Protein Hydrolysis of Black Soldier Fly Larvae (BSFL) by Using Enzyme and Fruit Waste Treatment

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DECLARATION

I hereby declare that this work is the result of my own work except for excerpts and summaries, each of which I have explained the source.

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Protein Hydrolysis of Black Soldier Fly Larvae (BSFL) by Using Enzyme and Fruit Waste Treatment

ABSTRACT

Black soldier fly larvae (BSFL), *Hermetia Illucens L*., thrive on organic waste, reducing environmental pollution, and converting waste material into insect-rich protein and fat. Thus, poultry feed formulations may help increase this insect species and make it one of the most capable for commercial production of BSFL's protein content. Furthermore, amino acid profiles 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 might be performed enzymatically to enhance broiler chicken digestibility and growth performance. In this work, multiple kinds of enzymes were utilized to hydrolyze protein in BSFL, including commercial protease and three types of fruit waste isolated from pineapple peel, bromelain, and date seed. The BSFL meal was treated with enzymes before the percentage of protein hydrolysis was determined. Three temperatures (30°C, 45°C, and 60°C) and four incubation times (0 hour, 1 hour, 3 hours, and 5 hours) were used to evaluate enzyme extracted from commercial protease, pineapple peel, bromelain, and date seed. The result reveals that pineapple peel (34%) has the highest percentage of protein reduction, followed by date seed (31%), bromelain (30%), and commercial protease (27%). The selected enzyme-treated BSFL was analysed using SDS-PAGE to prove this hydrolysis. This research demonstrates the ability of certain fruit waste to hydrolyze BSFL protein into smaller molecules. This discovery provides an opportunity for treated BSFL to become a low-cost and high-digestibility protein source for animal feed in the future.

Keywords: Black Soldier Fly Larvae, protein hydrolysis, broiler chicken, SDS-PAGE

Protein Hidrolisis Larva Lalat Askar Hitam (BSFL) dengan Menggunakan Rawatan Enzim dan Sisa Buahan

ABSTRAK

Larva lalat askar hitam (BSFL), Hermetia Illucens L., hidup subur pada sisa organik, mengurangkan pencemaran alam sekitar, dan menukar bahan buangan kepada protein dan lemak yang kaya dengan serangga. Oleh itu, formulasi makanan ayam boleh membantu meningkatkan spesies serangga ini dan menjadikannya salah satu yang paling mampu untuk pengeluaran komersial kandungan protein BSFL. Tambahan pula, 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 mungkin dilakukan secara enzimatik untuk meningkatkan penghadaman ayam pedaging dan prestasi pertumbuhan. Dalam kerja ini, pelbagai jenis enzim telah digunakan untuk menghidrolisis protein dalam BSFL, termasuk protease komersial dan tiga jenis sisa buah yang diasingkan daripada kulit nanas, bromelain, dan biji kurma. Hidangan BSFL telah dirawat dengan enzim sebelum peratusan hidrolisis protein ditentukan. Tiga suhu (30°C, 45°C, dan 60°C) dan empat masa pengeraman (0 jam, 1 jam, 3 jam dan 5 jam) digunakan untuk menilai enzim yang diekstrak daripada protease komersial, kulit nanas, bromelain, dan biji kurma. Hasilnya menunjukkan bahawa kulit nanas (34%) mempunyai peratusan pengurangan protein tertinggi, diikuti oleh biji kurma (31%), bromelain (30%), dan protease komersial (27%). BSFL yang dirawat enzim terpilih telah dianalisis menggunakan SDS-PAGE untuk membuktikan hidrolisis ini. Penyelidikan ini menunjukkan keupayaan sisa buah tertentu untuk menghidrolisis protein BSFL kepada molekul yang lebih kecil. Penemuan ini memberi peluang kepada BSFL yang dirawat untuk menjadi sumber protein kos rendah dan kebolehcernaan tinggi untuk makanan haiwan pada masa hadapan.

Kata kunci: Larva Lalat Askar Hitam, hidrolisis protein, ayam pedaging, SDS-PAGE

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LIST OF ABBREVIATIONS

BSFL	Black Soldier Fly Larvae
UV-Vis Spectrophotometer	Ultra-Violet Visible Spectrophotometer
ANOVA	Analysis of Variance
CRD	Customized Randomly Design
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
APS	Ammonium persulfate
TEMED	Tetramethyl ethylenediamine
BSA	Bovine Serum Albumin

LIST OF SYMBOLS

G	Gram
Kg	Kilogram
µL	Microlitre
mL	Millilitre
L	Litre
mg/mL	Milligram per millilitre
℃	Degree Celsius
M	Molar
µg/mL	Microgram per millilitre
hrs	Hour
mins	Minute
nm	Nanometre
%	Percent
&	And

CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to the United States Census Bureau, today's world population is growing by 74 million people, bringing the total world population in 2021 to 7.8 billion people, and the global population is expected to increase until 2100. By 2050, global food demand is predicted to grow by 70% to fulfill the needs of the 9.8 billion people who are expected to inhabit the planet by that time (Shumo et al., 2019). Cereal and meat output are believed to expand from 2.1 billion and 258 million tonnes per year between 2005 and 2007 to 3.0 billion and 455 million tonnes, respectively, by the middle of the century, enhancing global issues about food security (Shumo et al., 2019). Food production is straining to keep up with the needs of growing human populations, urbanization, changing dietary patterns, feed prices, and religious preferences. Feed expenses will increase for the livestock industry to thrive and fulfill expanding demand.

In Malaysia, it is made up of various ethnic groups, including Muslims and non-Muslims. Thus, certain religions, such as Hinduism forbid their adherents from consuming beef, whereas Islam forbids them from consuming pork. Thus, the demand for broiler chicken is growing since adherents of all religions can eat it. According to Feed Act 2020, any substance or mixture of substances, such as antioxidants, flavorings, carbohydrates, enzymes, mineral, and non-protein nitrogen products or proteins or vitamins that are used for livestock consumption to meet their nutritional needs and prevent or correct nutritional deficiencies, is defined as feed.

The poultry industry plays a significant role in enhancing consumers' worldwide food security status. Commercial livestock operations and smallholders are developing, expanding the need for poultry feed. Soybeans and fish meal have typically been essential sources of protein in livestock feeds, but with the rise of the poultry sector and the world's population, they have failed to fulfill the rising demand. The world's growing need for protein and lipids cannot be supplied by the current level of agricultural land utilization. As an alternative for soy and fish meals, insects' protein might be valuable.

Black soldier fly larvae (BSFL), *Hermetia illucens*, is known as one of the insects that rich source of protein and lipid contents. BSFL is typically rich in protein and lipids, with a protein content ranging from 30 to 53 g/100 g dry matter, while a lipid content ranging from 20 to 41 g/100 g dry matter, and chitin ranging from 2 to 9 g/100 g, which functions mainly as fiber in the human body (Bukkens, 1997). Insects are more efficient feed converters than pigs and cattle, create fewer ozone-depleting compounds, need less water, and pose a lower risk of zoonotic diseases (Müller et al., 2017). Because BSFL has numerous advantages over many other farmed insect species, they have been extensively studied and cultivated for their ability to convert a variety of "waste" streams into high-

quality proteins, fats, and minerals while showing their potential for efficient and scalable production in the livestock feed sector (Bessa et al., 2020).

Bacterial protease is one of the most potent modern chemicals, accounting for 60% of the total catalytic showcase in the world. Proteases are used in the food industry to improve the palatability, digestibility, and storage life of all accessible protein sources (Razzaq et al., 2019). Protease chemicals are essential in protein processing because they hydrolyze and segregate fewer edible proteins in poultry feed into more useable peptides (Marc, 2013). Efforts to improve dietary protein edibility using a high-quality protease may reduce feed costs by allowing the use of lower-cost crude protein feed ingredients with lower-quality amino acids, resulting in a 10% reduction in the amount of protein and edible amino acids needed from feedstuffs (Marc, 2013).

1.2 Problem Statement

Feed value, natural disintegration, and inadequate nutrient absorption of broiler chicken are some of the common concerns addressed in the poultry sector to keep the poultry industry growing. Protein is mainly derived from soybean and fish meal in common feed. BSFL, which can be utilized as a source of protein, biofuel generation, and waste management, may be used as a half-substitute for soybean meal and a complete substitute for fishmeal to lower the cost of poultry feed production and, as a result, lower the cost of poultry expenses (Mat et al., 2021).

Most chickens are maintained on larger, more specialized farms where insects are not a significant diet component. They are supplied commercial chicken feed, which often

includes protein derived from soybeans or fish (Meredith, 2018). The population is proliferating, making it difficult for the meat industry, notably the poultry industry, to expand as demand grows. This also will increase demand for soybean and fish meal for poultry feed production. Because soybean resources are limited, it must be imported, rising feed costs, while fish meal will cause overfishing in certain zones, leading the earth to deteriorate. As a result, BSFL is an excellent source to include in the feed since it can address the difficulties effectively.

The protein from feed, whether soybeans and fishmeal or BSFL, is not completely processed by the chicken since their stomach-related structure is not entirely functional. The use of BSFL in poultry farms diets as an alternative protein source to soybean and fishmeal requires the implementation. Broilers cannot process approximately a quarter of a feeding regimen due to poisonous hazardous variables in their contents, which may damage stomach-related processes and prohibit good broilers in connection to some of the catalysts needed to break down specific feed segments (Ojha et al., 2019). As a result, it is critical to developing a way to boost BSFL feed protein absorption in broilers by treating it with protease.

1.3 Objectives

1) To treat black soldier fly larvae (*Hermetia illucens sp*.) with commercial protease, pineapple peel powder, bromelain powder, and date seed powder at different temperatures and incubation times.

2) To determine the percentage of protein hydrolysis before and after the protease-treated black soldier fly larvae treatment through Bradford analysis.
3) To estimate the relative molecular weight of the protein sample by performing the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

1.4 Hypothesis

H_0: There is no significant percentage of protein hydrolysis in enzyme-treated black soldier fly larvae before and after treatment by Bradford analysis.

H_a: There is a significant percentage of protein hydrolysis in enzyme-treated black soldier fly larvae before and after the treatment by Bradford analysis.

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

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

1.5 Scope of Study

The purpose of this research is to improve the degree of hydrolysis in protease-treated black soldier fly larvae (*Hermetia illucens sp*.). The percentage of protein hydrolysis in black soldier fly larvae treated with different kinds of fruit waste and enzymes: pineapple peel powder, date seed powder, bromelain powder, and commercial protease will be compared before and after the treatment using Conventional Methodology. Each treatment temperature will be tested at 30°C, 45°C, and 60°C, with four different incubation durations, which are 0 hours, 1 hour, 3 hours, and 5 hours. Protein hydrolysis of protease-treated black soldier fly larvae (BSFL) will be determined using the Bradford assay technique 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 determined by doing the protein availability data that will be evaluated using Factorial in Customized Randomly Design (CRD) Analysis of Variance (ANOVA) and determining the relative molecular mass of protein sample after treatment by performing the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

1.6 Significances of Study

BSFL is rich in protein (40% to 44%). Therefore, it can be used to make high-quality feed. It has a lot of dry matter (35% to 45%), much lysine (6% to 8%), calcium (5% to 8%), and phosphorus (0.6% to 1.5%) in it (Jina et al., 2018). When BSFL meal is used as a feed component in broiler diets, it is regarded as an excellent source of energy and digestible amino acids for broilers (Jina et al., 2018). BSFL is also high in fat, which has a

wide quantitative (15 to 49%) and subjective variation depending on the concoction formulations of their rising substrates (Jina et al., 2018).

Furthermore, due to its excellent palatability, BSFL chitin helps to improve the immune system in animal exoskeletons, and larvae are also recognized as a natural chicken feed. Annually, the black soldier fly, the common housefly, and the yellow mealworm bio-convert around 1.3 billion tonnes of organic waste (Veldkamp, 2012). As a result, it may reduce nutrient waste in broiler dung while also helping to preserve the environment.

Furthermore, raising BSFL helps increase food security by avoiding overfishing by substituting fishmeal with BSFL meal. Overfishing may harm food security in the future, harm marine life, and pollute the environment. The capacity of BSFL and enzyme-treated BSFL in feeds might be identified and extensively exploited in feed later on as a result of this study. The use of chemicals in feed definition may aid in improving the protein absorbability of broiler chicken while eating feed. In contrast, enzyme treatment of BSFL may promote the synthesis of endogenous peptidase, lowering the vitality and amino acid requirements of broiler chicken. Protease chemicals play an essential role in protein absorption by hydrolyzing less solubilized proteins in animal diets and separating them into increasingly useable peptides (Marc, 2016).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Over the coming decades, food security problems will increase due to increased human growth worldwide. The Food and Agriculture Organization of the United Nations (FAO) estimates that nearly 900 million people suffer from chronic hunger due to food security problems. Global food consumption is predicted to grow by 70% by 2050 to fulfill the needs of the 9.7 billion people that would inhabit the planet at that time (Shumo et al., 2019). In the recent past, significant modifications in diets have occurred, preferring animal-based items such as milk, meat, fish, and eggs, and all these preferences are predicted to continue to grow. Economic expansion, combined with substantial migration from rural to urban regions, has expedited significant changes in dietarian patterns and an increased understanding of nutritional requirements.

Livestock is critical to the food system's response to these developing global concerns. Livestock is essential for smallholders as a source of income and a productive asset that reduces labor costs. Additionally, livestock helps with nutrition since animal-

derived food is essential as the primary source of protein for humans. On the other hand, the livestock industry in developing countries first should concentrate on enhancing nutrition and human health by encouraging diet diversity with different animal-sourced foods and decreasing the risk of food safety and zoonotic infections from livestock.

2.2 Global Food Security Index

The Global Food Security Index (GFSI) is a composite statistic that seeks to track country-level progress toward food security. These nations are meant to illustrate the geographical variety, economic prominence, and population size. The GFSI seeks to identify which nations are the most and least susceptible to food insecurity (Izraelov & Silber, 2019). The GFSI's conceptual framework is focused on three components of food security: cost, availability, and quality and safety. It employs a total of 28 measures divided into three domains: cost (6 indicators), availability (11 indications), and quality and safety (11 indicators) (11 indicators). The GFSI focuses on variables that contribute to food security rather than outputs such as dietary intake or population nutritional status (Thomas et al., 2017).

Global livestock and poultry systems are now under investigation due to increased livestock production's anticipated food and environmental system implications to fulfill the rising demand for animal-source diets (ASFs) (Delgado, 2013). Livestock production, which is heavily impacted by cultural preferences, will undoubtedly play a part in attaining this global food security (Devendra, 2001). In general, animal products account for around one-third of worldwide human protein consumption (Steinfeld et al., 2006). The poultry

(chicken) sector in Malaysia is separated into two key productions: broiler production and egg production. In Peninsular Malaysia, there are four grandparent (GP) farms and 22 parent stock (PS) farms for broiler production, as well as five layers of PS firms for egg production (FLFAM,2016). In recent years, the poultry industry has been the most critical contributor to the expansion of the animal industry. Since the 1960s, global chicken production has increased by more than 5% each year (Nabarro and Wannous, 2014). According to these writers, poultry's contribution to global meat production more than quadrupled from 15% in 1960 to 30% in 2000.

2.3 Poultry Feed

Poultry is one of the most advanced livestock industries. Feedstuffs must first be prepared to provide fowl with energy and protein to make poultry products. Malaysia's poultry industry is divided into two groups of producers. It is divided into commercial and traditional farms. Commercial farms operate on a contract farming arrangement with an integrator, while conventional farms are owned and operated by independent entrepreneurs (Ariffin et al., 2014). Natural resources derived from plant components such as corn, soybean, and rice are the primary constituents in poultry feedstuffs due to their high protein content for monogastric animals. However, the feedstuff used is determined by its availability, quality, and total manufacturing cost. Feed ingredients are primarily composed of corn and other substances to give comprehensive nutrition for chicken. In Malaysia, a quest for locally produced chicken feed has begun to offset the high cost of imported feedstuffs, primarily corn and soybeans.

Malaysia is heavily reliant on feed imports, particularly for the non-ruminant (poultry and swine) and aquaculture sectors. The primary imported carbohydrate and protein sources are corn and soya bean meals. The total imports are around RM 2.5 billion per year, with corn contributing to more than half of the total (Zahari & Wong 2009). The industry's reliance on imported feed is not a healthy development since it leads to price swings and excessive production costs. Various measures have been implemented to lower the import bill since feed represents around 70% of the price of livestock production (Zahari & Wong 2009).

Over the last three decades, local researchers have responded about the availability, nutritional value, optimal inclusion levels, and treatment methods that can improve the value of many locally available feed ingredients in poultry and ruminant rations, among other things. More research and development need to be done to make the feed more efficiently. According to Wong (2009), imported soya bean meal (SBM) and fishmeal are widely utilized in Malaysia as protein sources for non-ruminant and aquaculture feeding. SBM and fishmeal are required in 800,000 and 120,000 metric tonnes, respectively. 70% of production costs are devoted to chicken feed since a significant part of basic feed fixings are imported, and feed assets must meet global vitality requirements (Mohd Syauqi N. et al., 2015). According to Neumann, Harris, and Rogers (2002), animal-derived foodstuffs provide not only high-quality and easily digested protein and energy but also an efficient and compact source of immediately accessible micronutrients, which is one of the primary reasons for the growth in demand for poultry feed.

2.4 Black Soldier Fly Larvae (BSFL)

Hermetia illucens sp. often called a Black Soldier Fly, is a fly species in the Stratiomyidae family and the Diptera order. It is a medium-sized insect, measuring 16mm in length, with a primarily black body, blue or green metallic reflections on the thorax, a red abdomen, a broad head with large eyes that are twice the length of the head, a blackleg with white tarsi, and a membranous wing. It originated in the Neotropics but has now spread to some nations, which is also in great demand.

A mature female may lay around 206 to 639 eggs every day (Jeffery et al., 2002). These eggs are often laid in cracks or on surfaces above or near decaying substances like dung or compost and hatch in approximately four days. Black soldier fly larvae are grey-white, segmented, approximately an inch long, and very active. They grow into a dark brown color. They are flattened and torpedo-shaped, with problematic skin. The larvae head is smaller and narrower than the body, and the body has no legs or any distinguishing characteristics other than hairs and spines. The body's back end is blunt and has respiratory pores (spiracles).

Black soldier flies in the compost are black and roughly 5 to 8 inch long as adults. Their wings are smoky black. When at repose, their wings are draped over the back. There are apparent sections in the first abdominal segment. Adults eat and lay eggs on food waste, particularly in damp circumstances. In two days, the adults will emerge, mate, and die. The adult flies are black and are often confused for black wasps. They do not bite or spread infection since their legs are hairless.

The existence of the black soldier fly is intriguing because it can be utilized to address many of the issues associated with massive waste accumulations in restricted

livestock feeding activities (Sheppard and Newton, 1994). Black soldier flies are believed to be capable of replacing standard chicken feed components with a significant environmental effect, such as soybean meal (Newton et al., 2005). Because it is higher in protein (37% to 63%) and has a higher amino acid (AA) profile than soybean meal, BSFL may supply high-value nutrients (Barragan et al., 2017). Furthermore, it includes more lipids (15% to 49%), which may be separated and utilized to make biodiesel, while the remainder of the defatted foods could be used as a great source of protein for the food industry. BSFL also includes chitin, which is indigestible for monogastric animals and may impair protein digestion (Sánchez, 2014).

2.5 Enzyme

An enzyme is a biomolecule that can be produced biologically (naturally) or by other means (synthetically). Its primary role is to act as a catalyst, accelerating a certain chemical reaction while remaining unchanged in the process. Enzymes are typically protein molecules with a unique sequence of amino acids that fold to form a distinct three-dimensional structure that confers unique capabilities on the molecule (Smith, 2021).

Any protein that acts as a catalyst, increasing the reaction's rate, is referred to as an enzyme (Miller and Marie, 2003). Biochemical reactions do not proceed significantly in the absence of an enzyme. Enzymes function by binding molecules in such a way that they are retained in a precise geometric configuration that allows the response to occur (Miller and Marie, 2003). Catalysts are certain since they accelerate a certain type of concoction

reaction between two closely related mixtures known as substrates (Miller and Marie, 2003).

The feed, food, specialty, and detergent industries are the four commonly used catalyst enterprises. A compound is added to feed to consume fewer calories as feed added substances, promoting diet and metabolism and aids in enhancing feed fixes absorbability. There are six types of catalysts: hydrolases, oxidoreductases, transferases, lyases, isomerases, and ligases, and each class catalyzes a different type of reaction.

2.5.1 Protease

Proteases are a wide set of enzymes that catalyze the breakdown of peptide bonds in proteins and polypeptides. They are also known as proteinases or proteolytic enzymes. Proteases are classified as acid (pH 2.0 – 5.0), neutral (pH 7.0), or alkaline (pH 8.0 – 11.0) (Zhu et al., 2019). Plants, animals, and microbes may produce these enzymes under various situations, including high salt concentrations. Bromelain, chymotrypsin, papain, serrapeptase, and trypsin are some protease enzymes that can be found in supplements (Flores-Gallegos et al., 2019).

The interest in protease is growing because protease is now a critical component in many businesses that lead to progress and continuous innovative work. Protease can be found in plants such as papaya and pineapple, animals such as the pancreas and the digestive tract, and microorganisms. Microbial enzymes have gained popularity for their broad applications in ventures and medicine over the plant and animal catalysts because of their consistency, reactant movement, and simplicity of production by decreasing

processing times, low vitality input, cost viability, non-hazardous, and eco-friendly qualities (Singh et al., 2016).

2.5.2 Pineapple Peel

Pineapple peel is a by-product of pineapple processing that accounts for around 10% (w/w) of the weight of the original fruit. In industrial pineapple processing, almost 75 % of the fruit is not utilized and is discharged as waste, generating improper disposal, which can cause major environmental concerns. The most typical wastes are seeds, peels, husk, and pulp residues. As a result, in addition to acquiring the completed product, the necessity to create manufacturing methods that involve sustainable development utilizing wastes has grown in popularity over the years (Timofiecsyk and Pawlowsky, 2000). Pineapple waste has a dry matter composition of roughly 10%, with 96% organic matter and 4% inorganic substance. There is a significant concentration of biodegradable organic substances and suspended solids in the waste.

The use of pineapple peel wastes by transforming them into value-added products like manure or livestock feed is gaining popularity as a potential solution to their disposal issues. Pineapple fruit wastes can be used as a low-cost alternative feed for many livestock classes.

2.5.3 Bromelain

Bromelain, a proteolytic enzyme, is found in plants of the Bromeliaceae family, of which pineapple (*Ananas comosus*) is a well-known source. It is also present in pineapple wastes such as cores, peels, and leaves in lesser amounts than stems and fruits. Commercially accessible pineapple stem and fruit are derived from the pineapple stem and pulp. These wastes were often utilized as animal feed or fertilizer. The concentration of bromelain in the pineapple stem is high, necessitating its extraction since, unlike the pineapple fruit commonly consumed, the stem is a by-product and cheap.

2.5.4 Date Seed

Date seed is a waste from date fruit and generally contains about 10-15% of total date fruit mass, depending on its ripeness, variety, and grade. Date seed weights ranging from 0.5 g to 4 g, lengths from 1.2 to 3.6 cm, and widths from 0.6 to 1.3 cm. Date seed is typically oblong, ventrally fluted, and has a firm endosperm formed of a cellulose layer on the interior of the cell walls. Given that worldwide date fruit output exceeded 8 million tonnes in 2018, date seed provides a massive amount of trash (FAOSTAT 2018). According to a chemical composition study by Najjar et al., 2020, date seed includes 60-80% fiber, 4-14% oil, and a low protein.

Date seeds possess a high concentration of beneficial bioactive substances (Al–Farsi and Lee, 2011) and dietary fiber, making them ideal for producing fiber-rich foods (Hamada et al., 2002). Consequently, utilizing this low-cost agricultural by-product is

critical to the data industry in data-producing countries (Golshan et al., 2017). Date seeds have been the topic of several studies. This research has mostly focused on the chemical composition of date seeds.

CHAPTER 3

MATERIALS AND METHODS

3.1 Protein Hydrolysis

3.1.1 Materials and Apparatus

a) Material

The Source of 50g powder fatted fifth instar BSFL was from Miss Athirah, a postgraduate student of UMK Jeli Campus. BSFL is the main ingredient used in the experiment. For protein analysis, the material needed was Bovine Serum Albumin (BSA) powder, Bradford reagent, Disodium Phosphate (Na_2HPO_4), Monosodium phosphate (NaH_2PO_4), Sodium Hydroxide (NaOH), distilled water, Bromelain powder, date seed powder, pineapple peel powder and commercial protease (*Streptomyces griseus sp*.).

b) Apparatus

Apparatus used in the experiment were airtight zipper bags (A3 and A4), spatula, electronic weighing scale, drying oven, aluminium foil, beakers (25mL, 250mL, 500mL, and 1L), measuring cylinder (100mL and 500mL), micropipette (10µL, 100µL, and 1000µL), micropipette tips, falcon tube (50mL), specimen container (60mL), microcentrifuge tubes (2mL), centrifuge rack, test tube with cap (10mL), test tube rack, distilled water bottle, tissue, glove, face mask, plastic dropper (3mL), glass dropper, sprayer, media bottle (1000mL), cuvettes (2.5mL), Ultra-Visible Spectrophotometer, incubator shaker, micro-centrifuge machine, pH meter, vortex, and autoclave sterilizer.

3.1.2 Method

a) Experimental Design

Table 3.1 shows the experimental design of enzyme-treated black soldier fly larvae (BSFL), comprises different temperatures; 30°C, 45°C, and 60°C, and incubation times; T0 (0 hours), T1 (1 hour), T2 (3 hours), and T3 (5 hours). Weight of BSFL, the weight of each enzyme, the volume of distilled water, and the volume of Phosphate Buffer Solution (PBS) in each temperature were the same, which were 0.9g, 0.1g, 30mL, and 400µL, 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 Enzyme-treated BSFL

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

b) Protein Analysis

i) Preparation of Phosphate Buffer Solution in pH 8

To get the standard curve displayed in Table 3.2, the standard concentration of Bovine Serum Albumin (BSA) must be modified. The solution was added to the test tube, which had already been filled with Bradford reagent, PBS, and BSA. Following that, it was vortexed for 30 seconds and had to be left for 5 minutes. After 5 minutes, the absorbance was read using a UV-Vis Spectrophotometer at wavelength 595nm. Three replications were performed for each standard concentration of BSA. Then, a protein standard curve graph was created, with the standard concentration of BSA (μg/mL) on the x-axis and the absorbance value on the y-axis. The data collected in triplicate was then subjected to linear

regression, which was utilized to calculate the concentration of unknown samples. The correlation coefficient, R_2, should be near 1.00 to have a fit model. The protein hydrolysis of protease treated BSFL before and after treatment with various variables and parameters was estimated based on the protein standard curve.

Table 3.2: 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

3.2 SDS-PAGE

3.2.1 Material and Apparatus

a) Material

In this experiment, the material used is distilled water, 30% Acrylamide solution, stocking buffer, separating buffer, 10% Ammonium persulfate (APS), Tetramethyl ethylenediamine (TEMED), molecular weight protein marker, running buffer solution, Coomassie blue staining solution, de-staining solution, and sample solution.

b) Apparatus

Apparatus used in this experiment are glove, weighing scale, aluminium foil, spatula, measuring cylinder, microcentrifuge tube 1.5ml, microcentrifuge tube rack, micropipette 10µl, 100µl and 1000µl, micropipette tips, plastic dropper, beaker 5ml, and 10ml, casting frame, glass plate, 1mm spacer plate, square petri dish, comb, filter papers, tweezer, tank, microcentrifuge, dry bath incubator, rocking shaker, gel releaser, running module, and power supply.

3.2.2 Method

a) Preparation of Resolving and Stacking Gel

The component in table 3.3 and 3.4 was mixed in the beaker 10ml and 5ml. TEMED was added right before pouring the gel into the plate.

Table 3.3 Parameter for resolving gel preparation

Components	Amount (µl)
Distilled water	800
30% Acrylamide solution	4000
Separating Buffer	5000
10% Ammonium Persulfate solution	100
TEMED	10

Table 3.4 Parameter for stacking gel preparation

Components	Amount (µl)
Distilled water	1400
30% Acrylamide solution	500
Stacking Buffer	2000
10% Ammonium Persulfate solution	40
TEMED	4

b) Casting the Gel

A short plate and 1mm spacer plate was inserted into casting plates, and the casting plates were installed onto the casting stand. Before pouring the gel into the plate, we need to check the seal by pipetting distilled water halfway up the plate. If there is no leaking, distilled water was removed using filter paper. After that, the resolving gel mixture was added with 10µl TEMED and immediately pipetted in between casting plates using micropipette up to the line marked on the plate and let the resolving gel polymerize for 1 hour. The bubbles on the plate were removed using distilled water. After the resolving gel was polymerized, the stacking gel mixture was added with 4µl TEMED and mixed gently and quickly pipetted stacking gel solution on top of the resolving gel in casting plate, filling until the top of the plate the bubble was removed using distilled water. The comb was inserted carefully in the casting plate to avoid the bubbles trapped in the plates and allow the gel to polymerize for 30 to 50 minutes.

c) Running the SDS-PAGE Gel

After the gel was formed, the casting plates were unclipped from the casting stand, and the comb was removed. The plate was inserted into the running module, and the running module was placed into the tank. The running buffer solution was filled into the running module between two plates until wells on the two plates were fully submerged. Then, fill the buffer solution halfway in the tank. Molecular weight protein marker and

sample solution were carefully pipetted into each well using a micropipette. After that, the power cable was attached to the tank and power supply with matching red and black slots. The power was set for 200 volts and ran for 2 hours. When the blue dyes had reached close to the bottom of the gel, the run was stopped. The power supply was unplugged, and the running module was removed from the tank. The gel was removed from the glass plate by using a gel releaser.

After that, the gel was transferred into a square petri dish. In the petri dish, the Coomassie blue staining solution was inserted. Petri dish was placed on the rocking shaker overnight (10 – 15 hours). The gel was removed from the petri dish overnight using a tweezer and was transferred to another petri dish containing de-staining solution until a band on the gel was visible. When de-staining was complete, the gel was stored in distilled water and was observed.

3.3 Statistical Analysis

The findings of enzymes-treated BSFL concentration before and after treatment were interpreted using IBM SPSS Statistics 26 software utilizing a Factorial in Completely Randomized Design (CRD) of Analysis of Variance (ANOVA) with a significant difference ($p<0.05$). Factorial is performed when two or more variables need to be analyzed, whereas CRD is utilized in a laboratory experiment with homogeneous experimental units where environmental influences may be readily controlled. The average and standard deviation were calculated and tabulated. To observe mean between homogeneous subsets of groups of treatments whether they had significant difference or

not, it referred to the letter: a, b, and c, 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. The experiment data was graphed using Excel 2016 software since the data set is easy to analyze.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Protein Analysis

In this study, the effect of temperature and incubation time towards the protein hydrolysis of enzyme-treated BSFL 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 0.9789 which is close to 1.00.

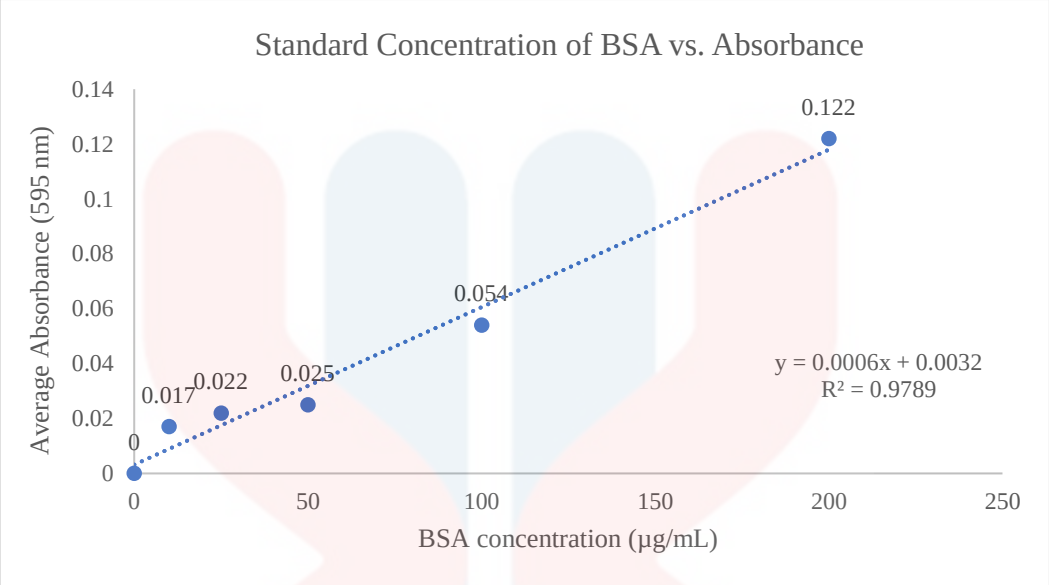

Figure 4.1 Protein Standard Curve

Figure 4.2, 4.3, 4.4, and 4.5 shows the graph of the percentage of protein hydrolysis versus incubation times; T1 (0 hours), T2 (1 hour), T3 (3 hours), T4 (5 hours) of commercial protease, pineapple peel, bromelain and date seed that treated with BSFL at different temperature; 30℃, 45℃, and 60℃.

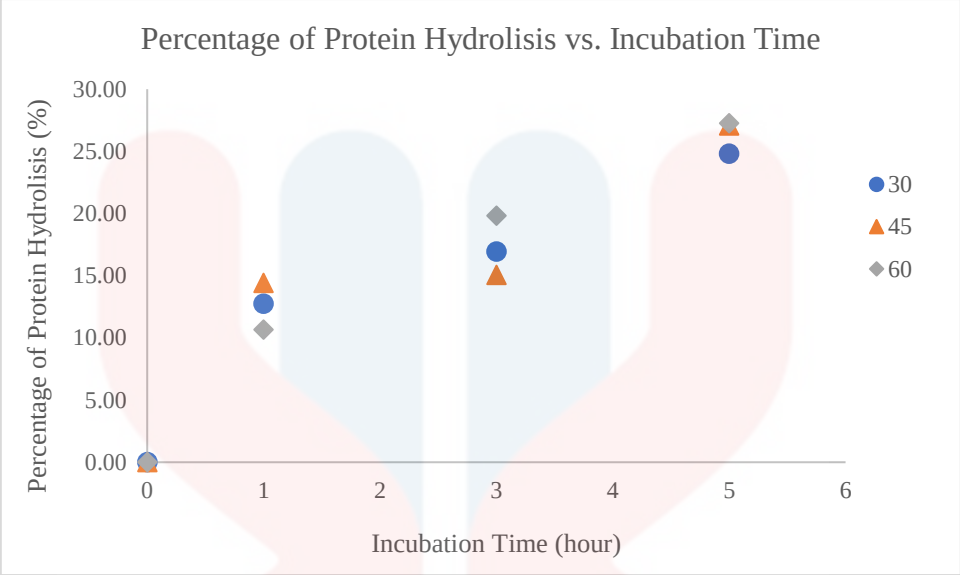

Figure 4.2: Percentage of protein hydrolysis vs. Incubation time of protease-treated BSFL

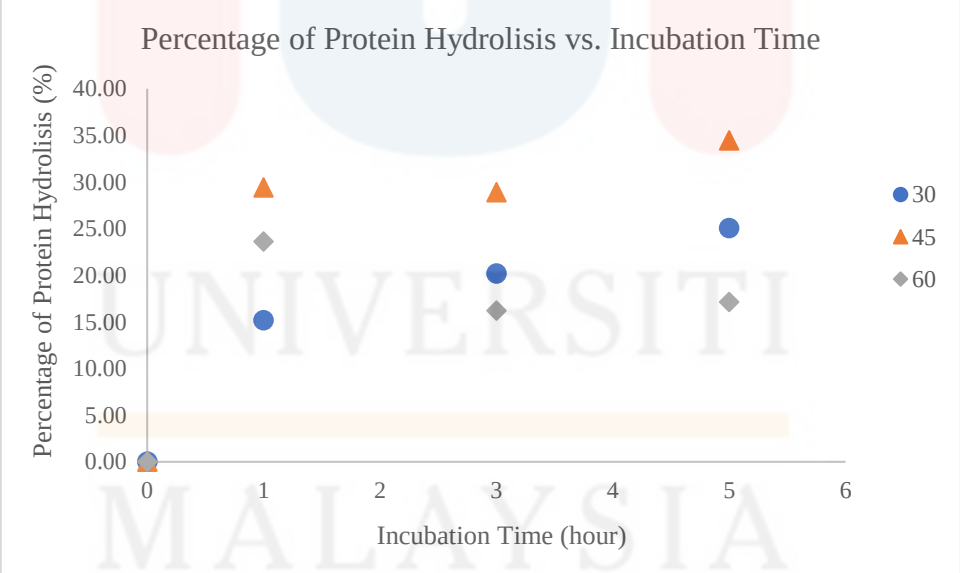

Figure 4.3: Percentage of protein hydrolysis vs. incubation time of pineapple peel treated with BSFL

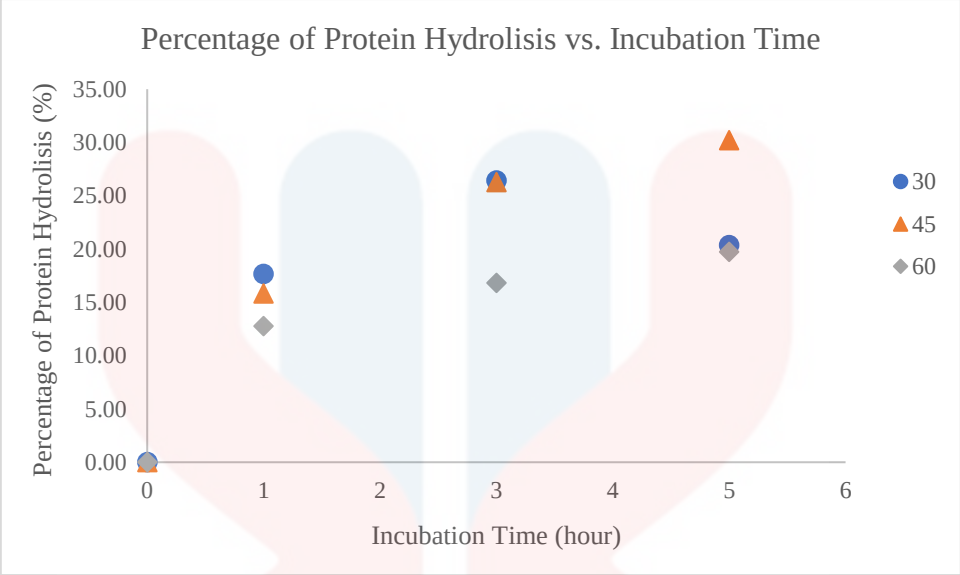

Figure 4.4: Percentage of protein hydrolysis vs. incubation time of Bromelain treated with BSFL

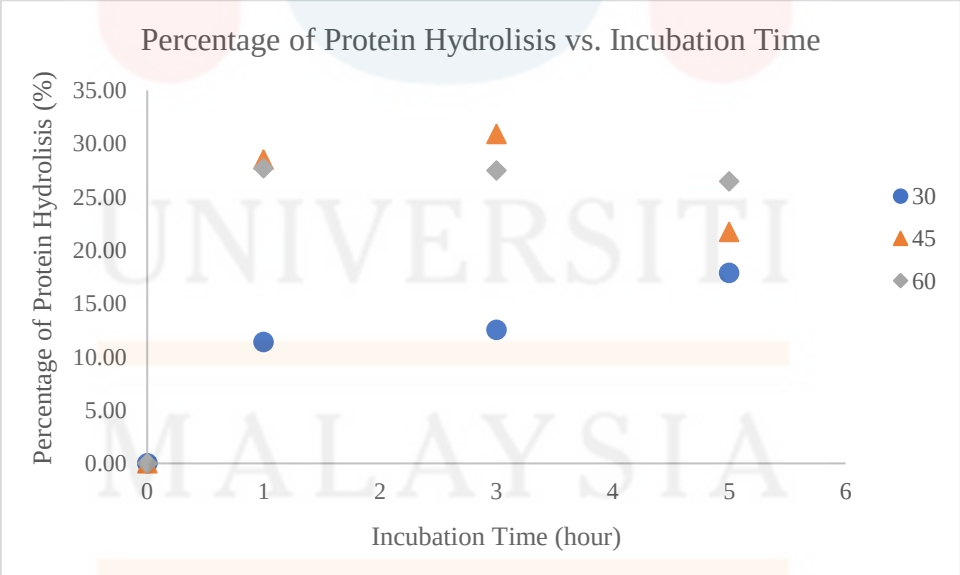

Figure 4.5: Percentage of protein hydrolysis vs. incubation time of date seed treated with BSFL

For protease-treated BSFL, at 30℃, 45°C, and 60°C, there is a significant difference between the incubation times at each temperature in which p=0.01 ($p<0.05$), p=0.00 ($p<0.05$) and p=0.02 ($p<0.05$) respectively. For pineapple peel treated with BSFL, bromelain treated with BSFL, and date seed treated with BSFL, at 30℃, 45°C, and 60°C, there is a significant difference between the incubation times at all group temperatures which is p=0.00 ($p<0.05$).

Based on Figure 4.2, the highest percentage of protein hydrolysis of protease-treated BSFL at 30°C for hours 5 which is 25%, while at 45℃ and 60°C shares the same highest percentage of protein availability that also at hours 5 (27%). Next, in Figure 4.3, the highest percentage of pineapple peel treated with BSFL is at 45℃ for 1 hour, 3 hours, and 5 hours which is 29%, 29%, and 34% respectively. Figure 4.4 describes the percentage of protein hydrolysis of bromelain treated with BSFL and the highest percentage is at 45℃ for hour 5 which is 30%. Lastly, for the percentage of protein hydrolysis of date seed treated with BSFL as stated in Figure 4.5, 45℃ for hour 3 are the highest which is 31%.

Table 4.1, 4.2, 4.3 and 4.4 shows the concentration of commercial protease, pineapple peel, Bromelain, and date seed treated with BSFL in triplicate and its mean ± standard deviation at three different temperatures; 30°C, 45°C, and 60°C, and four different incubation times; T1 (0 hours), T2 (1 hour), T3, (3 hours), and T4 (5 hours).

Table 4.1: Concentration and mean ± standard deviation of protease- treated with BSFL

Temperature (°C)	Incubation time (hrs)	Concentration of protease-treated BSFL (µg/mL)	Mean ± Standard deviation of protease-treated BSFL
30	0	1559.67 1499.67 1453.00	1504.11±53.47[a]
	1	1653.00 1703.00 1731.33	1695.78±39.66[ab]
	3	1688.00 1778.00 1811.33	1759.11±63.80[ab]
	5	1823.00 1739.67 2073.00	1878.56±173.47[b]
45	0	1878.00 1789.67 1779.67	1815.78±54.12[a]
	1	1998.00 2081.33 2156.33	2078.55±79.20[b]
	3	2116.33 2121.33 2031.33	2089.66±50.58[b]
	5	2244.67 2431.33 2246.33	2307.44±107.29[c]
60	0	1896.33 1811.33 1923.00	1876.89±58.32[a]
	1	1759.67 2304.67 2164.67	2076.34±283.03[ab]
	3	2386.33 2244.67 2256.33	2295.78±78.64[ab]
	5	2219.67 2451.33 2494.67	2388.56±147.86[b]

Table 4.2: Concentration and mean ± standard deviation of pineapple peel treated with BSFL

Temperature (°C)	Incubation time (hrs)	The concentration of pineapple peel treated with BSFL (µg/mL)	Mean ± Standard deviation of pineapple peel treated with BSFL
30	0	1966.33 2048.00 2174.67	2063.00±104.98[a]
	1	2474.67 2206.33 2449.67	2376.89±148.24[b]
	3	2536.33 2336.33 2568.00	2480.22±125.61[b]
	5	2588.00 2641.33 2509.67	2579.67±66.22[b]
45	0	2044.67 2011.33 1996.33	2017.44±24.74[a]
	1	2428.00 2688.00 2816.33	2644.11±197.85[b]
	3	2496.33 2558.00 2751.33	2601.89±133.04[b]
	5	2724.67 2699.67 2716.33	2494.25±12.73[b]
60	0	2148.00 2171.33 2076.33	2131.89±49.51[a]
	1	2856.33 2669.67 2378.00	2634.67±241.08[b]
	3	2444.67 2588.00 2541.33	2524.67±73.10[b]
	5	2549.67 2524.67 2414.67	2496.67±71.82[b]

Table 4.3: Concentration and mean ± standard deviation of Bromelain treated with BSFL

Temperature (°C)	Incubation time (hrs)	The concentration of Bromelain treated with BSFL (µg/mL)	Mean ± Standard deviation of Bromelain treated with BSFL
30	0	2216.33 2086.33 2124.67	2142.44±66.80[a]
	1	2489.67 2499.67 2574.67	2521.34±46.46[b]
	3	2796.33 2658.00 2674.67	2709.67±75.51[b]
	5	2709.67 2474.67 2453.00	2545.78±142.35[b]
45	0	2216.33 2086.33 2124.67	2200.78±17.82[a]
	1	2489.67 2499.67 2574.67	2551.89±98.34[ab]
	3	2796.33 2658.00 2674.67	2779.67±249.94[b]
	5	2709.67 2474.67 2453.00	2865.78±77.50[b]
60	0	2216.33 2086.33 2124.67	2230.78±91.84[a]
	1	2489.67 2499.67 2574.67	2515.78±25.07[b]
	3	2796.33 2658.00 2674.67	2654.11±25.07[b]
	5	2709.67 2474.67 2453.00	2670.78±108.04[b]

Table 4.4: Concentration and mean ± standard deviation of date seed treated with BSFL

Temperature (°C)	Incubation time (hrs)	The concentration of date seed treated with BSFL (µg/mL)	Mean ± Standard deviation of date seed treated with BSFL
30	0	2306.33 2226.33 2324.67	2285.78±52.29[a]
	1	2661.33 2496.33 2483.00	2546.89±99.33[ab]
	3	2629.67 2596.33 2493.00	2573.00±71.26[b]
	5	2728.00 2834.67 2519.67	2694.11±160.21[b]
45	0	2306.33 2226.33 2324.67	2081.33±39.30[a]
	1	2661.33 2496.33 2483.00	2674.67±230.15[b]
	3	2629.67 2596.33 2493.00	2724.67±118.51[b]
	5	2728.00 2834.67 2519.67	2532.44±132.02[b]
60	0	2306.33 2226.33 2324.67	2148.00±57.83[a]
	1	2661.33 2496.33 2483.00	2743.00±87.39[b]
	3	2629.67 2596.33 2493.00	2786.33±36.09[b]
	5	2728.00 2834.67 2519.67	2715.78±184.70[b]

Table 4.5: Percentage of protein hydrolysis and mean ± standard deviation of pineapple peel treated with BSFL at hours 5

Temperature (°C)	Percentage of protein hydrolysis (%)	Mean ± Standard deviation for the concentration of pineapple peel treated with BSFL
30	25	2579.67±66.22[b]
45	34	2494.25±12.73[b]
60	17	2496.67±71.82[b]

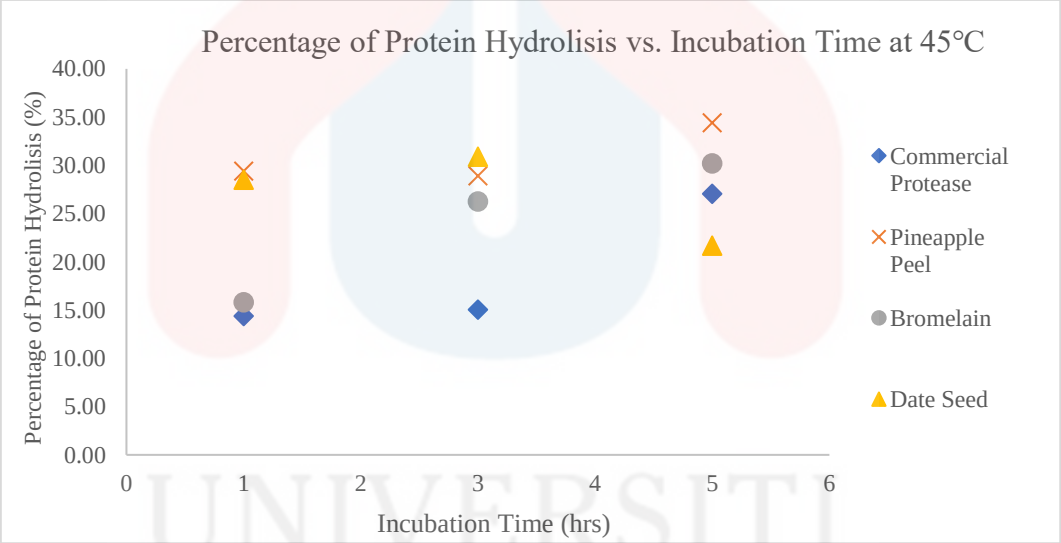

Figure 4.6: Percentage of protein availability vs. incubation time of enzyme-treated BSFL at 45°C

Based on Table 4.5 and Figure 4.6, pineapple peel treated with BSFL at hours 5, at the temperature of 45°C has the highest percentage of protein hydrolysis compared to at the temperature 30°C and 60°C which are 34%, 25%, and 17% respectively.

Referred in Table 4.5, pineapple peel treated with BSFL at 45°C has the highest percentage of protein hydrolysis (34%). The treatment was done at the same temperature with untreated, commercial protease, bromelain, and date seed. Through this study, it can determine the best enzyme to use to have the highest percentage of protein hydrolysis. The comparison is made between the commercial protease, pineapple peel, bromelain, and date seed as the type of enzymes used to determine the acceleration of protein hydrolysis reaction.

There is a significant difference in the incubation times between all groups of treatment in Table 4.6 in which $p=0.00$ ($p<0.05$). For the commercial protease, the highest percentage of protein hydrolysis is at hours 5 (27%), for pineapple peel, the highest percentage of protein hydrolysis is at hours 5 (34%), for bromelain, the highest percentage of protein hydrolysis is at hours 5 (30%) and for date seed, the highest percentage of protein hydrolysis is at hours 3 (31%).

Table 4.6 shows the concentration of enzyme-treated BSFL in triplicate and its mean ± standard deviation at the temperature of 45°C in untreated BSFL and four different types of enzyme used; commercial protease, pineapple peel, bromelain, and date seed in four different incubation times, T1 (0 hours) (control), T2 (1 hour), T3 (3 hours), and T4 (5 hours).

Table 4.6: Concentration and mean ± standard deviation of enzyme-treated BSFL at 45°C

Temperature (℃)	Incubation time (hrs)	The concentration of enzyme-treated BSFL (µg/mL)	Mean ± Standard deviation For concentration of enzyme-treated BSFL
45 (Untreated)	0	2368.00 2294.67 2174.67	2279.11±97.60[a]
	1	2808.00 2894.67 2564.67	2755.78±171.09[ab]
	3	2634.67 2761.33 2646.33	2680.78±70.00[b]
	5	3074.67 2503.00 2854.67	2810.78±288.35[b]
45 (Commercial Protease)	0	1878.00 1789.67 1779.67	1815.78±54.12[a]
	1	1998.00 2081.33 2156.33	2078.55±79.20[b]
	3	2116.33 2121.33 2031.33	2089.66±50.58[b]
	5	2244.67 2431.33 2246.33	2307.44±107.29[c]
45 (Pineapple Peel)	0	2044.67 2011.33 1996.33	2017.44±24.74[a]
	1	2428.00 2688.00 2816.33	2644.11±197.85[b]
	3	2496.33 2558.00 2751.33	2601.89±133.04[b]
	5	2724.67 2699.67 2716.33	2494.25±12.73[b]
45 (Bromelain)	0	2216.33 2086.33 2124.67 2489.67	2200.78±17.82[a]

	1	2499.67 2574.67 2796.33	2551.89±98.34[ab]
	3	2658.00 2674.67 2709.67	2779.67±249.94[b]
	5	2474.67 2453.00	2865.78±77.50[b]
45 (Date Seed)	0	2306.33 2226.33 2324.67	2081.33±39.30[a]
	1	2661.33 2496.33 2483.00	2674.67±230.15[b]
	3	2629.67 2596.33 2493.00	2724.67±118.51[b]
	5	2728.00 2834.67 2519.67	2532.44±132.02[b]

4.2 SDS-PAGE

The SDS-PAGE profile of protein hydrolysis of the untreated BSFL at 45℃ at hour 5 (as control) (UT), date seed treated with BSFL at 45℃ at hour 3 (DS) and pineapple peel treated with BSFL at 45℃ at hour 5 (PP) are shown in Figure 4.7.

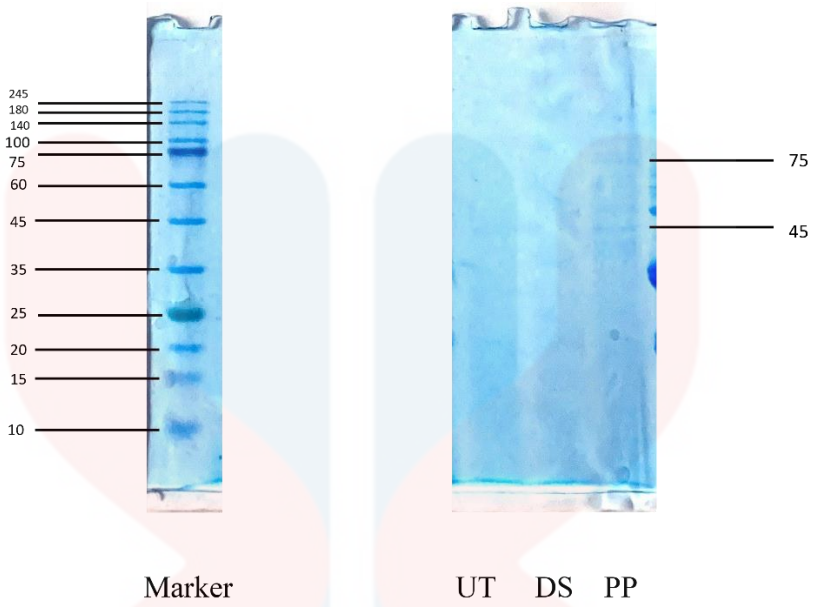

Figure 4.7: SDS-PAGE analysis of enzyme-treated BSFL; where UT = untreated BSFL, DS = date seed treated BSFL, and PP = Pineapple peel treated BSFL

In Figure 4.7, the image of the marker from the same gel was put on the side of the protein extract to help determine the bands. SDS–PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) is a form of electrophoresis that is often used to separate proteins based on their molecular weights. SDS is used to reduce the impact of a protein's structure and charge when it migrates or travels across the gel. As we can see in Figure 4.7, the band at pineapple peel treated with BSFL occurs at 45kDa and 75kDa. For another two protein samples, the band did not appear. It is because of some errors or factors that influence the appearance of the band such as lower denaturing temperature of protein samples, incorrect gel concentration, not enough protein was loaded on the gel, and many more.

Higher denaturation temperatures may produce more protein disintegration, resulting in more SDS binding and a more negatively charged and bigger protein. Given its size, one would infer that the protein is now migrating more slowly as a consequence of the filtering effect, causing the protein peaks to move to a higher migration time and hence a larger molecular weight estimate (Weber & Osborn, 1969).

Limitations SDS-PAGE is a reliable technique for determining molecular weight. Denaturing samples, decreasing proteins, leveling the charge-to-mass ratio, and electrophoresing under certain circumstances may all help to reduce protein-to-protein variance. A significant restriction of SDS-PAGE is the intentional denaturation of proteins before electrophoresis. Enzymatic activity, protein-protein interactions, and the identification of protein cofactors, for example, cannot be identified on SDS-PAGE-purified proteins (Nowakowski, 2014).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

To conclude, the preliminary research determined various data about the effects of different temperatures and incubation times on protein hydrolysis by treating BSFL with a different kind of enzyme.

The percentage of protein hydrolysis of enzyme-treated BSFL before and after treatment was analyzed, and it was discovered that at hour 5, 45°C of pineapple peel treated with BSFL has the highest percentage of protein hydrolysis, which is 34%, when compared to the other incubation times and temperatures, and that pineapple peel is better than other enzymes because it hydrolyzes protein faster.

Statistical analysis employing the Tukey Post Hoc Test in Factorial in CRD of ANOVA was effective in verifying the differences that occurred between treatment groups. There is a substantial difference in temperature between 30°C, 45°C, and 60°C, with

p=0.00 ($p<0.05$). There is a significant difference in the incubation times T1, T2, T3, and T4 for the temperatures 30°C, 45°C, and 60°C, with p=0.00 ($p<0.05$).

By studying the SDS-PAGE, the relative molecular weight of the protein sample was estimated by choosing the highest percentage of protein samples got from protein hydrolysis.

5.2 Recommendations

A future study might involve a 60-day feeding trial to evaluate broiler chicken growth performance, feed conversion rate, survival rate, and mortality rate by designing feed formulations and substituting enzyme-treated BSFL for protein sources.

Next, protein samples can be analyzed using mass spectrometry. Mass spectrometry is a technique used in analytical chemistry for determining the mass-to-charge ratio (m/z) of one or more molecules in a sample. Often, these data may also be utilized to determine the precise molecular weights of the sample components. Typically, mass spectrometers are used to measure the molecular weight of unknown substances, quantify known compounds, and analyze the structure and chemical characteristics of molecules.

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APPENDIX A

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
30	0	2099.1100	155.58048	3
	1	2547.4433	107.31812	3
	3	2792.4433	78.76242	3
	5	2738.0000	146.06587	3
	Total	2544.2492	304.27542	12
Total	0	2099.1100	155.58048	3
	1	2547.4433	107.31812	3
	3	2792.4433	78.76242	3
	5	2738.0000	146.06587	3
	Total	2544.2492	304.27542	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	891896.431[a]	3	297298.810	18.798	.001
Intercept	77678445.865	1	77678445.865	4911.599	.000
Temperature	.000	0	.	.	.
Incubation_time	891896.431	3	297298.810	18.798	.001
Temperature * Incubation_time	.000	0	.	.	.
Error	126522.445	8	15815.306		
Total	78696864.740	12			
Corrected Total	1018418.875	11			

a. R Squared = .876 (Adjusted R Squared = .829)

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

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2099.1100	
1	3		2547.4433
5	3		2738.0000
3	3		2792.4433
Sig.		1.000	.157

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 15815.306.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.3: Homogeneous subsets by Tukey HSD at 30°C of untreated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
45	0	2279.1133	97.59933	3
	1	2755.7800	171.08535	3
	3	2680.7767	70.00442	3
	5	2810.7800	288.35117	3
	Total	2631.6125	265.64838	12
Total	0	2279.1133	97.59933	3
	1	2755.7800	171.08535	3
	3	2680.7767	70.00442	3
	5	2810.7800	288.35117	3
	Total	2631.6125	265.64838	12

Figure A.4: Descriptive statistics with mean and standard deviation for incubation time at 45°C of untreated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	522574.017[a]	3	174191.339	5.493	.024
Intercept	83104612.202	1	83104612.202	2620.711	.000
Temperature	.000	0	.	.	.
Incubation_time	522574.017	3	174191.339	5.493	.024
Temperature * Incubation_time	.000	0	.	.	.
Error	253685.682	8	31710.710		
Total	83880871.900	12			
Corrected Total	776259.698	11			

a. R Squared = .673 (Adjusted R Squared = .551)

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

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2279.1133	
3	3	2680.7767	2680.7767
1	3		2755.7800
5	3		2810.7800
Sig.		.093	.808

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 31710.710.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6: Homogeneous subsets by Tukey HSD at 45°C of untreated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
60	0	2449.6667	58.40139	3
	1	2720.7767	217.19572	3
	3	2605.7767	203.68930	3
	5	2838.0000	38.11219	3
	Total	2653.5550	198.68407	12
Total	0	2449.6667	58.40139	3
	1	2720.7767	217.19572	3
	3	2605.7767	203.68930	3
	5	2838.0000	38.11219	3
	Total	2653.5550	198.68407	12

Figure A.7: Descriptive statistics with mean and standard deviation for incubation time at 60°C of untreated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	247175.796[a]	3	82391.932	3.524	.069
Intercept	84496249.656	1	84496249.656	3613.786	.000
Temperature	.000	0	.	.	.
Incubation_time	247175.796	3	82391.932	3.524	.069
Temperature * Incubation_time	.000	0	.	.	.
Error	187053.141	8	23381.643		
Total	84930478.593	12			
Corrected Total	434228.937	11			

a. R Squared = .569 (Adjusted R Squared = .408)

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

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1
0	3	2449.6667
3	3	2605.7767
1	3	2720.7767
5	3	2838.0000
Sig.		.057

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 23381.643.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.6: Homogeneous subsets by Tukey HSD at 60℃ of untreated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
30	0	1504.1133	53.47363	3
	1	1695.7767	39.66144	3
	3	1759.1100	63.79809	3
	5	1878.5567	173.47083	3
	Total	1709.3892	164.43550	12
Total	0	1504.1133	53.47363	3
	1	1695.7767	39.66144	3
	3	1759.1100	63.79809	3
	5	1878.5567	173.47083	3
	Total	1709.3892	164.43550	12

Figure A.10: Descriptive statistics with mean and standard deviation for incubation time at 30°C of protease-treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	220239.817[a]	3	73413.272	7.609	.010
Intercept	35064135.877	1	35064135.877	3634.080	.000
Temperature	.000	0	.	.	.
Incubation_time	220239.817	3	73413.272	7.609	.010
Temperature * Incubation_time	.000	0	.	.	.
Error	77189.570	8	9648.696		
Total	35361565.265	12			
Corrected Total	297429.387	11			

a. R Squared = .740 (Adjusted R Squared = .643)

Figure A.1: Significant difference between all incubation times at 30°C of protease-treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	1504.1133	
1	3	1695.7767	1695.7767
3	3	1759.1100	1759.1100
5	3		1878.5567
Sig.		.052	.182

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 9648.696.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.12: Homogeneous subsets by Tukey HSD at 30°C of protease-treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
45	0	1815.7800	54.11558	3
	1	2078.5533	79.20151	3
	3	2089.6633	50.57997	3
	5	2307.4433	107.29221	3
	Total	2072.8600	193.26039	12
Total	0	1815.7800	54.11558	3
	1	2078.5533	79.20151	3
	3	2089.6633	50.57997	3
	5	2307.4433	107.29221	3
	Total	2072.8600	193.26039	12

Figure A.13: Descriptive statistics with mean and standard deviation for incubation time at 45°C of protease-treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	364302.698[a]	3	121434.233	20.873	.000
Intercept	51560982.955	1	51560982.955	8862.577	.000
Temperature	.000	0	.	.	.
Incubation_time	364302.698	3	121434.233	20.873	.000
Temperature * Incubation_time	.000	0	.	.	.
Error	46542.656	8	5817.832		
Total	51971828.309	12			
Corrected Total	410845.354	11			

a. R Squared = .887 (Adjusted R Squared = .844)

Figure A.14: Significant difference between all incubation times at 45°C of protease-treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	2	3
0	3	1815.7800		
1	3		2078.5533	
3	3		2089.6633	
5	3			2307.4433
Sig.		1.000	.998	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 5817.832.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.15: Homogeneous subsets by Tukey HSD at 45°C of protease-treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
60	0	1876.8867	58.31878	3
	1	2076.3367	283.03416	3
	3	2295.7767	78.63790	3
	5	2388.5567	147.85675	3
	Total	2159.3892	251.64620	12
Total	0	1876.8867	58.31878	3
	1	2076.3367	283.03416	3
	3	2295.7767	78.63790	3
	5	2388.5567	147.85675	3
	Total	2159.3892	251.64620	12

Figure A.16: Descriptive statistics with mean and standard deviation for incubation time at 60°C of protease-treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	473474.020[a]	3	157824.673	5.659	.022
Intercept	55955538.877	1	55955538.877	2006.385	.000
Temperature	.000	0	.	.	.
Incubation_time	473474.020	3	157824.673	5.659	.022
Temperature * Incubation_time	.000	0	.	.	.
Error	223109.900	8	27888.738		
Total	56652122.798	12			
Corrected Total	696583.920	11			

a. R Squared = .680 (Adjusted R Squared = .560)

Figure A.17: Significant difference between all incubation times at 60°C of protease-treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	1876.8867	
1	3	2076.3367	2076.3367
3	3	2295.7767	2295.7767
5	3		2388.5567
Sig.		.060	.180

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 27888.738.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.18: Homogeneous subsets by Tukey HSD at 60°C of protease-treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
30	0	2063.0000	104.97685	3
	1	2376.8900	148.23726	3
	3	2480.2200	125.61447	3
	5	2579.6667	66.22441	3
	Total	2374.9442	225.07371	12
Total	0	2063.0000	104.97685	3
	1	2376.8900	148.23726	3
	3	2480.2200	125.61447	3
	5	2579.6667	66.22441	3
	Total	2374.9442	225.07371	12

Figure A.19: Descriptive statistics with mean and standard deviation for incubation time at 30°C of pineapple peel treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	450921.757[a]	3	150307.252	11.310	.003
Intercept	67684317.537	1	67684317.537	5092.963	.000
Temperature	.000	0	.	.	.
Incubation_time	450921.757	3	150307.252	11.310	.003
Temperature * Incubation_time	.000	0	.	.	.
Error	106318.185	8	13289.773		
Total	68241557.480	12			
Corrected Total	557239.943	11			

a. R Squared = .809 (Adjusted R Squared = .738)

Figure A.20: Significant difference between all incubation times at 30°C of pineapple peel treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2063.0000	
1	3		2376.8900
3	3		2480.2200
5	3		2579.6667
Sig.		1.000	.216

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 13289.773.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.21: Homogeneous subsets by Tukey HSD at 30℃ of pineapple peel treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
45	0	2017.4433	24.74305	3
	1	2644.1100	197.85044	3
	3	2601.8867	133.04428	3
	5	2713.5567	12.72865	3
	Total	2494.2492	308.02755	12
Total	0	2017.4433	24.74305	3
	1	2644.1100	197.85044	3
	3	2601.8867	133.04428	3
	5	2713.5567	12.72865	3
	Total	2494.2492	308.02755	12

Figure A.22: Descriptive statistics with mean and standard deviation for incubation time at 45℃ of pineapple peel treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	928451.049[a]	3	309483.683	21.485	.000
Intercept	74655346.865	1	74655346.865	5182.616	.000
Temperature	.000	0	.	.	.
Incubation_time	928451.049	3	309483.683	21.485	.000
Temperature * Incubation_time	.000	0	.	.	.
Error	115239.626	8	14404.953		
Total	75699037.540	12			
Corrected Total	1043690.675	11			

a. R Squared = .890 (Adjusted R Squared = .848)

Figure A.23: Significant difference between all incubation times at 45°C of pineapple peel treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2017.4433	
3	3		2601.8867
1	3		2644.1100
5	3		2713.5567
Sig.		1.000	.677

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 14404.953.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.24: Homogeneous subsets by Tukey HSD at 45°C of pineapple peel treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
60	0	2131.8867	49.50737	3
	1	2634.6667	241.07846	3
	3	2524.6667	73.10350	3
	5	2496.3367	71.82154	3
	Total	2446.8892	227.85334	12
Total	0	2131.8867	49.50737	3
	1	2634.6667	241.07846	3
	3	2524.6667	73.10350	3
	5	2496.3367	71.82154	3
	Total	2446.8892	227.85334	12

Figure A.25: Descriptive statistics with mean and standard deviation for incubation time at 60°C of pineapple peel treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	428944.078[a]	3	142981.359	8.047	.008
Intercept	71847199.127	1	71847199.127	4043.614	.000
Temperature	.000	0	.	.	.
Incubation_time	428944.078	3	142981.359	8.047	.008
Temperature * Incubation_time	.000	0	.	.	.
Error	142144.515	8	17768.064		
Total	72418287.720	12			
Corrected Total	571088.593	11			

a. R Squared = .751 (Adjusted R Squared = .658)

Figure A.26: Significant difference between all incubation times at 60°C of pineapple peel treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2131.8867	
5	3		2496.3367
3	3		2524.6667
1	3		2634.6667
Sig.		1.000	.604

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 17768.064.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.27: Homogeneous subsets by Tukey HSD at 60℃ of pineapple peel treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
30	0	2142.4433	66.79759	3
	1	2521.3367	46.45787	3
	3	2709.6667	75.51405	3
	5	2545.7800	142.34587	3
	Total	2479.8067	230.28649	12
Total	0	2142.4433	66.79759	3
	1	2521.3367	46.45787	3
	3	2709.6667	75.51405	3
	5	2545.7800	142.34587	3
	Total	2479.8067	230.28649	12

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

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	518180.580[a]	3	172726.860	21.203	.000
Intercept	73793293.249	1	73793293.249	9058.568	.000
Temperature	.000	0	.	.	.
Incubation_time	518180.580	3	172726.860	21.203	.000
Temperature * Incubation_time	.000	0	.	.	.
Error	65169.941	8	8146.243		
Total	74376643.769	12			
Corrected Total	583350.520	11			

a. R Squared = .888 (Adjusted R Squared = .846)

Figure A.29: Significant difference between all incubation times at 30°C of bromelain treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2142.4433	
1	3		2521.3367
5	3		2545.7800
3	3		2709.6667
Sig.		1.000	.124

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 8146.243.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.30: Homogeneous subsets by Tukey HSD at 30°C of bromelain treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
45	0	2200.7767	17.81905	3
	1	2551.8900	98.33614	3
	3	2779.6667	246.94012	3
	5	2865.7767	77.50342	3
	Total	2599.5275	293.53307	12
Total	0	2200.7767	17.81905	3
	1	2551.8900	98.33614	3
	3	2779.6667	246.94012	3
	5	2865.7767	77.50342	3
	Total	2599.5275	293.53307	12

Figure A.31: Descriptive statistics with mean and standard deviation for incubation time at 45°C of bromelain treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	793830.890[a]	3	264610.297	13.751	.002
Intercept	81090518.679	1	81090518.679	4213.933	.000
Temperature	.000	0	.	.	.
Incubation_time	793830.890	3	264610.297	13.751	.002
Temperature * Incubation_time	.000	0	.	.	.
Error	153947.433	8	19243.429		
Total	82038297.002	12			
Corrected Total	947778.323	11			

a. R Squared = .838 (Adjusted R Squared = .777)

Figure A.32: Significant difference between all incubation times at 45°C of bromelain treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2200.7767	
1	3	2551.8900	2551.8900
3	3		2779.6667
5	3		2865.7767
Sig.		.058	.092

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 19243.429.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.33: Homogeneous subsets by Tukey HSD at 45°C of bromelain treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
60	0	2230.7767	91.83746	3
	1	2515.7800	25.07481	3
	3	2654.1100	150.01365	3
	5	2670.7767	108.04295	3
	Total	2517.8608	204.40305	12
Total	0	2230.7767	91.83746	3
	1	2515.7800	25.07481	3
	3	2654.1100	150.01365	3
	5	2670.7767	108.04295	3
	Total	2517.8608	204.40305	12

Figure A.34: Descriptive statistics with mean and standard deviation for incubation time at 60°C of bromelain treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	373106.208[a]	3	124368.736	11.505	.003
Intercept	76075478.112	1	76075478.112	7037.470	.000
Temperature	.000	0	.	.	.
Incubation_time	373106.208	3	124368.736	11.505	.003
Temperature * Incubation_time	.000	0	.	.	.
Error	86480.482	8	10810.060		
Total	76535064.802	12			
Corrected Total	459586.690	11			

a. R Squared = .812 (Adjusted R Squared = .741)

Figure A.35: Significant difference between all incubation times at 60°C of bromelain treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2230.7767	
1	3		2515.7800
3	3		2654.1100
5	3		2670.7767
Sig.		1.000	.329

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10810.060.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.36: Homogeneous subsets by Tukey HSD at 60°C of bromelain treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
30	0	2285.7767	52.29262	3
	1	2546.8867	99.33468	3
	3	2573.0000	71.25931	3
	5	2694.1133	160.21074	3
	Total	2524.9442	179.01878	12
Total	0	2285.7767	52.29262	3
	1	2546.8867	99.33468	3
	3	2573.0000	71.25931	3
	5	2694.1133	160.21074	3
	Total	2524.9442	179.01878	12

Figure A.37: Descriptive statistics with mean and standard deviation for incubation time at 30°C of date seed treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	265830.409[a]	3	88610.136	8.177	.008
Intercept	76504116.537	1	76504116.537	7059.648	.000
Temperature	.000	0	.	.	.
Incubation_time	265830.409	3	88610.136	8.177	.008
Temperature * Incubation_time	.000	0	.	.	.
Error	86694.533	8	10836.817		
Total	76856641.480	12			
Corrected Total	352524.943	11			

a. R Squared = .754 (Adjusted R Squared = .662)

Figure A.38: Significant difference between all incubation times at 30°C of date seed treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2285.7767	
1	3	2546.8867	2546.8867
3	3		2573.0000
5	3		2694.1133
Sig.		.060	.369

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 10836.817.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.39: Homogeneous subsets by Tukey HSD at 30°C of date seed treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
45	0	2081.3333	39.30041	3
	1	2674.6667	230.15293	3
	3	2724.6667	118.51170	3
	5	2532.4433	132.01522	3
	Total	2503.2775	292.91648	12
Total	0	2081.3333	39.30041	3
	1	2674.6667	230.15293	3
	3	2724.6667	118.51170	3
	5	2532.4433	132.01522	3
	Total	2503.2775	292.91648	12

Figure A.40: Descriptive statistics with mean and standard deviation for incubation time at 45°C of date seed treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	771824.806[a]	3	257274.935	11.968	.003
Intercept	75196778.904	1	75196778.904	3498.015	.000
Temperature	.000	0	.	.	.
Incubation_time	771824.806	3	257274.935	11.968	.003
Temperature * Incubation_time	.000	0	.	.	.
Error	171975.870	8	21496.984		
Total	76140579.580	12			
Corrected Total	943800.676	11			

a. R Squared = .818 (Adjusted R Squared = .749)

Figure A.41: Figure A.38: Significant difference between all incubation times at 45°C of date seed treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2081.3333	
5	3		2532.4433
1	3		2674.6667
3	3		2724.6667
Sig.		1.000	.427

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 21496.984.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.42: Homogeneous subsets by Tukey HSD at 45°C of date seed treated BSFL

Descriptive Statistics

Dependent Variable: Concentration_of_Protein_Hydrolysis

Temperature	Incubation_time	Mean	Std. Deviation	N
60	0	2148.0000	57.82896	3
	1	2743.0000	87.38529	3
	3	2786.3333	36.09117	3
	5	2715.7800	184.69636	3
	Total	2598.2783	287.84310	12
Total	0	2148.0000	57.82896	3
	1	2743.0000	87.38529	3
	3	2786.3333	36.09117	3
	5	2715.7800	184.69636	3
	Total	2598.2783	287.84310	12

Figure A.43: Descriptive statistics with mean and standard deviation for incubation time at 60°C of date seed treated BSFL

Tests of Between-Subjects Effects

Dependent Variable: Concentration_of_Protein_Hydrolysis

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	818598.789[a]	3	272866.263	23.525	.000
Intercept	81012603.570	1	81012603.570	6984.493	.000
Temperature	.000	0	.	.	.
Incubation_time	818598.789	3	272866.263	23.525	.000
Temperature * Incubation_time	.000	0	.	.	.
Error	92791.393	8	11598.924		
Total	81923993.751	12			
Corrected Total	911390.182	11			

a. R Squared = .898 (Adjusted R Squared = .860)

Figure A.44: Figure A.38: Significant difference between all incubation times at 60°C of date seed treated BSFL

Concentration_of_Protein_Hydrolysis

Tukey HSD[a,b]

Incubation_time	N	Subset 1	Subset 2
0	3	2148.0000	
5	3		2715.7800
1	3		2743.0000
3	3		2786.3333
Sig.		1.000	.852

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 11598.924.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Figure A.45: Homogeneous subsets by Tukey HSD at 60°C of date seed treated BSFL

APPENDIX B

Figure B.1: Date seed sample that used in this experiment before sieved into powder form

Figure B.2: Pineapple peel sample that used in this experiment before drying and sieved into powder form

Figure B.3: Sample was weighed using an electronic weighing scale

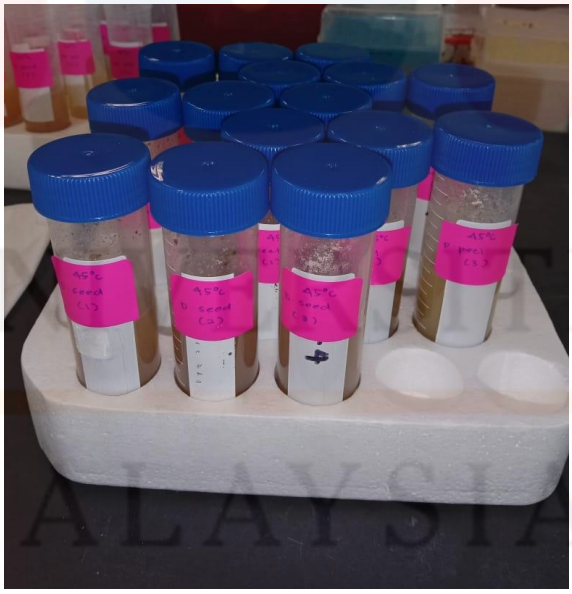

Figure B.4: The mixture of samples was inserted in a falcon tube before being placed in an incubation shaker

Figure B.5: Protein sample was prepared triplicate before start analyze in UV Spectrophotometer

Figure B.6: Protein sample was vortexed for 30 seconds

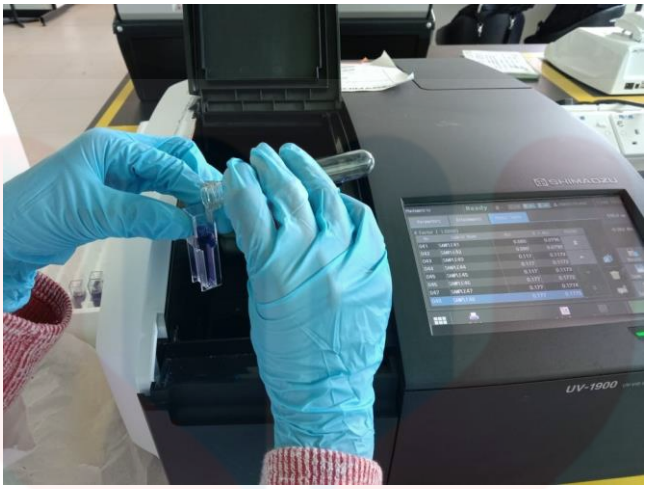

Figure B.7: The reading of the absorbance of protein sample was read using UV Spectrophotometer

Figure B.8: Preparation of stacking and resolving gel for SDS-PAGE

Figure B.9: Process of pipetting the gel into the casting plate for SDS-PAGE

Figure B.10: Process removing the gel from de-staining solution

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