian yang berkos cekap dan banyak yang kaya dengan protein dan serat. Kandungan protein biji labu dan profil asid amino adalah seperti pelbagai bahan makanan berprotein tinggi, seperti makanan ikan dan makanan kacang soya. Namun begitu, hanya separuh daripada kandungan protein yang diserap oleh usus ayam. Oleh itu, hidrolisis protein boleh dilakukan dengan kaedah enzimatik untuk meningkatkan kebolehcernaan dan prestasi pertumbuhan ayam pedaging. Dalam kajian ini, enzim protease komersial, serbuk kulit nanas, bromelain, dan serbuk biji kurma digunakan untuk pra-rawatan untuk menghidrolisis protein dalam biji labu. Biji labu telah dirawat oleh empat enzim ini untuk menilai peratusan hidrolisis protein dengan peningkatan suhu dan masa pengeraman. Terdapat tiga suhu yang diuji; 30°C, 45°C, dan 60°C, dan empat kali pengeraman masa yang diuji; T_o (0 jam), T_1 (1 jam), T_2 (3 jam), dan T_3 (5 jam) menggunakan empat jenis enzim ini. Keputusan menunjukkan peratusan protein yang sama menurun apabila biji kurma dan bromelain digunakan (42%), diikuti oleh kulit nanas (33%), dan protease komersial (28%). Untuk menyokong hidrolisis ini, serbuk labu yang dirawat enzim telah dianalisis dengan menggunakan SDS-PAGE. Keputusan daripada SDS-PAGE mengesahkan pengurangan saiz molekul protein labu. Penemuan ini membuka potensi labu yang dirawat untuk menjadi sumber protein yang cekap kos namun sangat mudah dihadam untuk makanan haiwan pada masa hadapan.

Kata kunci: biji labu, hidrolisis protein, SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE), makanan ayam pedaging

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LIST OF ABBREVIATIONS

UT	Untreated
CP	Commercial Protease
PP	Pineapple Peel
BR	Bromelain
DS	Date Seed
UV-Vis Spectrophotometer	Ultra-Violet Visible Spectrophotometer
ANOVA	Analysis of Variance
CRD	Customized Randomly Design
SDS-PAGE	SDS-Polyacrylamide Gel Electrophoresis

LIST OF SYMBOLS

g	gram
kg	kilogram
g kg^{-1}	Gram per kilogram
Cmol/kg	centimoles per kilogram
µL	Microliter
mL	milliliter
L	liter
°C	degree Celcius
M	Molar
hrs	hour
nm	nanometer
mins	minutes
ppm	parts per million
kDA	kiloDalton
%	percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to the Food and Agriculture Organization of the United Nations (FAO), animal health and welfare play a role in animal feeding as in the product feed. The production must be produced in good quality and safe products of animal origin. In livestock production, the demand for proteins of animal origin increases then leads to intensification. Basically, this was used in the industrial compound feed. In an upcoming world with an increasing human population day by day, livestock production must keep in line to keep up with the demands of the rise in urbanization, religious preference, associated shifts in diet habits and include the feed price.

Nevertheless, with increasing markedly there is also a need to cope with increasing safety concerns as the feed suppliers also must epitomize it. This is because of the mad cow disease crisis and the bovine spongiform encephalopathy (BSE), as we are connected with the administration of meat and bone meal (MBM). Generally, animal feed is concerned with the usage of high-protein crops such as maize and soybean but then there is anxiety about the use of genetically modified crops. Thus, this incident had a concern, especially when involving chemical contamination such as dioxin in feed. Then, the sources of the feed protein as in their suitability, safety, and high quality must be considered as there was always markedly increased demand for feed production

especially in livestock production for the sufficient future supply. Consumers are expected to want guarantees regarding food safety and feed production techniques up the connected food chain. There is therefore the need to source alternative feedstuff that is cheap, available, and less competed for by man and industry.

1.2 Problem Statement

Significant growth in worldwide demand for livestock, particularly chicken products, would necessitate increased feed protein supply, necessitating a constant examination of the sources and alternatives. Those were normal issues addressed in the broiler industry with regards to continuing the poultry business. The supply is basically of inefficient nutrient absorption of broiler chicken, the feed value, and the disintegration of nature.

Based on the demand, this sector has been facing problems in the high cost of feed consumption towards broilers. To encourage their quick growth, broilers should be fed a high-protein diet, as we all know. Furthermore, the feeding procedure must feed for 6 to 8 weeks to get the optimal weight for sale. During the first 6 weeks, each bird will consume around 10 pounds of grain. After 6 weeks, they will consume between 3 and 4 pounds of feed each week. They are modest in size, yet they are ferocious feeders. According to the National Academic Press (NAP), poultry diets are largely made up of a combination of feedstuffs such as animal by-product meal options, grain products, lipids, soybean meal, and vitamin and mineral premixes. These feedstuffs were high cost as creating a new modification using pumpkin seed as they also contain high in protein.

These feedstuffs are imported from other countries so this new modification will reduce the dependency on imported feed.

1.3 Objectives

1. To treat pumpkin seed powder with commercial protease, bromelain, pineapple peels powder, and date seed powder at different temperatures and incubation times.
2. To analyze the percentage of protein hydrolysis in four enzyme-treated pumpkin seed powder before and after the treatment through Bradford analysis.

1.4 Hypothesis

a) H_o: There is no significant value with the percentage of protein hydrolysis in four enzyme-treated pumpkin seed powder before and after the treatment through Bradford analysis

b) H_a: There is a significant value in the percentage of protein hydrolysis in four enzyme-treated pumpkin seed powder before and after the treatment through Bradford analysis

a) H_o: There are significant differences 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 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 Scope of Study

The study is focusing on optimizing the degree of hydrolysis of the pumpkin seed powder. The percentage of protein hydrolysis in four enzyme-treated pumpkin seeds before and after the treatment will be analyzed. The pumpkin seed powder will be treated with commercial protease, bromelain powder, pineapple peel powder, and date seed powder using Conventional Methodology. There will be three tested temperatures; 30°C, 45°C, and 60°C, and four tested incubation times; 0 hour, 1 hours, 3 hours, and 5 hours, for each temperature. Protein hydrolysis of four enzyme-treated pumpkin seed powder will be obtained before and after the treatment by the Bradford assay method using Ultra-Violet Visible Spectrophotometer (595 nm). The effects of different temperatures and incubation times towards the degree of protein hydrolysis will be identified and the data of protein availability will be analyzed using Factorial in Customized Randomly Design (CRD) of Analysis of Variance (ANOVA). Then, the high percentage from protein hydrolysis will be chosen to proceed in SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) to separate proteins according to their molecular weight. Through this method,

different proteins can be resolved, and the relative amount of each protein can be determined.

1.6 Significance of Study

Pumpkin seeds are high in nutrients and oil. Standard methods were used to determine the nutritional contents of pumpkin seeds. Total lipid 36.70 %, total protein 34.56 %, total soluble protein 18.10 %, moisture 4.06 %, ash 3.80 %, crude fibre 2.91 %, starch 2.15 % and sugar 1.08 %, were the approximate compositions of the powdered seed. Nitrogen 5.53 %, phosphorus 0.71 %, potassium 20.00 Cmol/kg, sodium 4.80 Cmol/kg, calcium 4.40 Cmol/kg, iron 290.0 ppm, magnesium 348.7 ppm, zinc 39.9 ppm, copper 70 ppm, and manganese 17.9 ppm were the mineral compositions of the seed. It's a potentially appealing source of fat, protein, and crude fiber. It is employed as a source of fat, protein, and crude fiber that has the potential to be appealing.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The human population has increased year by year since the beginning of the 20th century. With the increase of world population, food security is a great concern not only in developed country but more so in developing countries including Malaysia (Samsudin, Sharaai, & Ismail, 2015). The industry's growth and improvement would ensure the country's food security and reduce its reliance on imported food. Besides, Bello (2005) states that food security means that every person in the country has access to enough food per person and can access enough food for every person. In the FAO definition, food security is described as a situation in which the population, in addition to the quality of their food, has at any point social, economic, and physical access to an acceptable quantity to meet people's requirements and preferences for food every day (FAO, 2003).

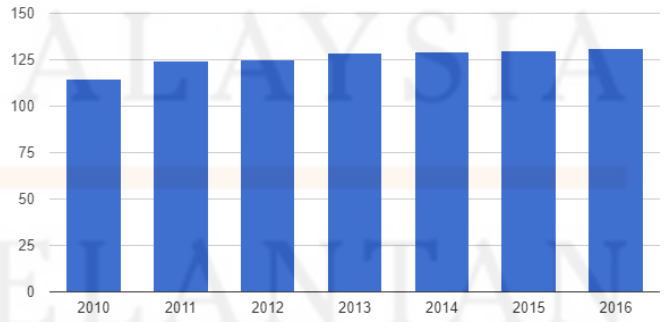


Figure 2.1: Malaysia’s Food production index in 2010 to 2016

Malaysia achieves self-sufficiency in poultry meat production (chicken, quails, and ducks). Malaysia's overall meat output is sufficient to fulfill domestic demand. Even though Malaysia has become self-sufficient in broiler meat production, food security remains a major challenge for the long-term viability of intensive agricultural systems (Samsuddin, Sharaai & Ismail, 2015).

2.2 Food Security Index

The Global Food Security Index (GFSI) examines food cost, availability, safety, and quality as well as natural resources and resilience, in 113 nations. The index is a dynamic quantitative and qualitative benchmarking model built from 58 distinct metrics that assess the determinants of food security in both emerging and developed nations. Malaysia's rank in the Global Food Security Index (GFSI) has declined from 28th in 2019 to 43rd in 2020, with a score of 67.9%, out of 113 nations, due to the Covid-19 epidemic. Malaysia scored 85.5% under affordability, mainly due to low food inflation, a small proportion of the population under the global poverty line, and food safety net programs.

However, lower scores in other categories are due to among others, low public expenditure on agricultural research and development (R&D), ineffective educational campaign on the importance of a balanced and nutritious diet, no dedicated agency responsible for food security, and high dependency on food imports. With the growing global population, food security is a major problem not just in wealthy countries, but also in emerging countries such as Malaysia (Samsudin, Sharaai, & Ismail, 2015).

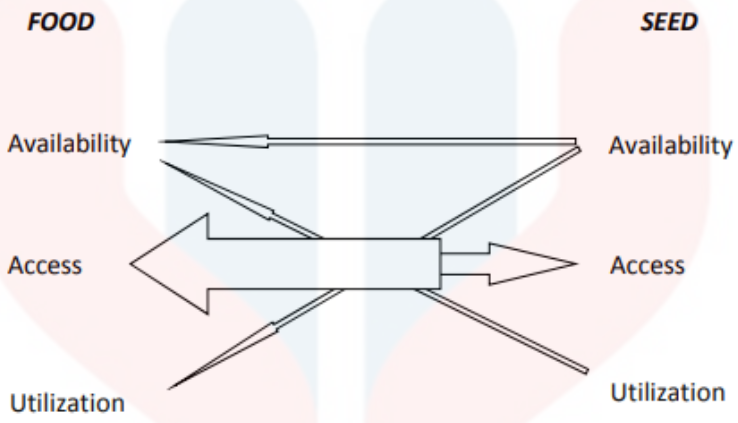
The Economist Intelligence Unit established the Global Food Security Index (GFSI) in 2016, which evaluates the basic concerns of food availability, price, and quality

across 113 nations, using 28 variables that quantify the drivers and underlying factors impacting food security. The GFSI, by analyzing situations at the national level, does not reflect local context or crucial cultural and political components, but it does give a valuable way to assess food security issues.

2.2.1 Abundant Seed and Food Security

Due to rising prices, the feed and food crisis has become a global concern. It prompted researchers to consider a non-traditional edible material as an alternative. Seed availability has a rare impact on food availability, as seed availability is usually not a concern for the reasons stated above (Remington et al., 2002; Sperling et al., 2008). Clearly, the seed is an important input, but small fluctuations in seed supply are more likely to affect farmers' production and food security than ownership especially land and the environment, assets, use of other inputs. Seed availability at the household level can be a constraint for very poor families in high-stress locations that employ high implantation rates or numerous sowings to reduce risk, although seed is generally always accessible at bigger sizes. As a result, for many crops, a significant drop in the harvest of more than 90% may still leave enough seed for future sowing or processing, but if local grain markets prices rise, seed access may be impacted.

Figure 2.2: Food security and seed security are linked in a two-way diagram. The arrow's relative width indicates the relative strength of the associations.



Source: Development in Practice (June 2011)

2.3 Dependency in Imported Feed

One of the most significant industries in the world is the broiler industry. Manufacturers throughout the world are concerned about the industry's expansion. Broiler meat output in Asia is predicted to grow due to the increase in demand. The industry has become one of the significant industries which can add to Malaysian GDP. Furthermore, it will become a standard meal in Malaysia since it is the cheapest and finest source of meat and protein. As a result, broiler meat output is likely to rise year after year, and demand is expected to rise until the year 2020.

Since 1984, Malaysia's broiler industry has reached self-sufficiency. The sector is vulnerable to a variety of issues that might have an impact on farmers' profit and loss

statements and viability, such as a strong reliance on imported raw materials for animal feed, the price of which is frequently volatile (Elsedig, Mohd & Fatimah, 2015). Elsedig, Mohd, and Fatimah (2015) also reported that chicken feed production accounts for more than 65% of broiler production costs. This is because the broiler industry consumes nearly four million tonnes of imported soybean and corn compound feed each year. Both components are imported from Argentina, and their prices are not regulated in accordance with global demand, which has an impact on chicken prices.

According to the article "Malaysian Poultry Industry Meeting" (2016), there are three reasons why broiler production costs are growing year after year. The first cause is a decrease in gasoline subsidies. As the price of gasoline rises, so will the cost of raw materials for chicken feed. Aside from that, the establishment of minimum wages for farmers has an impact on production costs. Ultimately, the devaluation of the Malaysian Ringgit contributed to an increase in operating costs.

2.4 Local Feed

Animal feed refers to any single or multiple items, whether processed, semi-processed, or raw, that are meant to be fed directly to food-producing animals, such as feedstuffs, ingredients, additives, and supplements. According to the Alltech global feed survey 2018, Malaysia has 45 feed mills that produce 4.4 million metric tonnes of feed yearly. According to Zailani, Arrifin, Wahid, Othman, and Fernando (2010), 62% of Malaysian livestock breeders employed plant-based feed for their farmed cattle. Broiler and layer feed is the most commonly used in plant-based feed. Livestock production and processing, cropping, storage, and retailing all contribute to the creation of animal feed.

The feed business has grown significantly and has become a single, important national industry. This industry is still expanding and improving (Zhang, Zhang, Chen, Yang, & Wang, 2007).

Feed resources are fundamental components and drivers of animal production systems, and the efficiency with which feed resources are used is extremely significant because it is the major predictor of animal performance and output. Feed is often regarded as the most significant component of livestock production systems, accounting for up to 70% of total production costs. Furthermore, the availability and consumption of animal feed have far-reaching consequences for farm economics, the environment, product quality, product safety, animal health, and animal welfare. As a result, local feed resources are under increasing strain to fulfill the demands of a growing animal population and a need for improved productivity. Regular and accurate assessment of national feed supplies is thus required for long-term livestock development. According to the Food and Agriculture Organization of the United Nations (FAO), country-level feed balance based on feedstock levels is important to facilitate livestock industry planning, such as determining how many animals can be embraced or produced based on existing feedstuffs and identifying what feed resources would and could be created to accomplish feed production.

2.5 Pumpkin

Pumpkins are abundant in vitamins A and E, as well as folate and fiber. Pumpkin seeds are high in oil and minerals. Even though they are primarily water, with an approximate moisture content of 85%, they can serve as a good supplementary protein source. The pumpkin is one of the most useful crops that may be cultivated for cattle, sheep, and pigs for fall feeding. They have a dietary analysis of roughly 91 % water, 5.8 % carbs, 1.3 % protein, and 1 % ash. As pasture grasses have a dietary analysis of 80% water, 10.6 % carbs, 3.5 % protein, and 2% ash.

Pumpkin is a *Cucurbitaceae* plant that grows as an annual or perennial. It is classified into three types: western pumpkin (*Cucurbita maxima*), oriental pumpkin (*Cucurbita moschata*), and peppo pumpkin (*Cucurbita pepo*). Alternatively, it is mostly a western pumpkin that has been processed into frozen or prepared cuisine. Oriental pumpkins are supposed to be Japanese pumpkins that are sticky, although Western pumpkins are nearly devoured presently. Pepo pumpkins vary from eastern and western pumpkins in that they contain shelled seeds, whereas pepo pumpkins are shellless and are rarely farmed in Japan. Any seed of eastern pumpkin, western pumpkin, or pepo pumpkin may be utilized to make the pumpkin seed meal employed in the present invention's animal feed.

2.5.1 Pumpkin Seed

Pumpkin seed is a low-cost, plentiful agricultural waste that is environmentally benign and outperforms commercial activated carbon. Cucurbitacin is a chemical found in pumpkin seeds that is not hazardous to cattle or humans. This chemical is found in many plant species and serves as a herbivore deterrent due to its bitter taste in big concentrations. Although pumpkin seeds are small, they are high in nutrients and nutraceuticals such as phytosterols, amino acids, unsaturated fatty acids, phenolic compounds, cucurbitacin, tocopherols, and important minerals. All of these bioactive substances are essential for living a healthy and balanced lifestyle.

When processed into frozen meals and the like, pumpkin seeds, which are raw materials for pumpkin seed meals to be combined in the animal feed of the present invention, are agricultural wastes. Pumpkin seeds and "wata" discharged during processing have previously been converted to compost, but it is not possible to say that they are compostable since they germinate for use as compost or require a long time of maturity. There wasn't any. As a result, pumpkin seeds were both unusable biomass and garbage. The current invention is also useful in terms of eliminating waste because it makes efficient use of available by using pumpkin seed meal after pressing pumpkin seed oil as a raw resource for food and drink or medical products for the prevention and treatment of prostate hypertrophy derived from this unused organic matter.

Pumpkin seed has the highest content protein concentration, around 35%, which translates to a significant and diversified amount of amino acids. Amino acids are important as both protein-building components and metabolic intermediates. An adequate number of high-quality essential amino acids are also required in the diet for physiological

activities. Pumpkin seeds also have a high concentration of essential nutrients. The seeds are high in calcium (Ca), magnesium (Mg), manganese (Mn), and phosphorus (P) but low in sodium (Na). Antioxidant-potential minerals such as Zn, Cu, Mn, and Fe function as coenzymes in antioxidation-dependent biocatalysts.

2.6 Treatment

Proteins, which are important components of all organisms, are incorporated into cellular structures and execute specialized tasks, such as hormones, antibodies, and enzymes. Enzymes are biocatalysts that catalyze biochemical changes that are essential to the proper functioning of all cellular metabolism (Silva, 2017). Protein hydrolysis is accomplished using chemical and enzymatic processes. The majority of the enzymes employed in protein hydrolysis are derived from animals or plants. The most prevalent method for producing protein hydrolysates is enzymatic proteolysis (Bhat and Kumar, 2015). Different oligopeptides can be generated through enzyme-substrate interaction depending on the catalytic characteristics of the peptidase in terms of substrate selectivity (Silva, 2018).

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a protein separation technology based on molecular weight. Protein separation by SDS-PAGE may be used to measure the relative molecular mass of proteins, their relative abundance in a sample, their distribution of the purity of protein samples, and across fractions. Smaller proteins move quicker when separated by electrophoresis across a gel matrix due to the lower resistance of the gel matrix. The structure and charge of the proteins also influence the

pace of migration across the gel matrix. Polymerized acrylamide forms a mesh-like matrix suitable for traditional protein separation. Because of the gel's elasticity, it is simple to handle. Researchers may separate proteins based on their length using SDS-treated protein polyacrylamide gel electrophoresis, which is a simple, inexpensive, and relatively accurate approach.

2.7 Enzyme

Enzymes play a variety of roles in our bodies. Chemical and enzymatic approaches are used to hydrolyze proteins. The majority of protein hydrolysis enzymes come from either animal or plant sources. The ability of enzymes to catalyze reactions without being consumed is one of their most significant characteristics. Enzymes are powerful catalysts that speed up biochemical reactions dramatically. Feed, food, specialized, and detergent businesses are the four major catalyst businesses that are commonly used. To increase the digestibility of feed ingredients, enzymes are now widely used in poultry feeds. Feed enzymes that function on carbohydrates, plant-bound minerals, and proteins are available in 16 generals. There are six types of catalysts: hydrolases, oxidoreductases, transferases, lyases, isomerases, and ligases, each of which catalyzes a different type of reaction. The present increasing demand for more effective use of renewable energy sources, as well as industrial pressure to function in an ecologically acceptable manner, inspired the creation of novel enzyme catalysts.

2.7.1 Introduction of Protease

Proteases are categorized as hydrolases in class 3 and peptide hydrolases or peptidases in classification 3.4, according to the International Union of Biochemistry and Molecular Biology's Nomenclature Committee. Exopeptidases and endopeptidases are the two major groups of proteases, with exopeptidases being the most common. Aspartic, cysteine, metallo, and serine proteases are the four groups of proteases based on their catalytic action. They can be used in a variety of industries to improve the taste, texture, and appearance of products as well as waste recovery. They are also used in the food sector, household cleaners, leather care, biosorption procedures, and medications. Their depolymerization action is also important in nutrition.

2.7.2 Pineapple Peel

Pineapple is a tropical and sub-tropical fruit with the world's third-highest output yield. Pineapple is rich in many nutrients and minerals, vitamins, and dietary fiber, and has antioxidant properties. The wastage from the pineapple industry consists of inedible pineapple peels and stems. Previously, these wastes were either discarded or utilized as feed or fertilizer. Pineapple peel waste has the potential to be a source of valuable bioactive chemicals. The separation of bromelain is one possible use for pineapple peels for value addition. Pineapple peels were used as materials, and the primary polyphenolic components and antioxidant interactions were studied.

2.7.3 Bromelain

Bromelain is collected throughout the pineapple plant to varying degrees and qualities depending on its source. Bromelain, which is obtained from pineapple stem and juice, was extracted from the plant in the late 1800s. It is classed as either stem bromelain or fruit bromelain, depending on its source, with all commercially available bromelain derived from the stem. Bromelain is a protein-digesting enzyme manufactured commercially from pineapple fruit or stem. Fruit and stem bromelain are manufactured differently and have distinct enzymatic compositions. The term "bromelain" typically refers to the "stem bromelain." Bromelain is a complex of thiol endopeptidases and other enzymes such as phosphatase, protease inhibitors, glucosidase, cellulase, peroxidase, and escharase.

Nevertheless, because pineapple stem is a waste product, most commercial enzyme products are manufactured from it. Bromelain may be used to treat osteoarthritis and to minimize edema and inflammation following surgery. Bromelain, a significant proteinase isolated from pineapple, is a major proteinase (*Ananas comosus*). Bromelain is collected in the plant to varying degrees and qualities depending on its source.

Bromelain's major constituent is a sulfhydryl proteolytic fraction. It also includes acid phosphatase, peroxidase, numerous protease inhibitors, and organically bound calcium. Bromelain is stable at pH 3.0 to 6.5, and once coupled with its substrate, its action is no longer affected by pH. The effective temperature range is 40°C - 65°C, with 50°C-60°C being the ideal. Enzyme or protein isolation, separation, and purification can be accomplished utilizing a variety of chromatography, electrophoretic, ultrafiltration, precipitation, and other methods.

2.7.4 Date Seed

Date seeds (Phoenix dactylifera L.) have piqued the interest of many researchers as a profitable byproduct of the date fruit industry and a valuable source of multifunctional and reactive chemicals. Date seeds (about 10% of the fruit weight) are a plentiful byproduct of this well-established industry (Besbes et al., 2004). Date seeds have been shown to have high levels of polyphenols (Al-Farsi & Lee, 2008). Plant phytochemicals are regarded to be the primary components responsible for many of the health benefits linked with eating fruits and seeds (Macedo et al., 2013; Mahbub et al., 2012; Gondoin, Grussu, Stewart, & Mc-Dougall, 2010). The health advantages of eating date seed products have long been recognized in Middle Eastern folk medicine. In Turkey and Arab nations, date seed infusions are used as aromatic coffees with memory-boosting properties. (Habib & Ibrahim, 2009).

Date seeds, commonly known as stones or pits, are a component of the fundamental date fruit, which consists of a succulent pericarp and seed that contributes for 10% to 15% of the date fruit's mass relying on quality and type (Hussein et al., 1998). Date seeds have higher levels of protein and fat than date flesh, which has values of 1.5 - 3.0 percent and 0.1 - 1.4%, respectively (Al-Farsi et al., 2007). In terms of the mineral composition of date seeds, Ali-Mohamed and Khamis (2004) reported on six types, with the following values (mg/100 g): 459.8 - 542.2 g potassium, 61.3 - 69.5 g magnesium, 21.7 - 26.1 g sodium, 6.5 - 11.3 g calcium, 1.3 - 1.7 manganese, 2.8 - 6.0 g iron, 0.4 - 0.6 copper and 1.0 - 1.4 zinc. The bulk of essential amino acids are found in date seed protein; glutamic acid was the most abundant amino acid in Deglet Nour and Allige date seeds, accounting for 17.8% and 16.8%, respectively (Bouaziz et al., 2008).

The seed technique can only be helpful for breeding reasons because of its diversity. As a result, date seeds are either tossed or fed to livestock, goats, camels, and fowl. The traditional use of date seed for animal feed is still likely the most widespread practice. Currently, seeds are mostly utilized for animal feed. Date seeds are used to make caffeine-free coffee by drying, roasting, and grinding them in the same manner as coffee beans are. The date seed oil has been utilized to replace the amounts of other vegetable oils in shaving soap formulations, shampoos, body lotions, and shaving soap formulations, and the quality of these cosmetic formulations is generally positive (Devshony et al., 1992).

CHAPTER 3

METHODOLOGY

3.1 Materials and Apparatus

3.1.1 Materials

Sources of 41 grams of pumpkin seed powder and 900 µL of commercial protease, 4.2 grams of pineapple peel powder, bromelain powder, and date seed powder. They were the main ingredients used in this experiment. For protein analysis, materials needed were Bovine Serum Albumin (BSA) powder, Bradford reagent, 14.19 grams of Di-sodium hydrogen phosphate (Na_2HPO_4), 12 grams of Sodium hydrogen phosphate (NaH_2PO_4), 4 grams of Sodium Hydroxide (NaOH) to produce Phosphate buffer solution (PBS), and distilled water. For (SDS-PAGE), 30% acrylamide, stacking buffer, 10% APS, TEMED, PM2700 ExcelBand™ 3-color Broad Range Protein Marker, sample buffer, sample dissociation buffer, diluted running buffer, de-staining.

3.1.2 Apparatus

Apparatus used in the experiment were airtight zipper bags (A3 and A4), spatula, electronic weighing scale, incubator shaker, aluminium foil, beakers (25 mL, 250 mL, and 500 mL), measuring cylinder (50 mL), falcon tube (50ml), micropipette (10 µL, 100 µL, and 1000 µL), volumetric flask (1000mL), micropipette tips, microcentrifuge tubes (1.5 mL), centrifuge rack, grinding machine, distilled water bottle, tissue, glove, face mask, plastic dropper (3 mL), sprayer, test tube with cap (10 mL) media bottle (1000 mL), cuvettes (1.5 mL to 2.5mL), ultra-visible spectrophotometer, microcentrifuge machine, pH meter, and autoclave sterilizer, short plate, 1mm and 0.75mm of the spacer glass plate, casting frame, filter paper, 1mm and 0.75mm comb, Thermo scientific™ compact digital rocker, digital dry bath, power supply.

3.2 Methods

3.2.1 Experimental Design

Table 3.1 shows the experimental design of three enzyme-treated pumpkin seed powder, comprises different temperatures; 30°C, 45°C, and 60°C, and incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours). Weight of pumpkin seed powder, the volume of Phosphate buffer solution (PBS), and weight of bromelain, pineapple peels powder, date seed powder, and commercial protease in each temperature were the same

which were 0.9 g, 30 mL, and 0.1 g@100 µL respectively. The experiment was run in triplicate to increase the precision of the results and to use statistical analysis to generate a p-value.

Table 3.1: Experimental design for four enzyme-treated pumpkin seed powder.

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

3.2.2 Protein Analysis

a) Preparation of Phosphate Buffer Solution (PBS) in pH 8

The number of moles and the molarity of Na_2HPO_4, NaH_2PO_4 and NaOH were being calculated to produce 1000 mL/0.1 M of Phosphate Buffer Solution in pH 8 by using equation 3.1 and equation 3.2 respectively where the molar mass of Na_2HPO_4 is 141.96 g/mol, the NaH_2PO_4 is 119.98 g/mol and the molar mass of NaOH is 40 g/mol. Both solutions were mixed to achieve pH 8 and distilled water was added until it achieved the volume of solution needed.

Equation 3.1

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

Equation 3.2

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

b) Preparation of Protein Standard Curve

The standard concentration of Bovine Serum Albumin (BSA) needed to be varied to get the standard curve as shown in Table 3.3. The solution was added into the test tube starting with Bradford reagent, PBS, and BSA. After that, it needed to vortexed for 30 seconds and needed to leave for 5 minutes. After 5 minutes, the absorbance reading at wavelength 595nm needs to be read through the UV-Spectrophotometer. Each standard concentration of BSA had three replications. Then, a graph for protein standard curve was prepared in which on the x-axis, it was the standard concentration of BSA (µg/mL) while on the y-axis, it was the absorbance reading. Then, linear regression will be performed on the data obtained in triplicate as it was used to calculate the concentration of unknown samples. To have a fit model, the value correlation coefficient, R^2 should be closed to 1.00. The protein hydrolysis of enzyme-treated pumpkin seed powder before and after treatment with different variables and parameters will be calculated based on the protein standard curve.

Table 3.3: Parameters to produce Protein Standard Curve

Standard concentration of BSA (µg / mL)	0	10	25	50	100	200
BSA stock solution for 1 mg / 1 mL (µL)	0	5	12.5	25	50	100
PBS (µL)	500	495	487.5	475	450	400
Bradford reagent (µL)	1000	1000	1000	1000	1000	1000

3.3 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

3.3.1 Preparation of Stacking and Resolving Gel

The percentage of gel concentration was determined with 4% for stacking gel and 12% for resolving gel. In a 50ml beaker separately, the appropriate volume of stacking gel containing the desired concentration of acrylamide was prepared using the values in Table 3.3.1a for stacking gel and Table 3.3.1b for resolving gel. the components mixed in the order listed. As soon as the TEMED has been added into the stacking beaker first, then the acrylamide will begin to polymerize. After adding the TEMED, the mixture was swirled for about 15 seconds and added into the spacer using a pipette. Then let it completely polymerize for 45 until 60 minutes.

Table 3.3.1a: Volume and ingredients of 4% stacking gel

Volume (µL)	Ingredients
1400	distilled water
500	30% acrylamide
2000	stacking buffer
40	10% APS (0.1g in 1ml)
4	TEMED

Table 3.3.1b: Volume and ingredients of 12% resolving gel

Volume (µL)	Ingredients
800	distilled water
4000	30% acrylamide
5000	stacking buffer
100	10% APS (0.1g in 1ml)
10	TEMED

3.3.2 Casting the gel

As the 4% of stacking gel has been completely polymerized, then TEMED was added into 12% of resolving gel then swirl the mixture. The mixture has been pipetted on top of the spacer then put the comb together. The gel has been left at room temperature for about 45 until 60 minutes to let them polymerize completely. While waiting for the gel to fully harden, protein samples from a high percentage of enzyme-treated were prepared by pipetting 10µL of each sample and mixed with sample buffer into a microcentrifuge tube. Then, the sample has been centrifuged for 5 minutes at 2000 rpm and then vortexed for 30 seconds.

After completely polymerizing the comb took off and the glass gel placed into the electrophoresis which contained a running buffer into the power supply.

3.3.3 Running the SDS-PAGE Gel

By using the pipette, the protein sample, and a marker placed into the well gently. The cell connected to the power supply and was run at a constant voltage of 200V for 120 minutes until the blue dye reached the bottom.

The cassette was removed, and the buffer solution was discarded. The top glass plate was lifted and left into the container filled with blue staining solution until it covered the gel completely. The container was closed tightly and left overnight on the mini rocking. After overnight, remove the excess dye and de-stain the gel for about 30 minutes. then the gel is placed into the zipper filled with a little bit of distilled water and the kDA will be observed.

3.4 Statistical Analysis

Factorial in Completely Randomized Design (CRD) of Analysis of Variance (ANOVA) was used to interpret the results of concentration of protease-treated pumpkin seed before and after the treatment using IBM SPSS Statistics 26 software with significant difference ($p < 0.05$). Factorial is used when there are two or more factors to be analyzed while CRD is used for the laboratory experiment with uniform experimental units where effects from the environment can be controlled easily. The mean and standard deviation were reported. To observe mean between homogeneous subsets of groups of treatments whether they had significant difference or not, it referred to the letter; a, b, c, and d on Tukey HSD in Post Hoc Test Multiple Comparisons of ANOVA as it had a significant

difference when any groups of treatment did not have the same letter and vice versa. Graph for the experiment data was done by using Excel 2016 software as it is easy to understand the data set.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Protein Hydrolysis

In this study, the effect of temperature and incubation time towards the protein hydrolysis of four enzyme-treated pumpkin seeds is analyzed by using Factorial in Completely Randomized Design (CRD) of Analysis of Variance (ANOVA). Figure 4.1 shows the linear regression of the protein standard curve obtained where the correlation coefficient, R^2 is 0.9878 which is closed to 1.00.

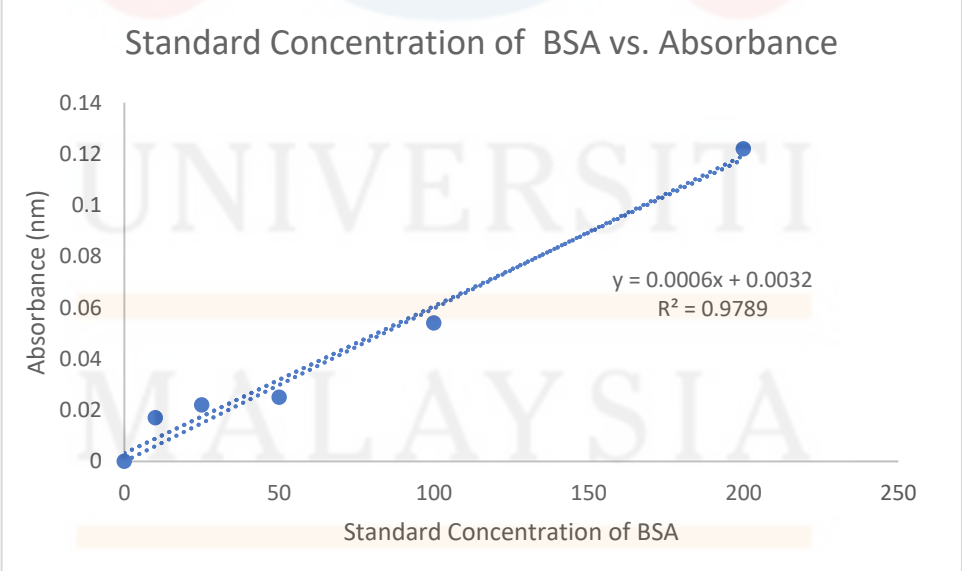


Figure 4.1 Protein Standard Curve

Figure 4.2 shows the percentage of protein hydrolysis versus incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours), in three different temperatures of enzyme-treated pumpkin seeds.

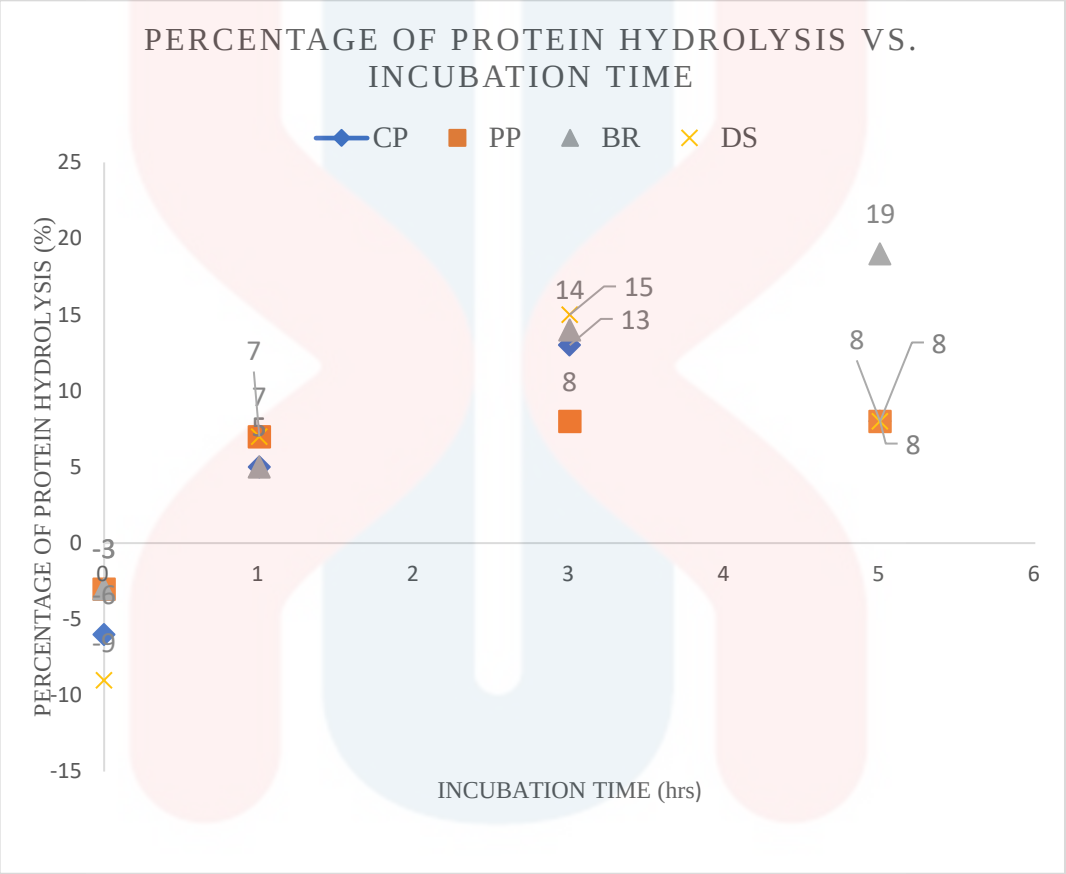


Figure 4.2a: The percentage of protein hydrolysis versus incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours), in three different temperatures of enzyme-treated pumpkin seeds at 30°C

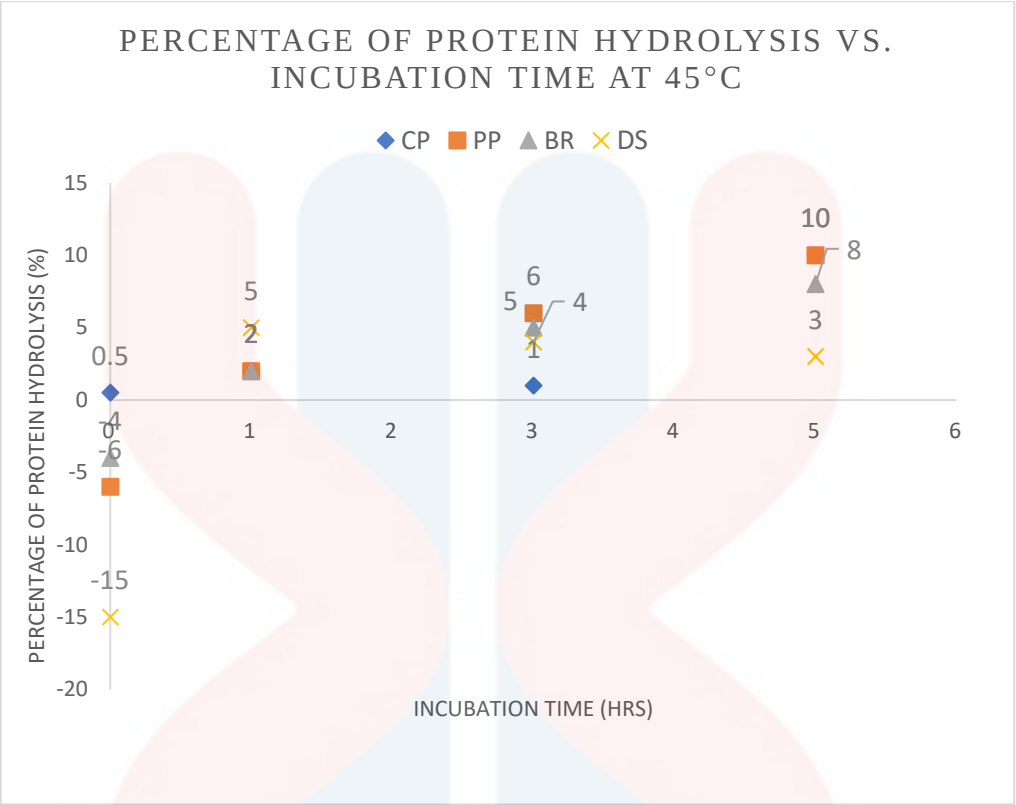


Figure 4.2b: the percentage of protein hydrolysis versus incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours), in three different temperatures of enzyme-treated pumpkin seeds at 45°C

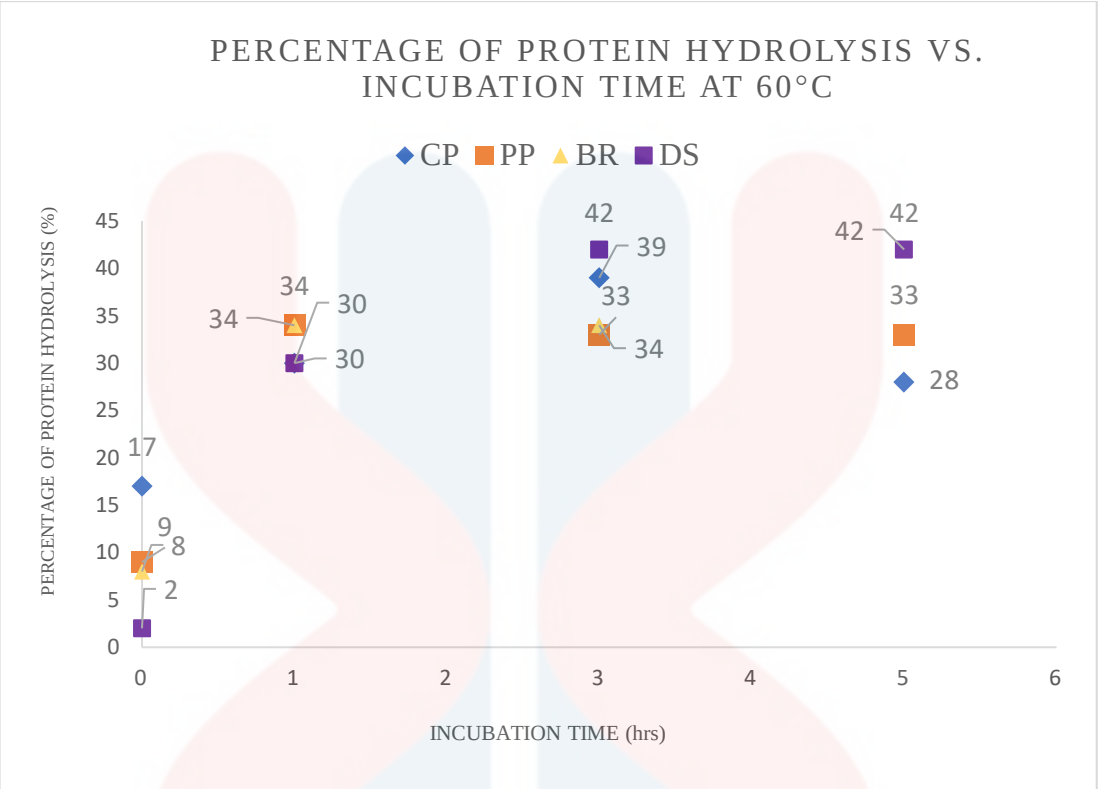


Figure 4.2c: the percentage of protein hydrolysis versus incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours), in three different temperatures of enzyme-treated pumpkin seeds at 60°C

Table 4.1 shows the concentration of four enzyme-treated pumpkin seeds in triplicate and its mean ± standard deviation at three different temperatures; 30°C, 45°C, and 60°C, and four different incubation times; T_o (0 hour), T_1 (1 hour), T_2 (3 hours), and T_3 (5 hours).

Table 4.1a: Concentration and mean ± standard deviation of commercial protease-treated pumpkin seed

Temperature (°C)	Incubation time (hrs)	The concentration of commercial protease-treated pumpkin seed (µg/mL)	Mean ± Standard deviation for the concentration of commercial protease-treated pumpkin seed
30	0	2208.33 2211.67 2435.00	2285.00 ± 81.14[a]
	1	2908.33 2843.33 2920.00	2890.55 ± 63.53[b]
	3	2838.33 2993.33 2885.00	2903.89 ± 16.91[b]
	5	3058.33 3043.33 3050.00	3050.55 ± 53.41[b]
45	0	2653.33 2611.67 2496.67	2587.22 ± 98.51[a]
	1	2870.00 2981.67 2978.33	2943.33 ± 161.44[a]
	3	3086.67 3075.00 3108.33	3090.00 ± 68.40[a]
	5	2933.33 2935.00 3026.67	2965.00 ± 29.27[b]
60	0	2640.00 2665.00 2698.33	2667.78 ± 29.26[a]
	1	2941.67 2943.33 3046.67	2977.33 ± 60.15[b]
	3	3011.67 3260.00 3081.67	3117.78 ± 128.04[bc]
	5	3285.00 3286.67 3163.33	3245.00 ± 70.73[c]

Table 4.1b: Concentration and mean ± standard deviation of pineapple peel-treated pumpkin seed

Temperature (°C)	Incubation time (hrs)	The concentration of pineapple peel-treated pumpkin seed (µg/mL)	Mean ± Standard deviation for the concentration of pineapple peel -treated pumpkin seed
30	0	2676.67 2655.00 2650.00	2660.56 ± 14.18[a]
	1	2903.33 2916.67 2981.67	2933.89 ± 41.91[b]
	3	3075.00 3100.00 3126.67	3052.22 ± 25.84[c]
	5	2960.00 2878.33 2990.00	2942.78 ± 57.79[b]
45	0	2775.00 2738.33 2736.67	2750.00 ± 21.67[a]
	1	2935.00 3113.333 2833.333	2960.55 ± 141.74[ab]
	3	2975.00 3138.33 3186.67	3029.44 ± 110.92[b]
	5	3178.33 3210.00 3201.67	3196.67 ± 16.42[b]
60	0	2470.00 2491.67 2515.00	2492.22 ± 22.51[a]
	1	3210.00 3093.33 3190.00	3164.44 ± 62.39[c]
	3	3115.00 3058.33 2925.00	3032.78 ± 97.54[bc]
	5	3015.00 2920.00 2966.67	2967.22 ± 47.50[b]

Table 4.1c: Concentration and mean ± standard deviation of bromelain-treated pumpkin seed

Temperature (°C)	Incubation time (hrs)	The concentration of bromelain-treated pumpkin seed (µg/mL)	Mean ± Standard deviation for the concentration of bromelain-treated pumpkin seed
30	0	2583.33 2755.00 2650.00	2662.78 ± 86.55[a]
	1	2853.33 2838.33 2923.33	2871.66 ± 45.37[b]
	3	3131.67 3110.00 3141.67	3127.78 ± 16.19[c]
	5	3215.00 3325.00 3240.00	3260.00 ± 57.66[c]
45	0	2771.67 2716.67 2888.33	2792.22 ± 87.66[a]
	1	2843.33 3140.00 2910.00	2964.44 ± 155.65[ab]
	3	3090.00 2951.67 3146.67	3062.78 ± 100.31[ab]
	5	3173.33 3120.00 3170.00	3154.44 ± 29.88[b]
60	0	2546.67 2446.67 2416.67	2470.00 ± 68.07[a]
	1	2931.67 2936.67 2921.67	2930.00 ± 7.64[b]
	3	3005.00 3066.67 3108.33	3060.00 ± 51.99[c]
	5	3251.67 3233.33 3240.00	3241.67 ± 9.28[d]

Table 4.1d: Concentration and mean ± standard deviation of date seed-treated pumpkin seed

Temperature (°C)	Incubation time (hrs)	The concentration of date seed-treated pumpkin seed (µg/mL)	Mean ± Standard deviation for the concentration of date seed -treated pumpkin seed
30	0	2620.00 2463.33 2405.00	2496.11 ± 111.19[a]
	1	2931.67 2916.67 2913.33	2920.56 ± 9.77[b]
	3	3091.67 3160.00 3171.67	3141.11 ± 43.21[b]
	5	2895.00 2985.00 2985.00	2955.00 ± 51.96[c]
45	0	2501.67 2458.33 2475.00	2478.33 ± 21.86[a]
	1	2968.33 3141.67 3050.00	3053.33 ± 86.72[b]
	3	3096.67 2990.00 2991.67	3026.11 ± 61.11[b]
	5	2971.67 2968.33 3035.00	2991.67 ± 37.56[b]
60	0	2321.67 2371.67 2328.33	2340.56 ± 27.15[a]
	1	3135.00 3011.67 3018.33	3055.00 ± 69.36[b]
	3	2968.33 3091.67 3091.67	3050.56 ± 71.21[b]
	5	3246.67 3251.67 3211.67	3236.67 ± 21.79[c]

Table 4.2: Percentage of protein hydrolysis and mean ± standard deviation of four enzyme-treated pumpkin seeds at hours 5

Temperature (°C)	Enzyme Treatment	Percentage of protein hydrolysis (%)	Mean ± Standard deviation for the concentration of enzyme-treated pumpkin seed
30	UT	6	2738.89 ± 126.08[a]
	CP	8	3050.55 ± 53.41[b]
	PP	8	2942.78 ± 57.79[b]
	BR	19	3260.00 ± 57.66[c]
	DS	8	2955.00 ± 51.96[c]
45	UT	5	2966.67 ± 67.10[b]
	CP	10	2965.00 ± 29.27[b]
	PP	10	3196.67 ± 16.42[b]
	BR	8	3154.44 ± 29.88[b]
	DS	3	2991.67 ± 37.5[b]
60	UT	23	3050.55 ± 7.52[a]
	CP	28	3245.00 ± 70.73[c]
	PP	33	2967.22 ± 47.50[b]
	BR	42	3241.67 ± 9.28[d]
	DS	42	3236.67 ± 21.79[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 or below the ($p<0.05$) in each enzyme-treated. Based on figure 4.1, at 30°C and 60°C, both shows a significant difference at p=0.00 ($p<0.05$) while at 45°C shows the p-value is p=0.025 in commercial protease, p=0.014 in bromelain and p=0.02 in date seed.

Based on Figure 4.2, at 60°C the highest percentage of protein hydrolysis is at hours 5, which in bromelain (BR), 42%, and date seed (DS) 42%. Between these two results show the reading is comparable. In an ANOVA on Tukey HSD, for 60°C, there is a significant difference between the percentage of protein availability at hours 5 with mean ± standard deviation 3241.67 ± 9.28^{d} and 3236.67 ± 21.79^{c} respectively as both incubation times are not in the same group of homogeneous subsets which is c and d.

In terms of temperature, there is no significant difference between all groups of temperatures with p=0.00 ($p<0.05$). Based on Table 4.2, at hours 5, the temperature of 60°C has the highest percentage of protein hydrolysis than 30°C, and 45°C which are 28% for commercial protease, 33% for pineapple peel, 42% for bromelain, and date seed respectively.

With this situation, the enzyme employed in this study might well be a suitable option for an industrial process that requires the enzyme to be able to hydrolyze proteins throughout a temperature range of 25–80°C. The enzyme preserved more than 40% of its maximal activity at temperatures ranging from 55 to 80°C. Because the enzyme was stable enough to be utilized at 70°C, the enzyme employed in this study was more thermotolerant and can be used in the industrial process, which is done at quite high temperatures. This reflects that the enzyme has outstanding heat-stable characteristics,

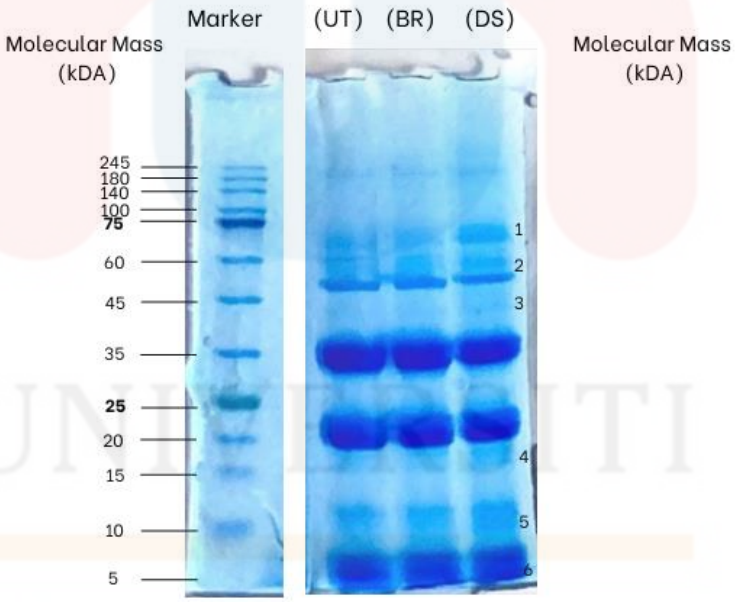
whereas most enzymes are destroyed or denatured when heated. According to (Ketnawa et al.,2012 and Koh et al.,2006), the optimal temperature for bromelain activity is 60°C.

In terms of thermostability between pineapple peel and commercial protease, at 60°C reaches its maximum protein hydrolysis at hours 5, 33% and 28%. However, the percentage of these enzymes could not be calculated because bromelain and date seed showed a higher percentage and caused them to be appropriately selected to perform SDS-PAGE.

4.2 SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A high percentage of protein hydrolysis was collected further in electrophoresis to analyze its molecular weight protein. The sample from bromelain and date seed at 5 hours of 60°C shows a high percentage in protein hydrolysis which both are 42%, and the untreated sample shows a percentage of 23%. Figure 4.2 shows the electrophoretic patterns of the high percentage of protein samples.

Figure 4.3: SDS-PAGE of pumpkin seed using a high percentage of protein hydrolysis of bromelain and date seed at 5 hours of incubation time at a temperature of 60°C.



As can be seen, the protein molecular weight indicators indicate a range of molecular mass from high to low. The SDS-PAGE results demonstrate that the band was present in the bromelain and date seed samples. Bands that consistently appear after electrophoresis of a specific sample are almost certainly typical of the polypeptides that describe the sample. Bands that show seldom and very faint, on the other hand, may

reflect essential polypeptides or something else The lower molecular weight components of the band did occur in the date seed sample with molecular weights ranging from 10 to 5 kDA. The main band did appear at 75 kDA and in the middle at 60 kDA. Finally, the band emerged between 20 and 15 kDA.

Bromelain shows in the SDS-PAGE identified a protein with main bands ranging from 10 to 5 kDa. Minor bands of higher and lower molecular weight components were also detected, but very marginally. When compared to the untreated sample as the control sample, the mobility of the bromelain sample does not display as much of the appearance of the band. The band in the control sample was likewise resolved into three large bands at 35 kDA, 25 kDA, and 10 – 5 kDA, as well as a few minor bands in the 75 – 45 kDA range.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

As a conclusion, the outcome of the preliminary study had shown some findings of the effects of different temperatures and incubation times towards the protein hydrolysis by treating pumpkin seed with different enzymes.

The percentage of protein hydrolysis of enzyme-treated pumpkin seed before and after treatment had been analyzed as it is shown that after 5 hours at 60°C of bromelain and date seed-treated pumpkin seed had the highest percentage of protein hydrolysis which is 42% compared to the other incubation times and temperatures as well as date seed and bromelain are better than the commercial protease and pineapple peels as they hydrolyze protein faster. The high percentage of the treatment has proceeded to do the SDS-PAGE and concluded the date seed sample shows the highest band appeared.

Statistical analysis by using the Tukey Post Hoc Test in Factorial in CRD of ANOVA has been carried out successfully by confirming the differences do not occur between groups of treatments. There is a significant difference between the temperatures; 30°C, 45 °C, and 60°C in which $p=0.00$, and below than the ($p<0.05$). At that three temperatures do not have any value that is more than the p-value, so this shows that this study shows only a significant difference among the groups of treatments.

5.2 Recommendations

A future study might involve a 42-day feeding trial to record broiler chicken growth performance, feed conversion rate, survival rate, and mortality rate by designing feed formulation and substituting protein sources with enzyme-treated pumpkin seed for production reasons. This study's results also demonstrate an excellent performance that may be used to do a feeding experiment, which had slightly better outcomes with the high percentage of protein analysis. The demand for protein replacement from soybeans or maize may be met by pumpkin seeds, which are a good plentiful seed that is suited for future research.

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APPENDIX A

Untreated Protein Sample 30°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
30 (UT)	0	2738.8900	126.07754	3
	1	2918.8900	44.39027	3
	3	3073.8900	78.92177	3
	5	3116.1133	39.38121	3
	Total	2961.9458	169.22594	12
Total	0	2738.8900	126.07754	3
	1	2918.8900	44.39027	3
	3	3073.8900	78.92177	3
	5	3116.1133	39.38121	3
	Total	2961.9458	169.22594	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	263720.472[a]	3	87906.824	13.711	.002	.837
Intercept	105277477.435	1	105277477.435	16420.377	.000	1.000
temperature	.000	0	.	.	.	.000
time	263720.472	3	87906.824	13.711	.002	.837
temperature * time	.000	0	.	.	.	.000
Error	51291.137	8	6411.392			
Total	105592489.045	12				
Corrected Total	315011.609	11				

a. R Squared = .837 (Adjusted R Squared = .776)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2738.8900	
1	3	2918.8900	2918.8900
3	3		3073.8900
5	3		3116.1133
Sig.		.094	.065

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6411.392.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3a: Homogeneous subsets by Tukey HSD

Commercial Protease at 30°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
30 (CP)	0	2587.2233	81.14073	3
	1	2943.3333	63.53048	3
	3	3090.0000	16.91268	3
	5	2965.0000	53.41431	3
	Total	2896.3892	201.68782	12
Total	0	2587.2233	81.14073	3
	1	2943.3333	63.53048	3
	3	3090.0000	16.91268	3
	5	2965.0000	53.41431	3
	Total	2896.3892	201.68782	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	419939.606[a]	3	139979.869	40.695	.000	.939
Intercept	100668842.457	1	100668842.457	29266.179	.000	1.000
temperature	.000	0	.	.	.	.000
time	419939.606	3	139979.869	40.695	.000	.939
temperature * time	.000	0	.	.	.	.000
Error	27518.137	8	3439.767			
Total	101116300.200	12				
Corrected Total	447457.743	11				

a. R Squared = .939 (Adjusted R Squared = .915)

Figure A.2b: Significant difference between all incubation times of commercial protease at 30°C

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2587.2233	
1	3		2943.3333
5	3		2965.0000
3	3		3090.0000
Sig.		1.000	.061

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3439.767.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3b: Homogeneous subsets by Tukey HSD

Pineapple peel at 30°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
30 (PP)	0	2660.5567	14.17673	3
	1	2933.8900	41.91283	3
	3	3100.5567	25.83950	3
	5	2942.7767	57.79299	3
	Total	2909.4450	168.54016	12
Total	0	2660.5567	14.17673	3
	1	2933.8900	41.91283	3
	3	3100.5567	25.83950	3
	5	2942.7767	57.79299	3
	Total	2909.4450	168.54016	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	300532.889[a]	3	100177.630	67.173	.000	.962
Intercept	101578442.496	1	101578442.496	68112.035	.000	1.000
temperature	.000	0	.	.	.	.000
time	300532.889	3	100177.630	67.173	.000	.962
temperature * time	.000	0	.	.	.	.000
Error	11930.748	8	1491.344			
Total	101890906.133	12				
Corrected Total	312463.637	11				

a. R Squared = .962 (Adjusted R Squared = .947)

Figure A.2c: Significant difference between all incubation times of pineapple peel at 30°C

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2	Subset 3
0	3	2660.5567		
1	3		2933.8900	
5	3		2942.7767	
3	3			3100.5567
Sig.		1.000	.992	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1491.344.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3c: Homogeneous subsets by Tukey HSD

Bromelain at 30°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
30	0	2662.7767	86.54525	3
	1	2871.6633	45.36886	3
	3	3127.7800	16.18939	3
	5	3260.0000	57.66281	3
	Total	2980.5550	245.70613	12
Total	0	2662.7767	86.54525	3
	1	2871.6633	45.36886	3
	3	3127.7800	16.18939	3
	5	3260.0000	57.66281	3
	Total	2980.5550	245.70613	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	637815.519[a]	3	212605.173	64.742	.000	.960
Intercept	106604497.296	1	106604497.296	32462.996	.000	1.000
temperature	.000	0	.	.	.	.000
time	637815.519	3	212605.173	64.742	.000	.960
temperature * time	.000	0	.	.	.	.000
Error	26271.019	8	3283.877			
Total	107268583.833	12				
Corrected Total	664086.537	11				

a. R Squared = .960 (Adjusted R Squared = .946)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	2662.7767		
1	3		2871.6633	
3	3			3127.7800
5	3			3260.0000
Sig.		1.000	1.000	.085

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3283.877.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3d: Homogeneous subsets by Tukey HSD

Date Seed at 30°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
30 (DS)	0	2496.1100	111.18519	3
	1	2920.5567	9.76824	3
	3	3141.1133	43.21492	3
	5	2955.0000	51.96152	3
	Total	2878.1950	252.70819	12
Total	0	2496.1100	111.18519	3
	1	2920.5567	9.76824	3
	3	3141.1133	43.21492	3
	5	2955.0000	51.96152	3
	Total	2878.1950	252.70819	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	668425.548[a]	3	222808.516	52.348	.000	.952
Intercept	99408077.496	1	99408077.496	23355.660	.000	1.000
temperature	.000	0	.	.	.	.000
time	668425.548	3	222808.516	52.348	.000	.952
temperature * time	.000	0	.	.	.	.000
Error	34050.189	8	4256.274			
Total	100110553.233	12				
Corrected Total	702475.737	11				

a. R Squared = .952 (Adjusted R Squared = .933)

Figure A.2e: Significant difference between all incubation times of date seed at 30°C

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	2496.1100		
1	3		2920.5567	
5	3		2955.0000	
3	3			3141.1133
Sig.		1.000	.914	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4256.274.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3e: Homogeneous subsets by Tukey HSD

Untreated at 45°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
45 (UT)	0	2917.7767	45.16447	3
	1	2731.1133	42.82849	3
	3	2812.2200	96.58440	3
	5	2966.6667	67.10382	3
	Total	2856.9442	111.24541	12
Total	0	2917.7767	45.16447	3
	1	2731.1133	42.82849	3
	3	2812.2200	96.58440	3
	5	2966.6667	67.10382	3
	Total	2856.9442	111.24541	12

Figure A.4a: Descriptive statistics with mean and standard deviation for incubation times at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	100719.809[a]	3	33573.270	7.585	.010	.740
Intercept	97945559.657	1	97945559.657	22127.617	.000	1.000
temperature	.000	0	.	.	.	.000
time	100719.809	3	33573.270	7.585	.010	.740
temperature * time	.000	0	.	.	.	.000
Error	35411.156	8	4426.394			
Total	98081690.622	12				
Corrected Total	136130.965	11				

a. R Squared = .740 (Adjusted R Squared = .642)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
1	3	2731.1133	
3	3	2812.2200	2812.2200
0	3		2917.7767
5	3		2966.6667
Sig.		.484	.083

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4426.394.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6a: Homogeneous subsets by Tukey HSD at 45°C

Commercial Protease at 45°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
45 (CP)	0	2933.3333	98.50570	3
	1	2982.2200	161.43635	3
	3	2935.0000	68.39583	3
	5	3218.8900	29.26919	3
	Total	3017.3608	150.65853	12
Total	0	2933.3333	98.50570	3
	1	2982.2200	161.43635	3
	3	2935.0000	68.39583	3
	5	3218.8900	29.26919	3
	Total	3017.3608	150.65853	12

Figure A.4b: Descriptive statistics with mean and standard deviation for incubation times at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	167078.432[a]	3	55692.811	5.394	.025	.669
Intercept	109253596.782	1	109253596.782	10581.528	.000	.999
temperature	.000	0	.	.	.	.000
time	167078.432	3	55692.811	5.394	.025	.669
temperature * time	.000	0	.	.	.	.000
Error	82599.485	8	10324.936			
Total	109503274.700	12				
Corrected Total	249677.918	11				

a. R Squared = .669 (Adjusted R Squared = .545)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2933.3333	
3	3	2935.0000	
1	3	2982.2200	2982.2200
5	3		3218.8900
Sig.		.933	.082

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10324.936.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6b: Homogeneous subsets by Tukey HSD at 45°C

Pineapple Peel at 45°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
45 (PP)	0	2750.0000	21.66654	3
	1	2960.5533	141.73824	3
	3	3100.0000	110.91861	3
	5	3196.6667	16.41713	3
	Total	3001.8050	191.74453	12
Total	0	2750.0000	21.66654	3
	1	2960.5533	141.73824	3
	3	3100.0000	110.91861	3
	5	3196.6667	16.41713	3
	Total	3001.8050	191.74453	12

Figure A.4c: Descriptive statistics with mean and standard deviation for incubation times at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	338162.356[a]	3	112720.785	13.609	.002	.836
Intercept	108129999.096	1	108129999.096	13054.595	.000	.999
temperature	.000	0	.	.	.	.000
time	338162.356	3	112720.785	13.609	.002	.836
temperature * time	.000	0	.	.	.	.000
Error	66263.259	8	8282.907			
Total	108534424.711	12				
Corrected Total	404425.615	11				

a. R Squared = .836 (Adjusted R Squared = .775)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2750.0000	
1	3	2960.5533	2960.5533
3	3		3100.0000
5	3		3196.6667
Sig.		.084	.052

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 8282.907.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6c: Homogeneous subsets by Tukey HSD at 45°C

Bromelain at 45°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
45 (BR)	0	2792.2233	87.65625	3
	1	2964.4433	155.64810	3
	3	3062.7800	100.30925	3
	5	3154.4433	29.87523	3
	Total	2993.4725	165.66835	12
Total	0	2792.2233	87.65625	3
	1	2964.4433	155.64810	3
	3	3062.7800	100.30925	3
	5	3154.4433	29.87523	3
	Total	2993.4725	165.66835	12

Figure A.4d: Descriptive statistics with mean and standard deviation for incubation times at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	216177.175[a]	3	72059.058	6.724	.014	.716
Intercept	107530531.299	1	107530531.299	10034.478	.000	.999
temperature	.000	0	.	.	.	.000
time	216177.175	3	72059.058	6.724	.014	.716
temperature * time	.000	0	.	.	.	.000
Error	85728.848	8	10716.106			
Total	107832437.322	12				
Corrected Total	301906.023	11				

a. R Squared = .716 (Adjusted R Squared = .610)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2792.2233	
1	3	2964.4433	2964.4433
3	3	3062.7800	3062.7800
5	3		3154.4433
Sig.		.050	.190

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10716.106.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6d: Homogeneous subsets by Tukey HSD at 45°C

Date Seed at 45°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
45(DS)	0	2478.3333	21.86143	3
	1	3053.3333	86.71806	3
	3	3026.1133	61.10957	3
	5	2991.6667	37.56491	3
	Total	2887.3617	252.48472	12
Total	0	2478.3333	21.86143	3
	1	3053.3333	86.71806	3
	3	3026.1133	61.10957	3
	5	2991.6667	37.56491	3
	Total	2887.3617	252.48472	12

Figure A.4e: Descriptive statistics with mean and standard deviation for incubation times at 45°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	674946.989[a]	3	224982.330	68.470	.000	.963
Intercept	100042288.730	1	100042288.730	30446.288	.000	1.000
temperature	.000	0	.	.	.	.000
time	674946.989	3	224982.330	68.470	.000	.963
temperature * time	.000	0	.	.	.	.000
Error	26286.893	8	3285.862			
Total	100743522.611	12				
Corrected Total	701233.882	11				

a. R Squared = .963 (Adjusted R Squared = .948)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2478.3333	
5	3		2991.6667
3	3		3026.1133
1	3		3053.3333
Sig.		1.000	.578

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 3285.862.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6e: Homogeneous subsets by Tukey HSD at 45°C

Untreated at 60°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
60(UT)	0	2285.0000	129.91454	3
	1	2890.5533	41.31077	3
	3	2905.5533	79.51779	3
	5	3050.5533	7.51529	3
	Total	2782.9150	314.56395	12
Total	0	2285.0000	129.91454	3
	1	2890.5533	41.31077	3
	3	2905.5533	79.51779	3
	5	3050.5533	7.51529	3
	Total	2782.9150	314.56395	12

Figure A.7a: Descriptive statistics with mean and standard deviation for incubation times at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1038527.389[a]	3	346175.796	55.468	.000	.954
Intercept	92935390.767	1	92935390.767	14891.149	.000	.999
temperature	.000	0	.	.	.	.000
time	1038527.389	3	346175.796	55.468	.000	.954
temperature * time	.000	0	.	.	.	.000
Error	49927.856	8	6240.982			
Total	94023846.011	12				
Corrected Total	1088455.244	11				

a. R Squared = .954 (Adjusted R Squared = .937)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	Subset 2
0	3	2285.0000	
1	3		2890.5533
3	3		2905.5533
5	3		3050.5533
Sig.		1.000	.138

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6240.982.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9a: Homogeneous subsets by Tukey HSD at 60°C

Commercial Protease at 60°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
60(CP)	0	2667.7767	29.26396	3
	1	2977.2233	60.14830	3
	3	3117.7800	128.04256	3
	5	3245.0000	70.73322	3
	Total	3001.9450	234.72690	12
Total	0	2667.7767	29.26396	3
	1	2977.2233	60.14830	3
	3	3117.7800	128.04256	3
	5	3245.0000	70.73322	3
	Total	3001.9450	234.72690	12

Figure A.7b: Descriptive statistics with mean and standard deviation for incubation times at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	554319.348[a]	3	184773.116	28.567	.000	.915
Intercept	108140085.396	1	108140085.396	16719.063	.000	1.000
temperature	.000	0	.	.	.	.000
time	554319.348	3	184773.116	28.567	.000	.915
temperature * time	.000	0	.	.	.	.000
Error	51744.567	8	6468.071			
Total	108746149.311	12				
Corrected Total	606063.915	11				

a. R Squared = .915 (Adjusted R Squared = .883)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	2667.7767		
1	3		2977.2233	
3	3		3117.7800	3117.7800
5	3			3245.0000
Sig.		1.000	.220	.286

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6468.071.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9b: Homogeneous subsets by Tukey HSD at 60°C

Pineapple Peel at 60°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
60(PP)	0	2492.2233	22.50510	3
	1	3164.4433	62.39254	3
	3	3032.7767	97.54348	3
	5	2967.2233	47.50242	3
	Total	2914.1667	270.52387	12
Total	0	2492.2233	22.50510	3
	1	3164.4433	62.39254	3
	3	3032.7767	97.54348	3
	5	2967.2233	47.50242	3
	Total	2914.1667	270.52387	12

Figure A.7c: Descriptive statistics with mean and standard deviation for incubation times at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	772673.785[a]	3	257557.928	63.710	.000	.960
Intercept	101908408.333	1	101908408.333	25208.445	.000	1.000
temperature	.000	0	.	.	.	.000
time	772673.785	3	257557.928	63.710	.000	.960
temperature * time	.000	0	.	.	.	.000
Error	32341.037	8	4042.630			
Total	102713423.156	12				
Corrected Total	805014.822	11				

a. R Squared = .960 (Adjusted R Squared = .945)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	2492.2233		
5	3		2967.2233	
3	3		3032.7767	3032.7767
1	3			3164.4433
Sig.		1.000	.609	.128

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 4042.630.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9c: Homogeneous subsets by Tukey HSD at 60°C

Bromelain at 60°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
60(BR)	0	2470.0033	68.06859	3
	1	2930.0033	7.63763	3
	3	3060.0000	51.98691	3
	5	3241.6667	9.28290	3
	Total	2925.4183	300.24357	12
Total	0	2470.0033	68.06859	3
	1	2930.0033	7.63763	3
	3	3060.0000	51.98691	3
	5	3241.6667	9.28290	3
	Total	2925.4183	300.24357	12

Figure A.7d: Descriptive statistics with mean and standard deviation for incubation times at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	976647.233[a]	3	325549.078	174.079	.000	.985
Intercept	102696869.100	1	102696869.100	54914.604	.000	1.000
temperature	.000	0	.	.	.	.000
time	976647.233	3	325549.078	174.079	.000	.985
temperature * time	.000	0	.	.	.	.000
Error	14960.956	8	1870.119			
Total	103688477.289	12				
Corrected Total	991608.189	11				

a. R Squared = .985 (Adjusted R Squared = .979)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3	4
0	3	2470.0033			
1	3		2930.0033		
3	3			3060.0000	
5	3				3241.6667
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 1870.119.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9d: Homogeneous subsets by Tukey HSD at 60°C

Date Seed at 60°C

Descriptive Statistics

Dependent Variable: Concentration of Protein Hydrolysis

Temperature	Incubation Time	Mean	Std. Deviation	N
60(DS)	0	2340.5567	27.14993	3
	1	3055.0000	69.36201	3
	3	3050.5567	71.21038	3
	5	3236.6700	21.79449	3
	Total	2920.6958	361.32282	12
Total	0	2340.5567	27.14993	3
	1	3055.0000	69.36201	3
	3	3050.5567	71.21038	3
	5	3236.6700	21.79449	3
	Total	2920.6958	361.32282	12

Figure A.7e: Descriptive statistics with mean and standard deviation for incubation times at 60°C

Tests of Between-Subjects Effects

Dependent Variable: Concentration of Protein Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1413907.716[a]	3	471302.572	169.929	.000	.985
Intercept	102365569.810	1	102365569.810	36908.025	.000	1.000
temperature	.000	0	.	.	.	.000
time	1413907.716	3	471302.572	169.929	.000	.985
temperature * time	.000	0	.	.	.	.000
Error	22188.252	8	2773.531			
Total	103801665.778	12				
Corrected Total	1436095.968	11				

a. R Squared = .985 (Adjusted R Squared = .979)

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

Concentration of Protein Hydrolysis

Tukey HSD[a,b]

Incubation Time	N	Subset 1	2	3
0	3	2340.5567		
3	3		3050.5567	
1	3		3055.0000	
5	3			3236.6700
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 2773.531.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.9e: Homogeneous subsets by Tukey HSD at 60°C

APPENDIX B

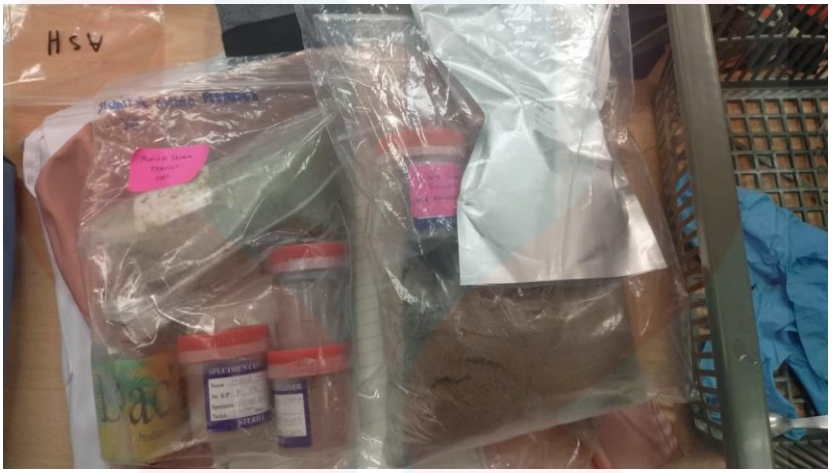

Figure B.1: Type of protein and enzyme used in this study

Figure B.2: Preparation of buffer solution in pH 8 using pH meter

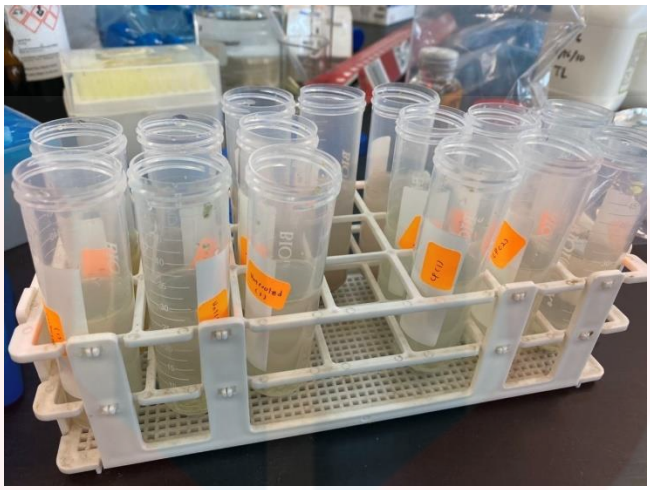

Figure B.3: Buffer solution was added into the falcon tubes filled with enzyme-treated treatment.

Figure B.4: Sample of treatment was put into the incubator shaker within the incubation time that had set for the experiment.

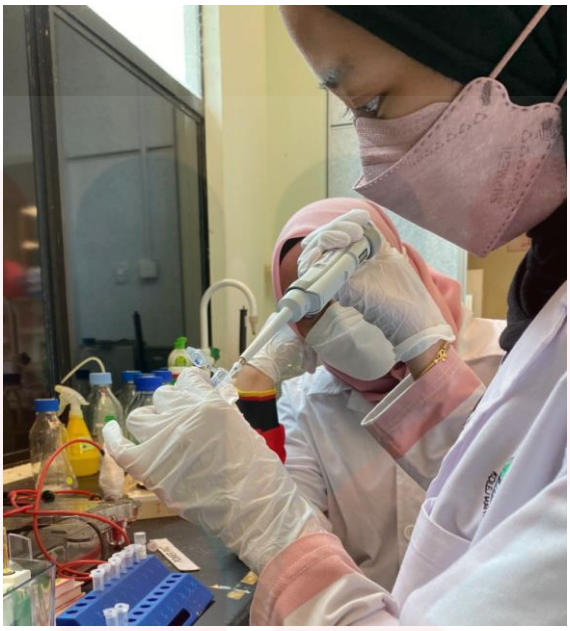

Figure B.5: Sample was transferred into the micro centrifuge tube every 1 hour, and 2 hours and then was put into microcentrifuge to get the supernatant.

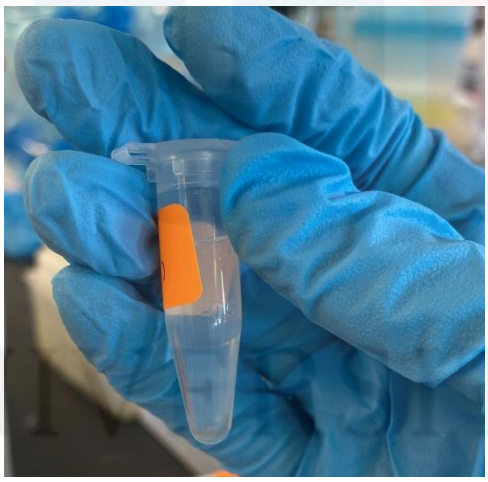

Figure B.6: The supernatant from the sample after 5 minutes of centrifuge at 4000rpm

Figure B.7: Bradford reagent was being pipetting into the microcentrifuge tubes.

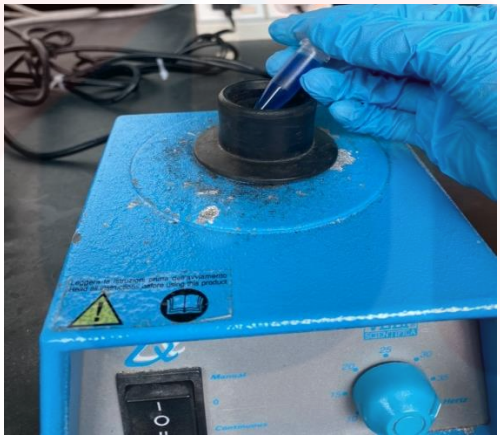

Figure B.8: Sample was being vortexed for 30 seconds to mix the solution well

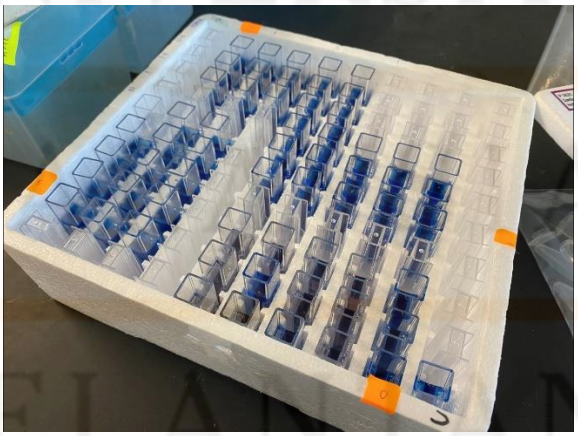

Figure B.10: The absorbance reading of sample was collected using UV-Spectrophometer

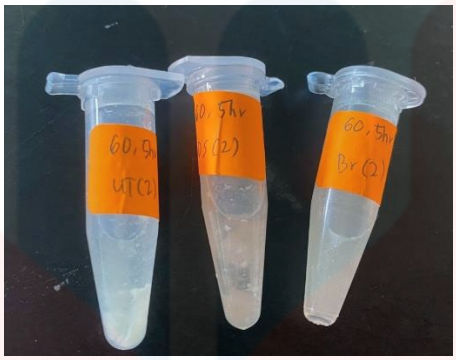

Figure B.11: High percentage of protein sample was proceeded to do the SDS-PAGE

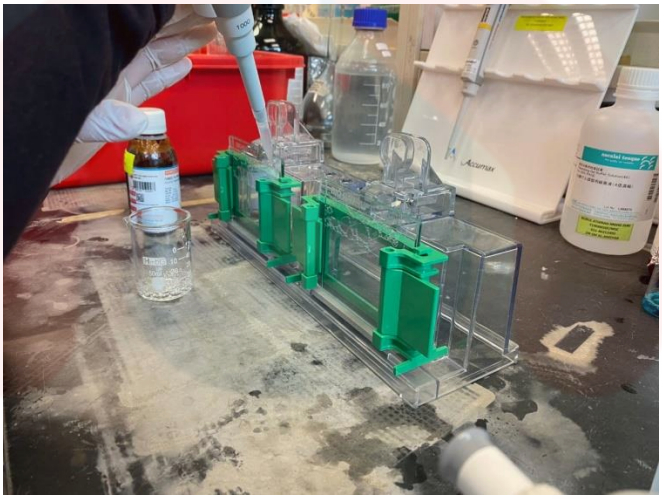

Figure B.12: The 12% of resolving gel was pipetted into the spacer

Figure B.13: The resolving gel has completely polymerized after 1 hours

Figure B.14: The stacking gel and resolving gel with the comb has completely polymerizedv

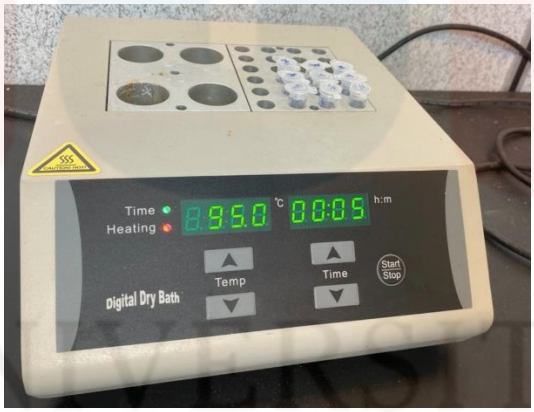

Figure B.15: The protein sample was prepared in the dry bath and then centrifuge for 5 minutes.

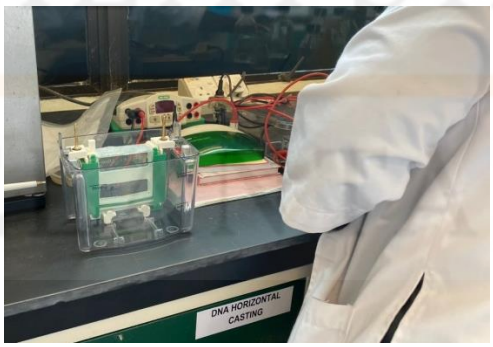

Figure B.16: The electrophoresis has been connected to power supply

Figure B.17: Protein sample was inserted into the well

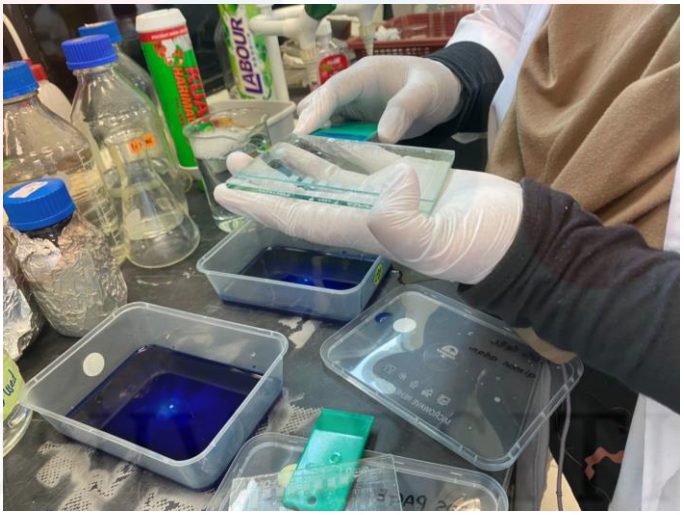

Figure B.18: After 2 hours, the gel was separated from the glass spacer and put into the staining container

Figure B.19: The gel has been placed in electronic rocking for over night

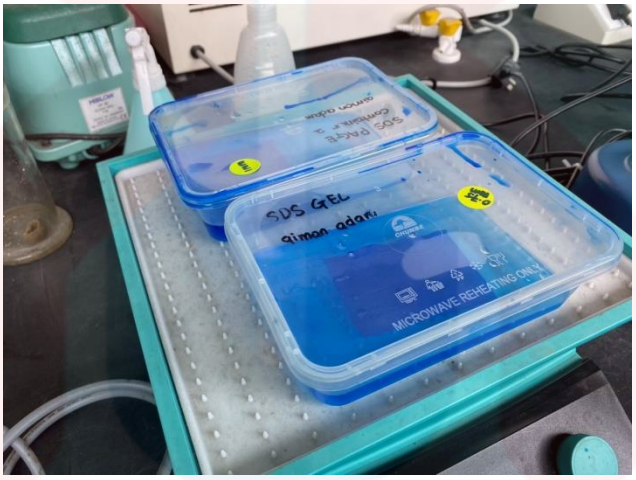

Figure B.20: After overnight, de-staining was placed and let they rocking for 10 minutes and then placed the gel into the zip lock.

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